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Posted Date: 9 October 2025

doi: 10.20944/preprints202510.0714.v1

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Review

# The Molecular Landscape and Utility of Multiomic Analyses in Triple Negative Breast Cancer: Further Subtyping and Exploring Novel Biomarkers and Therapeutic Targets

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## Simple Summary

Identification of targeted therapies and novel treatment strategies is critical for improving the survival of patients with triple-negative breast cancer (TNBC), which lacks ER, PR, and HER2 expression. Tumor resistance to chemotherapy and immunotherapy - the current systemic treatments for TNBC - significantly contributes to patient mortality. The biological complexity of TNBC, including its tumorigenesis, heterogeneity, and clinical behavior, remains insufficiently understood, despite the introduction of TNBC subtyping over a decade ago. Currently, multiomic technologies offer more powerful approaches to classify TNBC within a broad molecular landscape encompassing DNA, RNA, and proteins. These integrative analyses hold great promise for identifying novel biomarkers and actionable therapeutic targets to improve treatment outcomes.

## Abstract

Triple-negative breast cancer (TNBC) has the poorest prognosis among all breast cancer subtypes, largely due to the lack of targeted therapies and its resistance to both chemotherapy and immunotherapy. A deeper understanding of TNBC biology is therefore critical for identifying therapeutic targets. Molecular subtyping of TNBC, first introduced over a decade ago, has significantly advanced our knowledge of the disease's biology. However, tumor heterogeneity remains a major factor contributing to poor clinical outcomes and treatment resistance. The integration of multi-omics technologies including genomic, transcriptomic, and proteomic analyses, offers a powerful approach to further dissect tumor heterogeneity and accelerate the discovery of new biomarkers and therapeutic targets. This review aims to highlight the potential utility and evolving role of multiomics (-omics) in improving our understanding of TNBC biology - particularly tumor heterogeneity - ultimately facilitating the development of novel therapies and actionable strategies to treat the disease.

**Keywords:** triple-negative breast cancer; multiomics; tumor heterogeneity; biomarkers; therapeutic targets

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## 1. Introduction

Despite advancements in diagnosis and treatment, breast cancer has an incidence of over 2 million worldwide and remains the leading cause of cancer mortality for women [1,2]. Triple-negative breast cancer (TNBC) refers to a heterogeneous group of breast cancers that lack expression of ER, PR, and HER2. Despite recent advances with immunotherapy (IO) and antibody-drug

conjugates (ADCs), TNBC remains the most aggressive subtype, characterized by a high risk of recurrence and a short overall survival in the metastatic setting [3,4]. As a result, there continues to be a need to identify biomarkers for diagnosis, prognosis, and treatment of these aggressive breast cancers.

Multimics (also referred to as -omics) represent a potential avenue for interrogation of these novel gene targets. They are multimodal techniques that identify biological molecules (i.e., mRNA in the case of transcriptomics [5]) to gain a comprehensive understanding of biology and disease pathology. They generate tremendous amounts of data, which in the hands of skilled bioinformaticians can be used to comprehensively profile heterogeneous biologic samples. These techniques can be used to identify novel genes of interest from a background of 100s if not 1000s of genes, ultimately helping researchers work more efficiently to seek answers.

The purpose of this review is to explore current applications of -omics analyses and examine their potential role in the identification of novel biomarkers, with the goal of guiding the future of research on TNBC.

## 2. Multiomic Approaches to Cancer Research

Multimics technologies can be used to identify mRNA (transcriptomics), DNA (genomics), protein (proteomics), metabolites (metabolomics), and lipids (lipidomics). Transcriptomic approaches explore expression levels of different genes on a sample-wide (bulk RNA-seq) or single-cell (scRNA-seq) level. Recent advancements have allowed these principles to be applied directly onto histologic sections to assess gene expression with spatial context (spatial transcriptomics). Table 1 contains an overview of some of these different -omics approaches, along with their strengths and weaknesses. Integrated approaches (e.g., single-cell RNA-sequencing combined with spatial transcriptomics) optimize each approach's strengths to identify clinically relevant trends.

**Table 1.** Overview of Multiomic Approaches.

Technique	Description	Strength	Weakness
Single-cell transcriptomics (scRNA-seq)	Cellular level analysis of mRNA expression	High resolution. Identify cell populations in heterogeneous samples [6]	Cost [7]. Lacks spatial context.
Spatial transcriptomics	Analysis of mRNA from sample on slide	Trends with spatial context. Entire transcriptome probe sets available. Promising future as more advanced products are released	Cost [8]. Low resolution. Relatively low transcript capture in certain tissue types (e.g., mineralized tissue [9])
Microarray gene expression analysis	Collection of mRNAs using array of known probes	Targeted studies	Can only identify expression of known probes.
Bulk RNA-seq	Analysis of mRNA from whole sample	More sensitive than microarray and doesn't require probes.	Primarily for broad sample-wide trends
Proteomics	Analysis of pro-tein structure and function	Clinically relevant protein identification. Cost-effective methods [10]	Complexity of protein structure. Difficulty for studying post-translational modifications.
Genomics	Analysis of DNA sequencing	Whole-genome sequencing becoming more readily available. Identification of mutations. Exome sequencing can identify copy number variations.	Cost [11]. May not reflect transcribed/translated protein. Variants detected of may be of varying significance.

Multiomic techniques have been used to better understand the tumor biology of different cancers, from melanomas to gliomas and adenocarcinomas [12–15]. They have helped to better understand mechanisms behind response to checkpoint inhibitors [16] as well as assess why some tumors are less responsive to immunotherapy [17]. They can also be used to monitor tumorigenic signaling between malignant and non-malignant cells [18]. In breast cancer specifically, they have been used to identify transcriptionally divergent subtypes of breast cancers [19], and to analyze samples of HER2+ tumor in conjunction with histology [20]. Further, attempts have been made at using -omics to annotate HER2 status on histology [21] as well as to delineate the expression profiles of invasive ductal carcinoma and ductal carcinoma in-situ [22]. Recently, they have been used for the identification of novel biomarkers for breast cancer diagnosis and treatment [23].

### 3. Multiomic Approaches in Triple Negative Breast Cancer

Breast cancer has been classically divided into intrinsic subtypes based on expression of ER, PR, and HER2 in the PAM50 assay. These types include luminal A and B (both expressing hormone receptors, with Luminal B expressing high Ki-67), HER2-enriched, and basal-like (which is primarily composed of TNBCs). These molecular subtypes of breast cancer have clinical implications on risk and survival [24,25].

Triple negative breast cancer accounts for approximately 15% of breast cancers that lack expression of ER, PR, and HER2 [26]. They tend to be larger, higher in tumor grade, and are more aggressive than other breast cancer types [3]. Because of their lack of hormone receptors or HER2 expression, no targeted therapies currently exist. Currently treatment of TNBC relies on chemotherapy [26]. Only around 30% of TNBC cases respond to neoadjuvant chemotherapy [27], and even with successful chemotherapy, patients have higher risk of relapse and death after treatment [4]. Immunotherapies have demonstrated some effect, but some tumors are immune-cold and do not respond [28,29]. Although TNBC tumors are generally similar in their poor prognostic outcomes, there exists great heterogeneity between tumors – pathologically, mutationally, and transcriptionally [27]. Thus, there is a need to identify 1) prognostic indicators to stratify risk for TNBC cancers and 2) markers to target TNBC (and its different specific subtypes).

#### 3.1. Triple Negative Breast Cancer Molecular Subtypes Based on Gene Set Enrichment Analysis

Due to lack of non-cytotoxic therapies, concerted efforts have been made to classify TNBCs molecularly for possible identification of novel treatment targets. Six primary subtypes, collectively known as TNBCtype, were initially identified based on gene set enrichment analyses (GSEA), comparing relative expression of different canonical pathways from microarray data [26,30–32]. These initial subtypes are summarized in Table 2. Interestingly, while TNBCs overall do not express ER or PR, the LAR subtype was enriched in pathways associated with hormone response. Since initial stratification, these TNBCtypes have been refined to four current subtypes (TNBCtype-4), with laser microdissection of histology revealing the original immunomodulatory (IM) and mesenchymal stem-like (MSL) groups to be non-tumor associated cell lines.

**Table 2.** Original TNBCtype Classes.

	Subtype	Pathways
Basal-like	Basal-like 1 (BL1)	Proliferative gene pathways (cell cycle, DNA replication), usually associated with high Ki-67
	Basal-like 2 (BL2)	Growth factor genes
	Immunomodulatory (IM)*	Immune cell signaling
Mesenchymal	Mesenchymal-like (M)	Cell motility, cell differentiation, WNT, ALK, Extracellular matrix
	Mesenchymal stem-like (MSL)**	Growth factor and epithelial-to-mesenchymal transition

Luminal	Luminal androgen receptor (LAR)	Androgen/estrogen metabolism, Steroid biosynthesis, Porphyrin metabolism
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\*IM subtype has since been identified to be immune infiltrate into tumor on validation with laser microdissection methods [32] \*\*MSL subtype has been identified to be tumor associated stromal cells via validation with laser microdissection methods [32].

The molecular TNBCtype-4 classifications have been shown to be clinically relevant [33], notably as predictors of complete pathologic response rates to neoadjuvant chemotherapy, with basal-like 1 (BL1) having the best response and basal-like 2 (BL2) and luminal androgen receptor (LAR) the worst [31,34]. Further, multiomic investigations into these subtypes have revealed genes of interest that represent potential avenues of treatment for their associated subtypes.

**Table 3.** TNBCtype-4 Targets.

Subtype	Gene Findings	Citation
BL1	Upregulated DNA/RNA synthesis, cell division, and nuclear export	[35]
BL2	Upregulated extracellular matrix, collagen, cell junction, and cell membrane components	[35]
M	Lowly express PD-L1, making immunotherapy less effective	[36]
LAR	PRC-2, enhances chemotherapy response Genetic dependency on CCND1 GPX4, can be inhibited to cause ferroptosis Activating mutation in PIK3CA	[33,34,36,37]

The classification of these molecular subtypes of TNBC based on GSEA has been a landmark finding, however further studies reveal that TNBC complexity persists even past these subtypes. For example, while both “basal-like”, the BL1 and BL2 subtypes have notable differences that have been identified with further study. The BL2 subtype is enriched in genes that contribute to cancer invasion and motility [35], which could help explain why the BL2 subtype of TNBCs have worse observed responses to treatment.

MicroRNAs, which are single stranded, non-coding RNA that can post-transcriptionally modify mRNA and regulate cancer phenotype, represent an additional layer of complexity with these subtypes [38]. When compared with BL2, BL1 cancers have been demonstrated to upregulate the miR-17-92 cluster of microRNA [35], which are known to suppress E2F1 and PTEN, both of which are growth control proteins [39–42].

Even further, the LAR subtype of TNBC has been shown to exist on a spectrum. Distinct LAR subgroups (“sub-subtypes”) have been identified, with one being more biologically similar to mesenchymal TNBC and another being similar to basal TNBC [43]. Additionally, outside of these TNBCsubtype-4 analyses, comparisons of different TNBC groups have identified other markers that may help elucidate the mechanisms behind TNBC’s tumor heterogeneity.

### 3.2. Differentially Expressed Gene Analysis of Triple Negative Breast Cancer

In order to better understand mechanisms behind TNBC growth and tumorigenesis, research has been conducted comparing different phenotypes of TNBC to find differentially expressed genes (DEGs). DEG analysis is a method of identifying potential genes of interest when comparing different populations of study [44]. Out of 100s if not 1000s of genes, it identifies genes of interest that are over-expressed or under-expressed in one population versus another. Comparisons can be inter-sample

(between separate biological samples, i.e., comparing expression profiles between cancers with different TNM staging) or intra-sample (within the same sample, i.e., comparing different cell populations within the same tumor) [45].

The fundamental idea behind DEG analysis is that differences in gene expression ultimately contribute to differences in observed phenotype – whether that be disease aggressiveness or treatment response. Identification of these DEGs can be a vital step in understanding how different disease pathologies come to be and can be helpful when exploring phenomenon that are incredibly complex and driven by a variety of factors. In the case of triple negative breast cancer, DEG analysis can be performed with -omics techniques to investigate genes which underly differences in prognosis and treatment response.

### 3.2.1. Immune-Related

The cancer-immunity cycle, where the innate immune system catalyzes the adaptive response to tumor antigens, defines the body's response to cancers [46]. Mechanisms to evade this system [47] allow cancer cells to proliferate and for tumors to become malignant. Yet, even when the immune system is unable prevent cancer development, the advent of immunotherapies, like checkpoint inhibitors and monoclonal antibodies, have proven effective at harnessing the immune system's natural capabilities to treat cancer [48]. For example in high-risk, early stage TNBC, the immune checkpoint inhibitor pembrolizumab has achieved significant improvements in pathologic complete response[49,50]. In advanced metastatic disease with positive PD-L1 expression, pembrolizumab has also demonstrated significant effects on survival[51] and has become standard of care. Despite these advancements in treatment, TNBCs are heterogeneous, and a subset have molecular profiles that in some way impact their ability to respond to immunotherapy. -Omics techniques utilizing differential analysis have been used to identify key players in this chain, from immune response to immune therapy.

To evaluate the spectrum of immune reactions to TNBC development, differential gene expression analysis can be used to compare TNBC samples of different severities. These analyses help identify novel markers for downstream analysis. For example, macrophage inhibitory factor (MIF) is secreted by cancer cells and has been thought to drive immune evasion [52–54]. In Chen et al., RNA-sequencing across TNBC samples of varying severity revealed differential expression of MIF, with higher expression of this gene correlating with higher T stage. Furthermore, through the integration of other -omics approaches, Chen et al. were able to implicate a possible mechanism underlying this finding. Single-cell RNA-sequencing analysis, which can be used to infer cell-to-cell interactions, identified epithelial breast cancer cells which secrete MIF, potentially mediating the polarization of macrophages into the M2 phenotype. Spatial transcriptomics corroborated this result, with epithelial cell markers and MIF expression found to be expressed in concurrent regions [55].

Even with the adoption of novel immunotherapies, TNBC has proven to be a formidable foe, with many cases of TNBC being non-responsive to immune checkpoint inhibitors [28,29]. Much like with tumor severity, differential analysis of -omics data can be performed to better understand the genes that are involved in this phenomenon. In Liu et al., analysis of single-cell sequencing data from both immune checkpoint responders and non-responders, revealed SIGLEC9 (in myeloid cells) and TNFSF9 (in dendritic cells) to be expressed highly in non-responders compared to responders [56]. Interestingly, -omics techniques have similarly implicated SIGLEC9-expressing macrophages in other cancers that fail to respond to anti-PD-1 therapy [57], as well as TNFSF9 in metastatic progression [58], altogether suggesting that these two genes somehow contribute to immune-cold responses to immunotherapy in TNBC. On the other hand, investigations of immunotherapy response have also identified genes that could be important predictors of successful response to immune checkpoint inhibitors. In Zhang et al.'s comparison of single-cell RNA-sequencing from responders and non-responders, CXCL13 expressed in T cells was found to be enriched in responders prior to treatment. Additionally, for responders who received combination chemo- and immunotherapy, the population of CXCL13-expressing T-cells significantly expanded from baseline.

This pattern was not mimicked in patients who responded to monotherapy paclitaxel, who interestingly exhibited decreased levels of CXCL13+ T cells after treatment. These observations in single-cell sequencing suggest that not only is CXCL13 significant for TNBC response to immunotherapy – the addition of paclitaxel with these treatments may actually inhibit the expansion of CXCL13+ cells and dampen the effect of immune checkpoint blockers [59].

Differential analysis of gene expression and protein translation can be further applied to the intrinsic subtypes of breast cancer to explore immune therapy response. In Zhu et al., differential analysis of proteomics comparing all intrinsic breast cancer subtypes, revealed CD73, a protein which inhibits immune responses, to be enriched in TNBC. To explain this finding, the authors implicated OTUD4, which stabilizes CD73 [60] in TNBC cells, causing its eventual accumulation and downstream immunosuppressive effects. With additional spatial transcriptomic analysis, OTUD4 and CD73 were found to be positively correlated in malignant regions across samples of TNBC [61]. Here, -omics techniques allowed the authors to rapidly profile hundreds of proteins to identify a potential protein for further investigation. Armed with this knowledge, Zhu et al. investigated possible treatment modalities targeting the OTUD4/CD73 axis, by developing a pharmacologic inhibitor of OTUD4, ST80, which restored T-cell function and increased the efficacy of immune checkpoint therapy [61].

### 3.2.2. Epithelial Cells

The principles of differential gene expression have also been applied to the epithelial cells that make up TNBC tumors. Through single-cell RNA-sequencing, Kim et al. sought to establish markers that separate TNBC epithelial cells from epithelial cells from other breast cancers. Using this method, the authors identified 12 DEGs which were highly expressed in TNBC epithelial cells compared to normal breast tissue, ER+, and HER2+ breast cancer [62]. These included multiple genes that are involved in epithelial to mesenchymal transition: vimentin (VIM) and caldesmon (CALD1).

In epithelial cell cancers (carcinomas) the epithelial to mesenchymal transition has been heavily implicated in cancer progression and metastasis. During this process, epithelial cells shed their epithelial characteristics in favor of upregulating mesenchymal cell markers. Under typical conditions, this transition contributes to wound healing, however in carcinomas, it increases cellular motility and allows epithelial cell cancers to become invasive [63]. Further, genes involved in this process have been associated with drug-resistance [64], underlying the importance of studying these genes through techniques like -omics.

VIM, an intermediate filament protein found in mesenchymal cells [65], is required for the epithelial to mesenchymal transition and has been shown to promote TNBC invasion [66,67]. Silencing of the estrogen receptor leads to the development of the vimentin cytoskeleton [68], which is believed to be one of the driving factors behind the aggressiveness of TNBC. Further, pre-clinical experiments assessing the effect of vimentin knockdown on tumorigenicity have exhibited decreased metastatic progression, suggesting the possibility for VIM not only as a marker of TNBC, but also for future therapeutic investigation as well [67].

CALD1 encodes caldesmon, an actin-binding protein that regulates cell motility via actomyosin systems in both muscle and non-muscle cells [69,70]. Caldesmon has previously been implicated in other carcinomas, including colorectal cancer [69,71]. In breast cancer cells, silencing of the estrogen receptor led to CALD1 upregulation [68]. Further studies of CALD1 in breast cancer have suggested that CALD1 could be a marker for tamoxifen-resistance and cancer recurrence [72].

In sum, applying differential gene expression analysis to data from -omics techniques has identified many markers of TNBC – some associated with better or worse treatment response, cancer severity, or prognosis. These markers are shown in Table 4.

**Table 4.** Potential Markers of TNBC Identified by -Omics Techniques.

Markers	Techniques	Function	Citations
MIF	RNA-seq, scRNA-seq, Spatial transcriptomics	Regulates glucocorticoid immunosuppression mediating cell survival	[52–55]
CXCL13	scRNA-seq	Expressed in T cells to induce proinflammatory signaling in macrophages	[59]
CD73/OTUD4	Proteomics and Spatial transcriptomics	CD73 stabilizes OTUD4, causing accumulation and immunosuppression	[60,61]
VIM	scRNA-seq	Intermediate filament protein found in mesenchymal cells. Drives epithelial to mesenchymal transition	[65–68]
CALD1	scRNA-seq	Actin-binding protein involved in cell motility. Drives epithelial to mesenchymal transition.	[68–70]

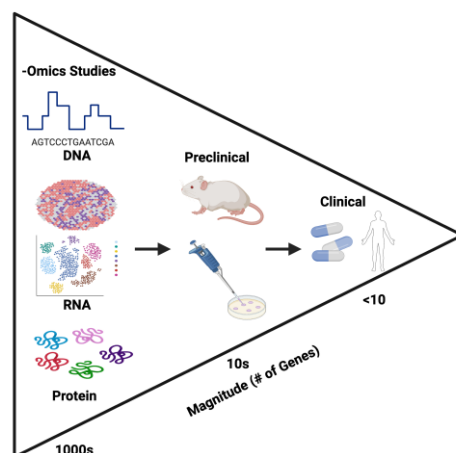
### 3.3. Additional Applications of -Omics in TNBC

Other -omic techniques, like whole genome sequencing or exome sequencing can be used to identify mutants and copy number variants that occur in TNBC. These techniques can be used to better understand how the mutation development contributes to TNBC development and response to treatment.

Kim et al. used exome sequencing to study the development of chemoresistance in treatment-naïve TNBC cases. They demonstrated broad categories of mutant responses to neoadjuvant chemotherapy: clonal persistence (where mutant frequencies were altered but mutants remained after therapy) and clonal extinction (where mutant clones were eradicated post-treatment). Further, Kim et al. performed single-nuclei RNA sequencing to identify phenotypic differences between chemoresistant and chemoresponsive tumors. They identified genes from several processes, including extracellular matrix degradation, angiogenesis, epithelial to mesenchymal transition, AKT1 signaling via mTOR, and hypoxia, that were upregulated in chemoresistant TNBC after neoadjuvant chemotherapy [73]. In this case, -omics were used to better understand the different phenotypic responses that TNBC can have to therapy.

## 4. Discussion

Because each of the multiomic approaches have their own strengths and weaknesses, the most compelling of research studies integrate multiple approaches in their investigations of triple negative breast cancer. Studies like Chen et al. [55] and Kim et al. [73] draw upon multiple -omics techniques to provide the most thorough analysis of TNBCs. Such approaches can be used to profile 100s if not 1000s of genes in order to identify potential novel biomarkers of TNBC. These isolated biomarkers can then be further validated preclinically using *in vitro* cell lines and animal models. Finally, the most promising of these genes can finally be evaluated clinically, to explore potential new avenues of TNBC classification, risk-stratification, and treatment. This proposed pipeline of -omics to bedside investigation is illustrated in Figure 1.



**Figure 1.** Proposed Pipeline Using -Omics Techniques to Identify Biomarkers in TNBC.

While the downstream effects of -omics implementation in TNBC research have yet to be fully realized, these techniques have already advanced our understanding of TNBC. For instance, the discovery of the TNBCtype-4 subtypes of triple negative breast cancer has led to investigations of new targeted therapies, exploring individualized therapies against genes enriched in each of the molecular subtypes of TNBC [74]. The knowledge that basal-like TNBCs upregulate growth factor genes has led to investigations into the efficacy of targeting epidermal growth factor receptor (EGFR) [75,76], vascular endothelial growth factor receptor (VEGFR) [77,78], and fibroblast growth factor receptor (FGFR) [33,79]. Furthermore, the identification of the LAR subtype has driven research into androgen receptor targeting [33] as well as into the effects of PI3K inhibitors [74], which have been shown to make cancers more susceptible to DNA-damaging agents [80].

The TNBCtype-4 subtypes of triple negative breast cancer have also demonstrated some potential for stratifying responses to chemotherapy regimens. For instance, the p53 family of tumor suppressors has been shown to be associated with the basal-like classes of TNBC, suggesting its status as a potential predictive marker for response to paclitaxel/cisplatin with everolimus [74].

Further applications of -omics techniques using differential gene analysis have identified key genes that distinguish TNBC from other types of breast cancer. These analyses can also identify how different subtypes (and even sub-subtypes) of TNBC differ from each other, to help us understand the heterogeneity that underlies all the different phenotypes of TNBC that are observed. However, because of this complexity, true understanding of TNBC remains elusive. Even within the TNBCsubtype-4 classifications, sub-subtypes exist. Additional markers that have been discovered independently of the TNBCsubtype-4 classifications are further evidence of the deep heterogeneity of TNBCs. It is unlikely that a “one size fits all” or even a “one size fits most” is possible with TNBC – as treatment responses have been demonstrated to be multifactorial, and not wholly dependent on any single known marker, an idea that has been exemplified with programmed death ligand 1 (PD-L1). The advent of immunotherapies has been an exciting advancement in oncology, as immunotherapies like atezolizumab and pembrolizumab can be used in a targeted fashion against PD-L1/PD-1. In the case of TNBC however, PD-L1 status is a poor predictor of tumor response to atezolizumab with carboplatin. Although the drug directly targets PD-L1, certain subtypes of TNBC can respond to immunotherapy regardless of their PD-L1 status, suggesting that this one gene is not capable of stratifying TNBC into responders and non-responders [81].

It is clear that triple negative breast cancers have vast complexity. In spite of the nature of the disease, -omics techniques have incredible potential to tackle this challenge. In particular, spatially resolved -omics technologies enable researchers to understand TNBC tumor cells in the context of their microenvironment, providing insights into cell-to-cell interactions, immune infiltration, and stromal contributions to tumor behavior. Advancements and further applications of -omics, like the

advent of sub-cellular resolution for spatial transcriptomics with the Xenium platform [82] or the profiling of non-coding sequences like microRNA [83] or long non-coding mRNA [84] make the field even more exciting. While cost remains a concern for multiomics studies (the techniques themselves are often costly to run even without considering labor costs), as with any new and emerging technology, the expenses will continue to decline as more innovations are made to improve these techniques. Finally, as -omics methods and analysis improve, it is our hope that they can be further integrated into our studies of TNBC.

## 5. Conclusions

Triple negative breast cancer remains one of the most complex and poorly understood cancers to afflict humanity. It has significant epidemiological consequences, and because of its aggressiveness and lack of expression of targetable ER, PR, or HER2, it continues to be poor prognostically. As such, there is a need to continue exploring novel biomarkers for classification and treatment of TNBC. Multiomics (or -omics) are multimodal techniques that are used to identify, profile, and understand biological molecules, such as DNA, RNA, or proteins. These molecules define biology and drive the mechanisms behind survival as well as disease, including TNBC.

In the hands of skilled bioinformaticians, multiomics can be used to tackle the complexity of TNBC, in a way that was not previously possible. These techniques can be used to explore 100s if not 1000s of genes between multiple experimental conditions, to better understand TNBC broadly and potentially identify novel biomarkers for downstream experimentation. As these technologies continue to advance, they should be further integrated as tools used to study TNBC, just as mouse models or clinical trials have been.

**Author Contributions:** Conceptualization, Y.P.; writing—original draft preparation, C.J.; writing—review and editing, Y.P.; visualization, C.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Wilkinson, L.; Gathani, T. Understanding breast cancer as a global health concern. *Br J Radiol* **2022**, *95*, 20211033, doi:10.1259/bjr.20211033.
2. Ginsburg, O.; Bray, F.; Coleman, M.P.; Vanderpuye, V.; Eniu, A.; Kotha, S.R.; Sarker, M.; Huong, T.T.; Allemani, C.; Dvaladze, A.; et al. The global burden of women's cancers: A grand challenge in global health. *The Lancet* **2017**, *389*, 847-860, doi:10.1016/S0140-6736(16)31392-7.
3. Carey, L.A.; Dees, E.C.; Sawyer, L.; Gatti, L.; Moore, D.T.; Collichio, F.; Ollila, D.W.; Sartor, C.I.; Graham, M.L.; Perou, C.M. The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res* **2007**, *13*, 2329-2334, doi:10.1158/1078-0432.Ccr-06-1109.
4. Dent, R.; Trudeau, M.; Pritchard, K.I.; Hanna, W.M.; Kahn, H.K.; Sawka, C.A.; Lickley, L.A.; Rawlinson, E.; Sun, P.; Narod, S.A. Triple-negative breast cancer: Clinical features and patterns of recurrence. *Clin Cancer Res* **2007**, *13*, 4429-4434, doi:10.1158/1078-0432.Ccr-06-3045.
5. Nassar, S.F.; Raddassi, K.; Wu, T. Single-Cell Multiomics Analysis for Drug Discovery. *Metabolites* **2021**, *11*, doi:10.3390/metabo11110729.
6. Papalexi, E.; Satija, R. Single-cell RNA sequencing to explore immune cell heterogeneity. *Nat Rev Immunol* **2018**, *18*, 35-45, doi:10.1038/nri.2017.76.
7. Ziegenhain, C.; Vieth, B.; Parekh, S.; Reinius, B.; Guillaumet-Adkins, A.; Smets, M.; Leonhardt, H.; Heyn, H.; Hellmann, I.; Enard, W. Comparative Analysis of Single-Cell RNA Sequencing Methods. *Mol Cell* **2017**, *65*, 631-643.e634, doi:10.1016/j.molcel.2017.01.023.
8. Zheng, B.; Fang, L. Spatially resolved transcriptomics provide a new method for cancer research. *J Exp Clin Cancer Res* **2022**, *41*, 179, doi:10.1186/s13046-022-02385-3.

9. Xiao, X.; Juan, C.; Drennon, T.; Uytingco, C.R.; Vishlaghi, N.; Sokolowskei, D.; Xu, L.; Levi, B.; Sammarco, M.C.; Tower, R.J. Spatial transcriptomic interrogation of the murine bone marrow signaling landscape. *Bone Research* **2023**, *11*, 59, doi:10.1038/s41413-023-00298-1.
10. Viode, A.; van Zalm, P.; Smolen, K.K.; Fatou, B.; Stevenson, D.; Jha, M.; Levy, O.; Steen, J.; Steen, H. A simple, time- and cost-effective, high-throughput depletion strategy for deep plasma proteomics. *Sci Adv* **2023**, *9*, eadf9717, doi:10.1126/sciadv.adf9717.
11. Nurchis, M.C.; Radio, F.C.; Salmasi, L.; Heidar Alizadeh, A.; Raspolini, G.M.; Altamura, G.; Tartaglia, M.; Dallapiccola, B.; Pizzo, E.; Gianino, M.M.; et al. Cost-Effectiveness of Whole-Genome vs Whole-Exome Sequencing Among Children With Suspected Genetic Disorders. *JAMA Netw Open* **2024**, *7*, e2353514, doi:10.1001/jamanetworkopen.2023.53514.
12. Zhang, Y.; Wang, D.; Peng, M.; Tang, L.; Ouyang, J.; Xiong, F.; Guo, C.; Tang, Y.; Zhou, Y.; Liao, Q.; et al. Single-cell RNA sequencing in cancer research. *J Exp Clin Cancer Res* **2021**, *40*, 81, doi:10.1186/s13046-021-01874-1.
13. Zhou, J.X.; Taramelli, R.; Pedrini, E.; Knijnenburg, T.; Huang, S. Extracting Intercellular Signaling Network of Cancer Tissues using Ligand-Receptor Expression Patterns from Whole-tumor and Single-cell Transcriptomes. *Scientific Reports* **2017**, *7*, 8815, doi:10.1038/s41598-017-09307-w.
14. Yuan, D.; Tao, Y.; Chen, G.; Shi, T. Systematic expression analysis of ligand-receptor pairs reveals important cell-to-cell interactions inside glioma. *Cell Communication and Signaling* **2019**, *17*, 48, doi:10.1186/s12964-019-0363-1.
15. Hosein, A.N.; Huang, H.; Wang, Z.; Parmar, K.; Du, W.; Huang, J.; Maitra, A.; Olson, E.; Verma, U.; Brekken, R.A. Cellular heterogeneity during mouse pancreatic ductal adenocarcinoma progression at single-cell resolution. *JCI Insight* **2019**, *4*, doi:10.1172/jci.insight.129212.
16. Wang, B.; Zhang, W.; Jankovic, V.; Golubov, J.; Poon, P.; Oswald, E.M.; Gurer, C.; Wei, J.; Ramos, I.; Wu, Q.; et al. Combination cancer immunotherapy targeting PD-1 and GITR can rescue CD8(+) T cell dysfunction and maintain memory phenotype. *Sci Immunol* **2018**, *3*, doi:10.1126/sciimmunol.aat7061.
17. Li, B.; Severson, E.; Pignon, J.-C.; Zhao, H.; Li, T.; Novak, J.; Jiang, P.; Shen, H.; Aster, J.C.; Rodig, S.; et al. Comprehensive analyses of tumor immunity: Implications for cancer immunotherapy. *Genome Biology* **2016**, *17*, 174, doi:10.1186/s13059-016-1028-7.
18. Calbo, J.; van Montfort, E.; Proost, N.; van Drunen, E.; Beverloo, H.B.; Meuwissen, R.; Berns, A. A Functional Role for Tumor Cell Heterogeneity in a Mouse Model of Small Cell Lung Cancer. *Cancer Cell* **2011**, *19*, 244-256, doi:10.1016/j.ccr.2010.12.021.
19. Wu, S.Z.; Al-Eryani, G.; Roden, D.L.; Junankar, S.; Harvey, K.; Andersson, A.; Thennavan, A.; Wang, C.; Torpy, J.R.; Bartonicek, N.; et al. A single-cell and spatially resolved atlas of human breast cancers. *Nat Genet* **2021**, *53*, 1334-1347, doi:10.1038/s41588-021-00911-1.
20. Vickovic, S.; Eraslan, G.; Salmén, F.; Klughammer, J.; Stenbeck, L.; Schapiro, D.; Äijö, T.; Bonneau, R.; Bergensträhle, L.; Navarro, J.F.; et al. High-definition spatial transcriptomics for in situ tissue profiling. *Nat Methods* **2019**, *16*, 987-990, doi:10.1038/s41592-019-0548-y.
21. Andersson, A.; Larsson, L.; Stenbeck, L.; Salmén, F.; Ehinger, A.; Wu, S.; Al-Eryani, G.; Roden, D.; Swarbrick, A.; Borg, Å. Spatial deconvolution of HER2-positive breast tumors reveals novel intercellular relationships. *bioRxiv* **2020**, 2020.2007. 2014.200600.
22. Yoosuf, N.; Navarro, J.F.; Salmén, F.; Ståhl, P.L.; Daub, C.O. Identification and transfer of spatial transcriptomics signatures for cancer diagnosis. *Breast Cancer Res* **2020**, *22*, 6, doi:10.1186/s13058-019-1242-9.
23. Chen, Y.C.; Sahoo, S.; Brien, R.; Jung, S.; Humphries, B.; Lee, W.; Cheng, Y.H.; Zhang, Z.; Luker, K.E.; Wicha, M.S.; et al. Single-cell RNA-sequencing of migratory breast cancer cells: Discovering genes associated with cancer metastasis. *Analyst* **2019**, *144*, 7296-7309, doi:10.1039/c9an01358j.
24. Prat, A.; Pineda, E.; Adamo, B.; Galván, P.; Fernández, A.; Gaba, L.; Díez, M.; Viladot, M.; Arance, A.; Muñoz, M. Clinical implications of the intrinsic molecular subtypes of breast cancer. *Breast* **2015**, *24* Suppl 2, S26-35, doi:10.1016/j.breast.2015.07.008.

25. Parker, J.S.; Mullins, M.; Cheang, M.C.U.; Leung, S.; Voduc, D.; Vickery, T.; Davies, S.; Fauron, C.; He, X.; Hu, Z.; et al. Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes. *J Clin Oncol* **2023**, *41*, 4192-4199, doi:10.1200/jco.22.02511.
26. Abramson, V.G.; Lehmann, B.D.; Ballinger, T.J.; Pietenpol, J.A. Subtyping of triple-negative breast cancer: Implications for therapy. *Cancer* **2015**, *121*, 8-16, doi:10.1002/cncr.28914.
27. Lehmann, B.D.; Pietenpol, J.A. Clinical implications of molecular heterogeneity in triple negative breast cancer. *Breast* **2015**, *24 Suppl 2*, S36-40, doi:10.1016/j.breast.2015.07.009.
28. Keenan, T.E.; Tolaney, S.M. Role of Immunotherapy in Triple-Negative Breast Cancer. *J Natl Compr Canc Netw* **2020**, *18*, 479-489, doi:10.6004/jnccn.2020.7554.
29. Steiner, M.; Tan, A.R. The evolving role of immune checkpoint inhibitors in the treatment of triple-negative breast cancer. *Clin Adv Hematol Oncol* **2021**, *19*, 305-315.
30. Lehmann, B.D.; Bauer, J.A.; Chen, X.; Sanders, M.E.; Chakravarthy, A.B.; Shyr, Y.; Pietenpol, J.A. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* **2011**, *121*, 2750-2767, doi:10.1172/jci45014.
31. Masuda, H.; Baggerly, K.A.; Wang, Y.; Zhang, Y.; Gonzalez-Angulo, A.M.; Meric-Bernstam, F.; Valero, V.; Lehmann, B.D.; Pietenpol, J.A.; Hortobagyi, G.N.; et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. *Clin Cancer Res* **2013**, *19*, 5533-5540, doi:10.1158/1078-0432.Ccr-13-0799.
32. Lehmann, B.D.; Jovanović, B.; Chen, X.; Estrada, M.V.; Johnson, K.N.; Shyr, Y.; Moses, H.L.; Sanders, M.E.; Pietenpol, J.A. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS ONE* **2016**, *11*, e0157368, doi:10.1371/journal.pone.0157368.
33. Lehmann, B.D.; Pietenpol, J.A. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. *J Pathol* **2014**, *232*, 142-150, doi:10.1002/path.4280.
34. Zhang, W.; Li, E.; Wang, L.; Lehmann, B.D.; Chen, X.S. Transcriptome Meta-Analysis of Triple-Negative Breast Cancer Response to Neoadjuvant Chemotherapy. *Cancers (Basel)* **2023**, *15*, doi:10.3390/cancers15082194.
35. Kalecky, K.; Modisette, R.; Pena, S.; Cho, Y.R.; Taube, J. Integrative analysis of breast cancer profiles in TCGA by TNBC subgrouping reveals novel microRNA-specific clusters, including miR-17-92a, distinguishing basal-like 1 and basal-like 2 TNBC subtypes. *BMC Cancer* **2020**, *20*, 141, doi:10.1186/s12885-020-6600-6.
36. Lehmann, B.D.; Colaprico, A.; Silva, T.C.; Chen, J.; An, H.; Ban, Y.; Huang, H.; Wang, L.; James, J.L.; Balko, J.M.; et al. Multi-omics analysis identifies therapeutic vulnerabilities in triple-negative breast cancer subtypes. *Nat Commun* **2021**, *12*, 6276, doi:10.1038/s41467-021-26502-6.
37. Yang, F.; Xiao, Y.; Ding, J.H.; Jin, X.; Ma, D.; Li, D.Q.; Shi, J.X.; Huang, W.; Wang, Y.P.; Jiang, Y.Z.; et al. Ferroptosis heterogeneity in triple-negative breast cancer reveals an innovative immunotherapy combination strategy. *Cell Metab* **2023**, *35*, 84-100.e108, doi:10.1016/j.cmet.2022.09.021.
38. Hussen, B.M.; Hidayat, H.J.; Salihi, A.; Sabir, D.K.; Taheri, M.; Ghafouri-Fard, S. MicroRNA: A signature for cancer progression. *Biomed Pharmacother* **2021**, *138*, 111528, doi:10.1016/j.biopha.2021.111528.
39. Knoll, S.; Emmrich, S.; Pützer, B.M. The E2F1-miRNA cancer progression network. *Adv Exp Med Biol* **2013**, *774*, 135-147, doi:10.1007/978-94-007-5590-1\_8.
40. O'Donnell, K.A.; Wentzel, E.A.; Zeller, K.I.; Dang, C.V.; Mendell, J.T. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* **2005**, *435*, 839-843, doi:10.1038/nature03677.
41. Chawra, H.S.; Agarwal, M.; Mishra, A.; Chandel, S.S.; Singh, R.P.; Dubey, G.; Kukreti, N.; Singh, M. MicroRNA-21's role in PTEN suppression and PI3K/AKT activation: Implications for cancer biology. *Pathol Res Pract* **2024**, *254*, 155091, doi:10.1016/j.prp.2024.155091.
42. Zhou, W.; Shi, G.; Zhang, Q.; Wu, Q.; Li, B.; Zhang, Z. MicroRNA-20b promotes cell growth of breast cancer cells partly via targeting phosphatase and tensin homologue (PTEN). *Cell Biosci* **2014**, *4*, 62, doi:10.1186/2045-3701-4-62.
43. Jovanovic, B.; Church, S.E.; Gorman, K.M.; North, K.; Richardson, E.T.; DiLullo, M.; Attaya, V.; Kasparian, J.; Mohammed-Abreu, A.; Kirkner, G.; et al. Integrative multi-omic profiling of triple-negative breast cancer for identifying suitable therapies. *Clinical Cancer Research* **2024**, doi:10.1158/1078-0432.Ccr-23-1242.

44. Khozyainova, A.A.; Valyaeva, A.A.; Arbatsky, M.S.; Isaev, S.V.; Iamshchikov, P.S.; Volchkov, E.V.; Sabirov, M.S.; Zainullina, V.R.; Chechekhin, V.I.; Vorobev, R.S.; et al. Complex Analysis of Single-Cell RNA Sequencing Data. *Biochemistry (Mosc)* **2023**, *88*, 231-252, doi:10.1134/s0006297923020074.
45. Potter, S.S. Single-cell RNA sequencing for the study of development, physiology and disease. *Nat Rev Nephrol* **2018**, *14*, 479-492, doi:10.1038/s41581-018-0021-7.
46. Demaria, O.; Cornen, S.; Daëron, M.; Morel, Y.; Medzhitov, R.; Vivier, E. Harnessing innate immunity in cancer therapy. *Nature* **2019**, *574*, 45-56, doi:10.1038/s41586-019-1593-5.
47. Vinay, D.S.; Ryan, E.P.; Pawelec, G.; Talib, W.H.; Stagg, J.; Elkord, E.; Lichtor, T.; Decker, W.K.; Whelan, R.L.; Kumara, H.; et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol* **2015**, *35 Suppl*, S185-s198, doi:10.1016/j.semcancer.2015.03.004.
48. Kumar, A.R.; Devan, A.R.; Nair, B.; Vinod, B.S.; Nath, L.R. Harnessing the immune system against cancer: Current immunotherapy approaches and therapeutic targets. *Mol Biol Rep* **2021**, *48*, 8075-8095, doi:10.1007/s11033-021-06752-9.
49. Puztai, L.; Denkert, C.; O'Shaughnessy, J.; Cortes, J.; Dent, R.; McArthur, H.; Kümmel, S.; Bergh, J.; Park, Y.H.; Hui, R.; et al. Event-free survival by residual cancer burden with pembrolizumab in early-stage TNBC: Exploratory analysis from KEYNOTE-522. *Ann Oncol* **2024**, *35*, 429-436, doi:10.1016/j.annonc.2024.02.002.
50. Schmid, P.; Cortes, J.; Puztai, L.; McArthur, H.; Kümmel, S.; Bergh, J.; Denkert, C.; Park, Y.H.; Hui, R.; Harbeck, N.; et al. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med* **2020**, *382*, 810-821, doi:10.1056/NEJMoa1910549.
51. Cortes, J.; Rugo, H.S.; Cescon, D.W.; Im, S.A.; Yusof, M.M.; Gallardo, C.; Lipatov, O.; Barrios, C.H.; Perez-Garcia, J.; Iwata, H.; et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. *N Engl J Med* **2022**, *387*, 217-226, doi:10.1056/NEJMoa2202809.
52. Yan, L.; Wu, M.; Wang, T.; Yuan, H.; Zhang, X.; Zhang, H.; Li, T.; Pandey, V.; Han, X.; Lobie, P.E.; et al. Breast Cancer Stem Cells Secrete MIF to Mediate Tumor Metabolic Reprogramming That Drives Immune Evasion. *Cancer Res* **2024**, *84*, 1270-1285, doi:10.1158/0008-5472.Can-23-2390.
53. Balogh, K.N.; Templeton, D.J.; Cross, J.V. Macrophage Migration Inhibitory Factor protects cancer cells from immunogenic cell death and impairs anti-tumor immune responses. *PLoS ONE* **2018**, *13*, e0197702, doi:10.1371/journal.pone.0197702.
54. Kang, I.; Bucala, R. The immunobiology of MIF: Function, genetics and prospects for precision medicine. *Nat Rev Rheumatol* **2019**, *15*, 427-437, doi:10.1038/s41584-019-0238-2.
55. Chen, M.; Liu, H.; Hong, B.; Xiao, Y.; Qian, Y. MIF as a potential diagnostic and prognostic biomarker for triple-negative breast cancer that correlates with the polarization of M2 macrophages. *Faseb j* **2024**, *38*, e23696, doi:10.1096/fj.202400578R.
56. Liu, P.W.; Lin, J.; Hou, R.; Cai, Z.; Gong, Y.; He, P.A.; Yang, J. Single-cell RNA-seq reveals the metabolic status of immune cells response to immunotherapy in triple-negative breast cancer. *Comput Biol Med* **2024**, *169*, 107926, doi:10.1016/j.combiomed.2024.107926.
57. Mei, Y.; Wang, X.; Zhang, J.; Liu, D.; He, J.; Huang, C.; Liao, J.; Wang, Y.; Feng, Y.; Li, H.; et al. Siglec-9 acts as an immune-checkpoint molecule on macrophages in glioblastoma, restricting T-cell priming and immunotherapy response. *Nat Cancer* **2023**, *4*, 1273-1291, doi:10.1038/s43018-023-00598-9.
58. Wu, J.; Wang, Y.; Yang, Y.; Liu, F.; Jiang, Z.; Jiang, Z. TNFSF9 promotes metastasis of pancreatic cancer by regulating M2 polarization of macrophages through Src/FAK/p-Akt/IL-1 $\beta$  signaling. *Int Immunopharmacol* **2022**, *102*, 108429, doi:10.1016/j.intimp.2021.108429.
59. Zhang, Y.; Chen, H.; Mo, H.; Hu, X.; Gao, R.; Zhao, Y.; Liu, B.; Niu, L.; Sun, X.; Yu, X.; et al. Single-cell analyses reveal key immune cell subsets associated with response to PD-L1 blockade in triple-negative breast cancer. *Cancer Cell* **2021**, *39*, 1578-1593.e1578, doi:10.1016/j.ccell.2021.09.010.
60. Ma, X.; Wan, R.; Wen, Y.; Liu, T.; Song, Y.; Zhu, Y. Deubiquitinating enzyme OTUD4 regulates metastasis in triple-negative breast cancer by stabilizing Snail1. *Exp Cell Res* **2024**, *434*, 113864, doi:10.1016/j.yexcr.2023.113864.

61. Zhu, Y.; Banerjee, A.; Xie, P.; Ivanov, A.A.; Uddin, A.; Jiao, Q.; Chi, J.J.; Zeng, L.; Lee, J.Y.; Xue, Y.; et al. Pharmacological suppression of the OTUD4/CD73 proteolytic axis revives antitumor immunity against immune-suppressive breast cancers. *J Clin Invest* **2024**, *134*, doi:10.1172/jci176390.
62. Kim, M.; Yang, W.; Hong, D.; Won, H.S.; Yoon, S. A Retrospective View of the Triple-Negative Breast Cancer Microenvironment: Novel Markers, Interactions, and Mechanisms of Tumor-Associated Components Using Public Single-Cell RNA-Seq Datasets. *Cancers (Basel)* **2024**, *16*, doi:10.3390/cancers16061173.
63. Lamouille, S.; Xu, J.; Derynck, R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* **2014**, *15*, 178-196, doi:10.1038/nrm3758.
64. Du, B.; Shim, J.S. Targeting Epithelial-Mesenchymal Transition (EMT) to Overcome Drug Resistance in Cancer. *Molecules* **2016**, *21*, doi:10.3390/molecules21070965.
65. Chen, Z.; Fang, Z.; Ma, J. Regulatory mechanisms and clinical significance of vimentin in breast cancer. *Biomed Pharmacother* **2021**, *133*, 111068, doi:10.1016/j.biopha.2020.111068.
66. Grasset, E.M.; Dunworth, M.; Sharma, G.; Loth, M.; Tandurella, J.; Cimino-Mathews, A.; Gentz, M.; Bracht, S.; Haynes, M.; Fertig, E.J.; et al. Triple-negative breast cancer metastasis involves complex epithelial-mesenchymal transition dynamics and requires vimentin. *Sci Transl Med* **2022**, *14*, eabn7571, doi:10.1126/scitranslmed.abn7571.
67. Winter, M.; Meignan, S.; Völkel, P.; Angrand, P.O.; Chopin, V.; Bidan, N.; Toillon, R.A.; Adriaenssens, E.; Lagadec, C.; Le Bourhis, X. Vimentin Promotes the Aggressiveness of Triple Negative Breast Cancer Cells Surviving Chemotherapeutic Treatment. *Cells* **2021**, *10*, doi:10.3390/cells10061504.
68. Al Saleh, S.; Al Mulla, F.; Luqmani, Y.A. Estrogen receptor silencing induces epithelial to mesenchymal transition in human breast cancer cells. *PLoS ONE* **2011**, *6*, e20610, doi:10.1371/journal.pone.0020610.
69. Alnuaimi, A.R.; Nair, V.A.; Malhab, L.J.B.; Abu-Gharbieh, E.; Ranade, A.V.; Pintus, G.; Hamad, M.; Busch, H.; Kirfel, J.; Hamoudi, R.; et al. Emerging role of caldesmon in cancer: A potential biomarker for colorectal cancer and other cancers. *World J Gastrointest Oncol* **2022**, *14*, 1637-1653, doi:10.4251/wjgo.v14.i9.1637.
70. Sobue, K.; Sellers, J.R. Caldesmon, a novel regulatory protein in smooth muscle and nonmuscle actomyosin systems. *J Biol Chem* **1991**, *266*, 12115-12118.
71. Ma, W.Q.; Miao, M.C.; Ding, P.A.; Tan, B.B.; Liu, W.B.; Guo, S.; Er, L.M.; Zhang, Z.D.; Zhao, Q. CALD1 facilitates epithelial-mesenchymal transition progression in gastric cancer cells by modulating the PI3K-Akt pathway. *World J Gastrointest Oncol* **2024**, *16*, 1029-1045, doi:10.4251/wjgo.v16.i3.1029.
72. De Marchi, T.; Timmermans, A.M.; Smid, M.; Look, M.P.; Stingl, C.; Opdam, M.; Linn, S.C.; Sweep, F.C.; Span, P.N.; Kliffen, M.; et al. Annexin-A1 and caldesmon are associated with resistance to tamoxifen in estrogen receptor positive recurrent breast cancer. *Oncotarget* **2016**, *7*, 3098-3110, doi:10.18632/oncotarget.6521.
73. Kim, C.; Gao, R.; Sei, E.; Brandt, R.; Hartman, J.; Hatschek, T.; Crosetto, N.; Foukakis, T.; Navin, N.E. Chemoresistance Evolution in Triple-Negative Breast Cancer Delineated by Single-Cell Sequencing. *Cell* **2018**, *173*, 879-893.e813, doi:10.1016/j.cell.2018.03.041.
74. Mayer, I.A.; Abramson, V.G.; Lehmann, B.D.; Pietenpol, J.A. New strategies for triple-negative breast cancer--deciphering the heterogeneity. *Clin Cancer Res* **2014**, *20*, 782-790, doi:10.1158/1078-0432.Ccr-13-0583.
75. Baselga, J.; Gómez, P.; Greil, R.; Braga, S.; Climent, M.A.; Wardley, A.M.; Kaufman, B.; Stemmer, S.M.; Pêgo, A.; Chan, A.; et al. Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. *J Clin Oncol* **2013**, *31*, 2586-2592, doi:10.1200/jco.2012.46.2408.
76. Carey, L.A.; Rugo, H.S.; Marcom, P.K.; Mayer, E.L.; Esteva, F.J.; Ma, C.X.; Liu, M.C.; Storniolo, A.M.; Rimawi, M.F.; Forero-Torres, A.; et al. TBCRC 001: Randomized phase II study of cetuximab in combination with carboplatin in stage IV triple-negative breast cancer. *J Clin Oncol* **2012**, *30*, 2615-2623, doi:10.1200/jco.2010.34.5579.
77. Burstein, H.J.; Elias, A.D.; Rugo, H.S.; Cobleigh, M.A.; Wolff, A.C.; Eisenberg, P.D.; Lehman, M.; Adams, B.J.; Bello, C.L.; DePrimo, S.E.; et al. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. *J Clin Oncol* **2008**, *26*, 1810-1816, doi:10.1200/jco.2007.14.5375.

78. von Minckwitz, G.; Eidtmann, H.; Rezai, M.; Fasching, P.A.; Tesch, H.; Eggemann, H.; Schrader, I.; Kittel, K.; Hanusch, C.; Kreienberg, R.; et al. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. *N Engl J Med* **2012**, *366*, 299-309, doi:10.1056/NEJMoa1111065.
79. Turner, N.; Lambros, M.B.; Horlings, H.M.; Pearson, A.; Sharpe, R.; Natrajan, R.; Geyer, F.C.; van Kouwenhove, M.; Kreike, B.; Mackay, A.; et al. Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. *Oncogene* **2010**, *29*, 2013-2023, doi:10.1038/onc.2009.489.
80. Ibrahim, Y.H.; García-García, C.; Serra, V.; He, L.; Torres-Lockhart, K.; Prat, A.; Anton, P.; Cozar, P.; Guzmán, M.; Grueso, J.; et al. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer Discov* **2012**, *2*, 1036-1047, doi:10.1158/2159-8290.Cd-11-0348.
81. Lehmann, B.D.; Abramson, V.G.; Dees, E.C.; Shah, P.D.; Ballinger, T.J.; Isaacs, C.; Santa-Maria, C.A.; An, H.; Gonzalez-Ericsson, P.I.; Sanders, M.E.; et al. Atezolizumab in Combination With Carboplatin and Survival Outcomes in Patients With Metastatic Triple-Negative Breast Cancer: The TBCRC 043 Phase 2 Randomized Clinical Trial. *JAMA Oncol* **2024**, *10*, 193-201, doi:10.1001/jamaoncol.2023.5424.
82. Marco Salas, S.; Kuemmerle, L.B.; Mattsson-Langseth, C.; Tismeyer, S.; Avenel, C.; Hu, T.; Rehman, H.; Grillo, M.; Czarnewski, P.; Helgadottir, S.; et al. Optimizing Xenium In Situ data utility by quality assessment and best-practice analysis workflows. *Nat Methods* **2025**, *22*, 813-823, doi:10.1038/s41592-025-02617-2.
83. Chen, C.; Li, Y.; Wei, W.; Lu, Y.; Zou, B.; Zhang, L.; Shan, J.; Zhu, Y.; Wang, S.; Wu, H.; et al. A precise microdissection strategy enabled spatial heterogeneity analysis on the targeted region of formalin-fixed paraffin-embedded tissues. *Talanta* **2024**, *278*, 126501, doi:10.1016/j.talanta.2024.126501.
84. Pinkney, H.R.; Black, M.A.; Diermeier, S.D. Single-Cell RNA-Seq Reveals Heterogeneous lncRNA Expression in Xenografted Triple-Negative Breast Cancer Cells. *Biology (Basel)* **2021**, *10*, doi:10.3390/biology10100987.

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