Tackling the Diversity of Triple-Negative Breast Cancer

Nicholas C. Turner1,2 and Jorge S. Reis-Filho3,4

Abstract

Triple-negative breast cancer (TNBC) comprises a highly diverse collection of cancers. Here, we review this diversity both in terms of gene expression subtypes and the repertoire of genetic events. Transcriptomic analyses of TNBC have revealed at least six subtypes, with the luminal androgen receptor (luminal AR) or molecular apocrine cancers forming a distinct group within triple-negative disease. Distinct from the gene expression subtypes, a diverse set of genetic events have been described in TNBC, with a number of potentially targetable genetic events found although all at relatively low frequency. Clinical trials to define the clinical utility of therapies targeting these low-frequency events will require substantial screening efforts to identify sufficient patients. Set against the diversity of TNBC, clinical studies of patients with triple-negative disease will need to be either focused on molecularly defined subsets with upfront molecular stratification, or powered for a secondary endpoint analysis of a molecularly defined subset. Such approaches will be crucial to realize the potential of precision medicine for patients with TNBCs. Clin Cancer Res; 19(23); 6380–8. ©2013 AACR.

Introduction

Substantial progress has been made in the management of breast cancer, and this has been reflected in substantial improvements in breast cancer mortality over the last 30 years. Such improvements have at least, in part, come from the development of combination chemotherapy regimens (1), endocrine therapies (2, 3), and HER2-targeting therapies (4). Additional steady improvements in outcome, both in the adjuvant and metastatic settings, can be anticipated for both estrogen receptor (ER)–positive breast cancer (5) and for HER2-positive cancers (6), given the progress currently being made with targeted therapies for these breast cancer subtypes. For patients with triple-negative breast cancers (TNBC), however, it is probable that the improvements in survival have currently plateaued. Although the outcome of patients with this disease has significantly improved with adjuvant chemotherapy, which reduces the risk of death by approximately 30% (7), there has been limited progress in incorporating additional systemic therapies into the management of TNBCs. Consequently, patients with TNBCs are currently the subgroup of patients with breast cancer with the worst outcome (8). However, there are no agents currently in phase III registration trials specifically for this group of cancers (9), with a number of recent phase II studies of targeted therapies generating results that did not support full development specifically to treat patients with triple-negative disease (10, 11).

The development of high-throughput genomic methods, and the implementation of bioinformatic tools, has revealed the heterogeneity of breast cancer subtypes. These approaches have resulted in profound changes in our understanding of TNBC, and the realization that this operational term comprises a spectrum of different entities with distinct molecular characteristics that can potentially be targeted in the clinic. In this review, we seek to outline the current understanding of what TNBCs represent, in particular focusing on the diversity of subtypes of TNBC, and how this diversity will need to be embraced in the design of future clinical trials.

Pathologic Heterogeneity

Although this review focuses on the recent data that describe the genetic and gene expression subtypes of TNBC, there is important histopathologic diversity in TNBCs. The vast majority of TNBCs are high-grade, invasive ductal carcinomas of no special type that are characterized by marked degrees of nuclear pleomorphism, lack of tubule formation, and high mitotic rates. A substantial proportion of the cases display brisk lymphocytic infiltrate, areas of central necrosis, and pushing borders (12). Medullary features, as well as areas of focal metaplastic differentiation in the form of squamous and spindle cells, can be found in a subset of these tumors (13–15). TNBCs, however, comprise a spectrum of lesions, some of which should be considered separately from other TNBC. Adenoid cystic carcinomas (16) and secretory carcinomas (17) of the breast, both of which characteristically display a...
Molecular Diversity of TNBC

Gene expression subtypes of TNBC

Following the seminal studies of Perou and colleagues that described the basic subtypes of breast cancer as a whole, often referred to as "intrinsic subtypes" (23), a number of studies identified additional subtypes of breast cancer by using microarray-based class discovery analyses. Some of these breast cancer subtypes have either been exclusively composed of TNBCs or are enriched for cancers with this phenotype. These subtypes include (i) claudin-low tumors (24–26), which are reported to be enriched for cells with stem cell–like properties and features of epithelial-to-mesenchymal transition; (ii) IFN-rich cancers (27), which comprise tumors with a significantly better outcome than the remaining TNBCs; and (iii) molecular apocrine cancers (28, 29), which are characterized by AR pathway activation (27–30).

The major advancement was made in the seminal study by Lehmann and colleagues that integrated some of these previous observations into a comprehensive subtyping specifically of TNBCs. This study, which focused solely on TNBCs, led to the realization that TNBCs are composed of multiple molecular subtypes (31). Through the analysis of publically available data from 587 TNBCs, six reproducible gene expression subtypes of TNBC were identified (i.e., two basal-like, an immunomodulatory, a mesenchymal, a mesenchymal stem-like, and a luminal AR subtype; Fig. 1). Overreaching these subgroups are three basic subsets of cancers: basal-like cancers, mesenchymal-like cancers, and a luminal AR subset. Of the many important findings arising from this study, potentially the most important is the recognition of the luminal AR subtype. This subtype represents approximately 10% of TNBC and forms a distinct gene expression subgroup that shares many gene expression features of ER-positive luminal breast cancer (31).

Overlap between TNBC subtypes and "intrinsic subtypes" as defined by PAM50

There is a complicated issue of terminology between the triple-negative subtypes defined by Lehmann and colleagues and the overlap with the "intrinsic gene" expression subtypes first described by Perou and colleagues (23) and, later, developed into the PAM50 assay (32). Both the PAM50 subtypes (also known as "intrinsic subtypes"), which describe subtypes of breast cancer as a whole, and the TNBC subtypes described by Lehmann and colleagues include a subgroup named "basal-like." However, this term does not necessarily describe exactly the same group of cancers in both classifiers (33). Cancers that are basal-like in the PAM50 assay encompass TNBC basal-like subtypes as well as some of the tumors classified as immunomodulatory and mesenchymal-like as defined by the Lehmann TNBC subtypes (Fig. 1; ref. 34). Similarly, the standard histologic definitions of basal-like TNBC, including positivity for the EGF receptor (EGFR) or basal-like cytokeratins, likely identify both basal-like and subsets of mesenchymal-like TNBC subtypes (Fig. 2).

In the classification proposed by Lehmann and colleagues (31), basal-like TNBC subtypes have been further subdivided into two subtypes, both of which are characterized by high levels of proliferation-related genes. Those cancers with basal-like 1 phenotype are enriched not only for proliferation-related genes, but also for the expression of genes involved in the DNA damage response. The basal-like 2 subtype, in contrast, was shown to be associated with gene expression patterns enriched for genes related to growth factor signaling. Basal-like 1 cancer cell lines are enriched for sensitivity to specific DNA-damaging agents such as cisplatin (31), although initial preliminary clinical data did not identify a clear association between this subtype and sensitivity to neoadjuvant gemcitabine and carboplatin chemotherapy (35).

Mesenchymal-like TNBC subtypes have been further subdivided into two subtypes: mesenchymal-like and mesenchymal stem-like (31). Both subtypes are so named because of enrichment for gene expression patterns associated with epithelial-to-mesenchymal transition (36). The mesenchymal stem-like subtype, in particular, describes a similar group of cancers previously described as claudin-low (24), that have lower proliferation relative to those of basal-like TNBCs, and are enriched for the expression of genes associated with a cancer stem cell–like (or tumor-initiating cell-like) phenotype (24). Multiple growth factor pathways are expressed at high levels in mesenchymal-like TNBCs, with the expression of the FGF2 ligand being an important autocrine growth factor in these cancers (37). Metaplastic breast cancers,
especially those composed of spindle cells, may frequently fall in this subtype (25).

The immunomodulatory subtype is dominated by the gene expression consequences of lymphocytic infiltration, which dominates the gene expression subtype of the individual cancers. Consequently, this gene expression subtype likely includes cancers that biologically are of basal-like and mesenchymal-like subtypes. A further subtype of immunomodulatory cancers reflects brisk lymphocytic infiltration and is not dominated by the gene expression characteristics of the tumor. With the advent of massively parallel sequencing (also known as next-generation sequencing) has transformed our ability to interrogate tumor genomes and investigate the biology of TNBCs (40, 41). Two large studies have, so far, addressed the genetics of TNBC, and these have revealed the diversity of genetic events in TNBC (Table 1). The only common genetic event of TNBC is a mutation of TP53 that occurs in approximately 80% of cancers (40, 41), with all other mutations occurring at a relatively low frequency. Among the relative low-frequency events are a number of potential targetable driver genetic events that are enriched in TNBC. PTEN mutations and/or homozygous deletions are reported to be found in approximately 10% of TNBCs, EGFR amplification in approximately 5%, and FGFR2 amplification in approximately 4%, all enriched in TNBC compared with other breast cancer subtypes (40–42). Mutations found more commonly in ER/HER2–positive breast cancer, such as the PIK3CA mutations, are found in 7% to 10% of TNBCs (43). A dysfunctional retinoblastoma (RB) pathway is observed in a substantial proportion of cases. In contrast with ER-positive cancers where this pathway is activated through CCND1 amplification and gains of CDK4, the inactivation of the RB pathway in TNBCs is driven by mutation or loss of RB1 (~20% of cases) and CCNE1 amplification (~9% of cases; ref. 41).

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Genetic drivers

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Furthermore, the repertoire of somatic mutations found in TNBCs encompasses alterations in genes involved in
cytoskeletal organization and extracellular matrix interactions, although each individual gene is mutated at only a low frequency (40, 41), and a substantial proportion of TNBCs do not have targetable genetic events, as these are currently understood. Interestingly, the mutational repertoire of TNBCs shows similarities with that of high-grade serous ovarian cancers (44), suggesting that these cancers may share some similarities.

Table 1. Common genetic events in TNBC along with actionable targets with a frequency of greater than 2%

<table>
<thead>
<tr>
<th>Target</th>
<th>Approximate frequency in TNBC</th>
<th>Gene expression subtype enrichment</th>
<th>References</th>
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<tr>
<td>Potentially actionable</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PTEN mutation/deletion</td>
<td>10%</td>
<td>Nonluminal TNBC enriched in BRCA1 germline mutant cancers</td>
<td>(40, 41, 47)</td>
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<tr>
<td>PIK3CA mutation</td>
<td>8%</td>
<td>Luminal AR</td>
<td>(40, 41, 43)</td>
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<tr>
<td>EGFR amplification</td>
<td>4%</td>
<td>?Basal-like</td>
<td>(72)</td>
</tr>
<tr>
<td>HER2 mutation</td>
<td>~2%</td>
<td></td>
<td>(40, 41)</td>
</tr>
<tr>
<td>FGFR2 amplification</td>
<td>~4%</td>
<td>?Luminal AR TNBC</td>
<td>(42, 72)</td>
</tr>
<tr>
<td>Germline BRCA1/2 mutation</td>
<td>~10%</td>
<td>Basal-like 1 TNBC subtype in TNBC cell lines</td>
<td>(31, 40, 41)</td>
</tr>
<tr>
<td>Sporadic BRCA1/2 mutation</td>
<td>~5%</td>
<td></td>
<td>(40, 41)</td>
</tr>
<tr>
<td>Currently not actionable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 mutation</td>
<td>80%</td>
<td></td>
<td>(40, 41)</td>
</tr>
<tr>
<td>Myc amplification</td>
<td>40%</td>
<td></td>
<td>(72)</td>
</tr>
<tr>
<td>CCNE1 amplification</td>
<td>9%</td>
<td></td>
<td>(43, 72)</td>
</tr>
<tr>
<td>MCL1 amplification</td>
<td>20%</td>
<td></td>
<td>(72)</td>
</tr>
<tr>
<td>RB1 mutation or loss</td>
<td>20%</td>
<td></td>
<td>(40, 41)</td>
</tr>
<tr>
<td>USH2A mutation</td>
<td>9%</td>
<td></td>
<td>(40, 41)</td>
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Associations between gene expression subtypes and genetic drivers

Initial analyses are revealing intriguing associations between gene expression TNBC subtypes and specific genetic driver events (Fig. 2). The luminal AR subtype seems to be heavily enriched for PIK3CA mutations (31, 45). Luminal AR and ER-negative/HER2-amplified breast cancers likely share substantial similarity with regard to apocrine morphology, AR expression, and relative enrichment for PIK3CA mutations compared with other ER-negative breast cancers (41, 46). Potentially, the luminal AR subtype may be enriched for amplification events affecting alternative tyrosine kinase receptors. For example, the high level FGFR2-amplified breast cell lines, SUM52 and MFM223, are of the luminal AR phenotype (42).

Contrastingly, in nonluminal TNBCs (those of basal-like or mesenchymal-like histology) the phosphoinositide 3-kinase (PI3K) pathway is activated more frequently by PTEN loss, caused by inactivating mutations and/or deletions (41), and also, potentially, through loss of an alternative member of the PI3K pathway, INPP4B (41). Whether PTEN loss is enriched in a particular gene expression subtype is unknown, but it is interesting that there seems to be an association between germline mutation in BRCA1 and PTEN loss (47). Cancers arising in women with germline mutations in BRCA1 have a TNBC phenotype in 70% to 85% of cancers. BRCA1 mutant cancer cell lines are heavily enriched for the basal-like 1 TNBC subtype (31), although it is unknown if this association will also be observed in cancers.

Subtyping by genomic scar

TNBCs with BRCA1 or BRCA2 germline mutations are highly sensitive to both platinum chemotherapy and PARP inhibitors, agents that target the underlying DNA double-strand-break repair defect in these cancers (48, 49). Sporadic cancers that share a similar defect in DNA repair, or BRCAAness, have the potential also to be highly sensitive to these treatments (50). A number of groups have suggested that cancers defective in BRCA1/2 mutations have a characteristic pattern of genomic instability that is definable in terms of loss of heterozygosity (51, 52), patterns of deletions flanked by microhomology (53), or the presence and frequency of large-scale transitions assessed by microarray-based comparative genomic hybridization (54). These approaches have been assessed in small, independent studies, suggesting that these genomic classifiers may identify sporadic cancers that are more sensitive to platinum chemotherapy (35, 55), potentially identifying a group of sporadic cancers that have BRCAAness.

Challenges in the Realization of Precision Medicine in TNBC

Subtype defined or genetic driver defined?

The realization that TNBCs are highly heterogeneous presents a major challenge to the development of targeted therapy in this group of cancers. Subgrouping into more homogeneous entities will be required for the development of targeted therapies with two complementary classifications emerging: one based on gene expression subtypes and the other defined by the genetic (or epigenetic) events that have occurred in the cancer.

Gene expression subtypes have failed to make a clinical impact in many cancers, in part because tractable mutations superseded gene expression classification. Arguably, the best example of the use of a mutation-based rather than gene expression profiling–based taxonomy is non–small cell lung cancer, in which although gene expression subtypes have been identified and validated, the molecular classification of these cancers is based on the presence of specific driving genetic aberrations (e.g., EGFR mutations, ALK and ROS1 gene rearrangements) that predict response to specific targeted therapies (56). Many TNBCs, however, do not have an actionable mutation (41), as we currently define them. This emphasizes the potential importance of taking both mutation and gene expression subtypes forward in parallel, potentially to investigate subtype-specific therapies. In addition, the identification of synthetic lethal strategies to target loss of function genetic events or non-druggable events may be required to implement effective targeted therapies for patients with triple-negative disease (57).

Bringing gene expression subtypes to the clinic

The initial identification of gene expression subtypes generally uses unsupervised clustering methods to identify subgroups, and this approach can be readily repeated when examining groups of cancer samples. However, this approach cannot easily be translated into a clinical diagnostic test (58) that seeks to classify a single cancer into a gene expression subtype (39, 60). The translation of the breast cancer “intrinsic gene” subtypes into a single sample predictor has proved challenging, although the PAM50 Nanostring-based commercially available test may provide an opportunity to implement this type of subtyping in clinical practice (32).

Luminal AR cancers have relatively distinctive gene expression patterns as compared with those of other triple-negative subtypes. Given this observation, it is likely that this subtype of cancers will be relatively simple to identify (31). This subtype likely overlaps strongly with those TNBCs identified to be AR positive by immunohistochemistry, which may represent a simple selection strategy. In addition, luminal AR cancers are likely to lack the expression of basal cytokeratins (i.e., cytokeratins 5/6, 14, and 17), which have been used in many published research studies as part of an immunohistochemistry-based surrogate panel to identify basal-like cancers. It should be noted that mesenchymal stem-like cancers are also likely to lack expression of basal cytokeratins, suggesting that two subtypes of TNBC are enriched for basal-cytokeratin–negative TNBCs (31). Emerging data suggest that luminal AR cancers may have a lower pathologic complete response (path CR) rate to neoadjuvant chemotherapy, compared with other TNBC subtypes (35). Given the paucity of data, it is, perhaps, too early to conclude that this implies less benefit
from chemotherapy. Similar to the relative lack of path CR in ER-positive luminal breast cancer, this may simply reflect differences in the prognostic importance of achieving path CR (61).

**Intratumor genetic heterogeneity**

TNBCs exhibit high levels of genomic instability, which has the potential to foster genetic diversity in cancer (62). Emerging data suggest that the majority of TNBCs are oligo- or multiclonal at diagnosis, and that this phenomenon may be more frequent than in other breast cancer subtypes (40, 63, 64), although the data at this time are relatively preliminary and more data are required to confirm the true incidence of oligo/multiclonality in TNBCs. The pattern of oligoclonality seen in TNBCs likely reflects relatively late establishment of multiple clones in the tumor, with some mutations present in all cancer cells, reflecting a shared origin of all clones in the cancer, but also with distinct mutations that have arisen later in tumor evolution. Whether this heterogeneity is reflective of distinct geographic heterogeneity, different clones are present in different sections of the tumor as seen in renal cancer (65), or the clones are intermixed remains unaddressed at this time.

Importantly, direct evidence to demonstrate that intratumor genetic heterogeneity affects even driver genetic aberrations in TNBCs has recently been reported, with a subset of these cancers displaying *TP53* and *PIK3CA* mutations in less than 50% of cancer cells (40). Data, so far, are largely limited to massively parallel sequencing and microarray-based comparative genomic hybridization analyses (40, 62), and it is unclear if intratumor heterogeneity is also observed for gene expression subtypes.

This oligo/multiclonal nature of many TNBCs presents a major challenge to the concept of precision medicine. Targeting an actionable mutation present only in a subclone in the cancer may not result in a significant clinical response. Likewise, genetic instability, which likely fosters clonal diversity and intratumor genetic heterogeneity, may create an optimal milieu for the outgrowth of resistant subclones that, although initially present at low frequency, are enriched under the Darwinian pressure of targeted therapy (62). The full clinical significance of such potential diversity will be a major area of research for this decade. It may be necessary to design combinatorial therapies targeting molecular aberrations restricted to different subclones of a TNBC, or to target the genetic aberrations that enable this heterogeneity to develop.

**Primary versus metastasis**

With substantial evidence of intratumoral heterogeneity in TNBCs, this raises the issue of whether biopsy of metastatic disease is required to assess biomarkers. It is well described that both ER and HER2 status can change from primary to metastasis (66), which would suggest that biopsy of all patients with TNBCs when they relapse should be done to assess these routine markers. The current data, however, suggest that the vast majority of patients with TNBCs remain triple-negative on recurrence (66), and there is a pressing need for clinical research to assess how frequently actionable mutations, and gene expression subtypes, change from primary to recurrence. Importantly, differences in the mutational repertoire of a primary and recurrent TNBC have been reported (63), although it is unclear as to whether cancer cells from different metastatic deposits would harbor private mutations, as recently described in renal cell carcinomas (65). This is important to describe because, if intratumor genetic heterogeneity is also present within and between metastatic deposits, single metastatic samples may be of limited clinical utility. Alternative approaches, based on massively parallel sequencing analysis of circulating tumor cells and/or cell-free plasma DNA, are likely to constitute useful alternatives to the analyses of primary and metastatic tumor samples.

**Moving Forward in Clinical Trials**

Many studies of targeted therapy for TNBC have now been reported or are under way. Two studies have investigated the therapeutic potential of targeting EGFR, which is expressed in approximately 60% of TNBCs. Targeting EGFR with cetuximab had only low activity, with a 6% response as a single agent (11) and, in a phase II randomized study of the addition of cetuximab to cisplatin chemotherapy, there was only a modestly improved response rate from 10.3% with cisplatin alone to 20% with the combination (67). The level of benefit from cetuximab in these studies was insufficient to justify future clinical development. Both studies were initiated before the full heterogeneity of TNBC was described, and investigating whether benefit from cetuximab would be restricted to or enriched in specific subtypes of TNBCs, or TNBCs harboring *EGFR* gene amplification, is warranted.

These trials illustrate the potential problem that is addressed in relatively few ongoing trials. TNBCs have such diversity that it is becoming clear that it is unlikely that a targeted therapy will have substantial activity in unselected TNBCs. For example 15% to 20% of TNBCs have genetic events in the PI3K pathway (40), either through mutations of *PIK3CA* that are heavily enriched in luminal AR cancer, or through losses of *PTEN* and *INPP4B* that are more frequent in nonluminal TNBCs. Trials of therapies targeting the PI3K pathway may have significant activity in this subset of TNBC; however, it is uncertain whether activity would be seen in patients with wild-type cancers. This emphasizes the importance of bringing sequencing strategies into the clinic, as discussed by Tabchy and colleagues in this edition of CCR Focus (68).

**Bringing forward molecular stratification to the clinic**

The challenge for future trials in TNBCs is to bring molecular stratification to the clinic potentially with both genetic and gene expression subtypes, and there is initial evidence of the potential benefit of this approach. One recently reported study screened TNBCs for AR expression to identify cancers of the luminal AR phenotype, and only AR-positive TNBCs were treated with the AR antagonist
bicalutamide (68). A 19% clinical benefit rate was seen with bicalutamide in patients with this subtype of triple-negative disease (69) and, although this rate is relatively low, trials with the more potent AR antagonist enzalutamide are underway (NCT01889238). The high level of PIK3CA mutations in this subtype suggests the potential for combination AR–PI3K targeting, which is being taken forward in clinical trials. The distinct biology of luminal AR TNBC does raise a question as to whether strenuous efforts should be made to consider this group of cancers separately from other TNBCs.

It is clear that the diversity of TNBCs must be addressed in trial designs. Unselected trials in TNBC have a place but with appropriately powered biomarker-defined secondary endpoints to assess efficacy in a predicted sensitive subset. Screening patients upfront to identify a predicted sensitive subset presents a more statistically robust approach, but is technically more challenging, often requiring the screening of a large number of patients. The neoadjuvant setting can present an ideal opportunity requiring the screening of a large number of patients. The neoadjuvant setting can present an ideal opportunity requiring the screening of a large number of patients. The neoadjuvant setting can present an ideal opportunity requiring the screening of a large number of patients.

Even approaches such as immune therapy that may cut across diversity to a certain extent are likely to need to consider the diversity of TNBCs. Such immune approaches are discussed in this CCR Focus section (71). For example, TNBCs with a brisk lymphocytic infiltrate and the immunomodulatory gene expression subtype likely present a subtype to enrich in these studies. Similarly, those TNBCs with genetic loss of the T-cell–receptor loci as defined by microarray-based comparative genomic hybridization (72) or massively parallel sequencing may also identify a group of these tumors with substantial lymphocytic infiltrate that may have a good clinical outcome and/or be sensitive to conventional chemotherapy regimens.

Conclusions

The complexity of TNBCs could lead to a nihilistic view of our ability to treat this subset of cancers. There is room for substantial optimism, however, if the diversity of TNBCs is accepted into clinical trial design and if recent advances in technology are incorporated into the armamentarium of oncologists and pathologists to define the genetic make-up of cancers and to identify the most appropriate targeted therapy for each individual patient. By combining pathology with cutting-edge next-generation sequencing, gene expression profiling, and bioinformatics, it will be possible to address the diversity of TNBCs on a large scale, and to start tackling the rare subtypes of TNBC. However, to do this, we will be assessing increasingly rare subsets of cancers, with all challenges that the rarity of the subtype poses. Therefore, to realize the potentials of precision medicine for patients with TNBCs, cooperative groups of trialists and even collaborations between cooperative groups will be essential.

Disclosure of Potential Conflicts of Interest

N.C. Turner has received a commercial research grant from AstraZeneca and is a consultant/advisory board member for AstraZeneca, Novartis, Roche, Clovis, Tesaro, and Servier. No potential conflicts of interest were disclosed by the other author.

Authors’ Contributions

Conception and design: N.C. Turner, J.S. Reis-Filho

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.S. Reis-Filho

Writing, review, and/or revision of the manuscript: N.C. Turner, J.S. Reis-Filho

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References


# Clinical Cancer Research

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