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Review

Multilevel classification framework for breast cancer cell selection and its integration with advanced disease models

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SUMMARY

Breast cancer cell lines are indispensable tools for unraveling disease mechanisms, enabling drug discovery, and developing personalized treatments, yet their heterogeneity and inconsistent classification pose significant challenges in model selection and data reproducibility. This review aims at providing a comprehensive and user-friendly framework for broadly mapping the features of breast cancer types and commercially available human breast cancer cell lines, defining *absolute criteria*, i.e., objective features such as origin (e.g., MDA-MB, MCF), histological subtype (ductal, lobular), hormone receptor status (ER/PR/HER2), and genetic mutations (*BRCA1, TP53*), and *relative criteria*, which contextualize functional behaviors such as metastatic potential, drug sensitivity, and genomic instability. It then examines how the proposed framework could be applied to cell line screening in advanced and emerging disease models. By supporting better informed choices, this work aims to improve experimental design and strengthen the connection between *in vitro* breast cancer studies and their clinical translation.

INTRODUCTION

Breast cancer cell lines are *in vitro* disease models widely used in biomedical research to gain insights into the pathophysiology of the disease, and to develop novel diagnostic and therapeutic strategies.[1] Derived from human tumors, they provide a renewable resource for investigating the cellular and molecular mechanisms underlying disease progression, enabling the search for new therapeutic agents and diagnostic markers, by recapitulating local conditions and allowing controlled perturbations *in vitro*.[2,3] In drug discovery and development, cell models are utilized to screen potential anti-cancer drugs for their efficacy and possible toxicity.[4] In personalized medicine, patient-derived cells allow to evaluate individual responses to specific treatments, aiming to improve therapeutic outcomes.[5] However, cell lines do not fully represent the heterogeneity of patient tumors, especially when employed in isolation, risking to oversimplify the biological environments characteristic of complex living systems.[6] Moreover, they may acquire genetic changes during long-term culture, leading to substantial alterations in both morphology and functionality.[6] In this scenario, selecting the optimal cancer cell line based on its properties and experimental objectives becomes critical toward obtaining reproducible and translatable results. This review presents a classification framework that distinguishes commercially available human breast cancer cell lines based on absolute criteria, such as origin and hormone receptor status, and relative criteria, such as metastatic potential and drug response. It illustrates how diverse cellular features can be systematically organized to optimize cell lines' use in translational research, alongside their integration with advanced disease models, from organoids to co-culture systems and patient-derived xenografts.

Absolute criteria form the foundational layer of cell lines classification, capturing static or semi-static features that define the cell line identity and heritage. The *origin* of the cell line, representing whether it is derived from a primary tumor or metastatic lesion, is a clear example of absolute criteria, and influences differentiation state and drug responsiveness. Histological subtype, including ductal, lobular, or metaplastic, offers another level of biological context that can dictate architectural and invasive properties.[7] Hormone receptor status (ER, PR, HER2), one of the most clinically relevant stratifications in breast oncology,[8]

drives therapeutic decisions and aligns with subtypes such as triple-negative or HER2-positive disease. Moreover, molecular subtypes, such as luminal A/B or basal-like, capture transcriptomic signatures that reflect biological states,[9] while common genetic mutations (e.g., *BRCA1/2, TP53, PTEN*) frame the cell within defined oncogenic trajectories.[10] Absolute parameters ensure that the use of a cell line is grounded in clinically and genetically meaningful choices; yet, relying solely on absolute descriptors neglects the functional plasticity of breast cancer cells.

The relative criteria component addresses this limitation by encompassing dynamic phenotypes and regulatory states that vary across contexts and influence experimental outputs. These include functional attributes such as metastatic ability, proliferation rate, and apoptotic resistance, which directly affect how a cell line behaves under experimental perturbation. For example, the propensity to metastasize to bone or brain, which is central to studying organotropism, is interlinked with signaling adaptations and cellular machinery, such as invadopodia formation and EMT programs.[11,12] Similarly, apoptotic resistance, shaped by alterations in caspase expression or FLIP activity, dictates the cell line survival under cytotoxic challenge and is therefore crucial for modeling drug resistance.[13] The relative aspects also incorporate advanced molecular and regulatory dimensions such as gene expression signatures (e.g., PAM50, MammaPrint), radiation response profiles, and drug sensitivity patterns that mirror therapeutic resistance observed in clinical settings. This stratification aligns with the contemporary view that cancer is a highly adaptive and evolving system.[14] Additionally, layers such as epigenomic modifications, stem cell properties, and inflammatory status bridge the molecular and microenvironmental axes of tumor biology. For instance, ALDH1 or CD44+/CD24− status has been linked to tumor-initiating potential and chemoresistance,[15] and immune signatures (e.g., PD-L1 or cytokine expression) can dictate immunotherapeutic outcomes.[16] Genomic instability adds another dimension, often indicating susceptibility to specific DNA-damaging agents or synthetic lethality strategies.[17]

This schematization emphasizes how integrating these criteria could drive the choice and development of more predictive and clinically relevant models. Multi-omics could then allow the simultaneous profiling of genomics, transcriptomics, and proteomics to refine cell line characterization, enabling matching to patient-derived data. AI and data fusion techniques could forecast drug responses or disease trajectories based on integrative datasets. Finally, informed baseline choices could guide the design of *in vitro* models. 3D tissue constructs and co-culture systems better recapitulate *in vivo* architectures and cell-cell interactions, serving as an essential bridge toward preclinical and patient-derived xenograft (PDX) models, ensuring that selected cell lines can perform in biologically complex environments or be employed for their development.

ABSOLUTE CLASSIFICATION CRITERIA

We define as *absolute criteria* those features that can be classified statically and objectively. As opposed to *relative criteria* (e.g., metastatic potential graded as *low* or *high*), absolute criteria are intrinsic attributes such as cellular origin (e.g., "MCF" origin, Michigan Cancer Foundation), histological subtype, hormone receptor status, genetic alterations, and molecular subtype. The following subsections address these characteristics.

Cell line origin

The origin of a cell line denotes its derivation from specific breast tumor tissues. This classification is rooted in the cell line provenance and drives its research application. For brevity, the origins of breast cancer cell line families, along with their associated experimental uses, are summarized in Table 1. In addition to tumor-derived breast cancer cell lines, engineered breast epithelial models have been developed to study specific processes such as transformation, epithelial-to-mesenchymal transition (EMT), and cancer stem cell behavior in a more controlled context. A widely used model is MCF10A, a non-tumorigenic human mammary epithelial cell line often used as the healthy reference in breast cancer studies.[18] More advanced systems include HMLE and HMLER cells. HMLE cells are obtained by introducing genes that prevent cellular aging (*hTERT*) and inhibit tumor suppressor activity (SV40 large T antigen), allowing long-term growth in culture.[19] When the HMLE model is further modified with an oncogene (*H-RAS*), it becomes tumorigenic (known as HMLER).[19] A variant called HMLER-shEcad, where the gene for E-cadherin is silenced, is commonly used to model EMT and the acquisition of cancer stem cell–like traits.[19] Despite lacking the genetic complexity of actual tumors, these models are valuable tools to dissect the functional impact of specific molecular changes and complement the use of patient-derived breast cancer cell lines in experimental research.

Histological subtype

Tumor morphology, its growth pattern, degree of differentiation, and resemblance to normal terminal duct-lobular units (TDLUs), determine if a lesion is *in situ* or invasive, with invasive tumors carrying a higher risk of metastasis.[46] Histological classifications also guide molecular profiling and subsequent targeted therapy selection. This section details the histological diversity of breast cancer and emphasizes clinic-pathological characteristics and correlations. Adenocarcinomas, comprising over 95% of breast cancer, arise from the glandular epithelium of ducts or lobules (Figure 1). They are characterized by glandular differentiation and mucin production, the latter being intracellular, as in signet-ring cells, or extracellular, as seen in mucinous carcinomas.[47] Such tumors are subclassified by their site of origin and invasiveness. Ductal carcinoma is the most prevalent breast cancer type, originating in the mammary milk ducts.[48] Ductal and lobular carcinoma *in situ* represent the two main forms of preinvasive breast adenocarcinomas. Ductal carcinoma *in situ* (DCIS) originates in the mammary ducts and remains confined to the ductal system, exhibiting a range of architectural patterns such as solid, cribriform, papillary, and micropapillary.[7] Lobular carcinoma *in situ* (LCIS), by contrast, arises in the terminal ductal lobular units and is characterized by the proliferation of neoplastic cells that distend and fill the acini. LCIS is considered a non-obligate precursor of invasive lobular carcinoma and is classified into three main histological subtypes: classic, pleomorphic, and florid. These variants differ in cytologic features, architectural patterns, and potential biological behavior.

Table 1. Cell line family, acronym origin, representative cell lines, and clinically relevant research applications of cell line families

Cell line family	Acronym origin	Representative cell lines		Key research applications of the cell line family	Reference
		Name	*Source*		
MDA-MB	M.D. Anderson Cancer Center - Mammary/Breast	MDA-MB-231	Metastatic sites, pleural effusions	Metastasis; chemoresistance; tumor-microenvironment interactions	Cailleau et al.[20]
		MDA-MB-468	Brain metastasis		
MCF	Michigan Cancer Foundation	MCF-7	Pleural effusions	HR+ Breast cancer progression; weakly metastatic control	Soule et al.[21]
		MCF-10A	Fibrocystic breast tissue		
HCC	Human Cancer Culture	HCC1937	Primary breast tumor carrying BRCA-1 mutation	DNA repair defects; targeted therapy resistance	Tomlinson et al.[22]
		HCC1954	HER2-positive metastatic site		
BT	Breast Tumor	BT-474	solid invasive ductal carcinoma, HER2 amplification	HER2-targeted therapies (drug testing, e.g., lapatinib)	Lasfargues et al.[23]
		BT-20	Primary TNBC, lacks functional TP53		
CAMA	Caucasian Malignant Adenocarcinoma	CAMA-1	Liver metastasis	Endocrine resistance mechanisms	Fogh et al.[24]
SK-BR	Sloan Kettering Institute – Breast	SK-BR-3	Pleural effusion, TP53 mutation	HER2-targeted therapies	Trempe[25]
ZR	Zurich/Michigan cancer foundation	ZR-75-1	Ascitic effusion, metastatic ductal carcinoma	Hormone receptor plasticity; metastatic adaptation	Engel et al.[26]
		ZR-75-30	Subline of the above, with reduced hormone dependence		
SUM	Dr. Stephen Ethier, University of Michigan	SUM-149PT	Primary inflammatory TNBC, BRCA-1 mutation	IBC-specific pathways	Forozan et al.[27]
		SUM-159PT	Metastatic site of inflammatory TNBC		
Hs	Human Somatic	Hs578T	Breast carcinosarcoma	Sarcomatoid differentiation; tumor-stroma crosstalk	Hackett et al.[28]
DU	Duke University	DU4475	Rare metastatic TNBC model	niche-specific metastasis mechanisms	van de Wetering et al.[29]
CAL	Cancer Associated Line	CAL-51	Ductal carcinoma, TP53-mutated	tumor heterogeneity	Neve et al.[30]
		CAL-120	Metastatic site, basal-like		
MFM	Max Faber Memorial laboratory	MFM-223	Pleural effusion with metaplastic TNBC	tumor-stroma interactions; drug sensitivity in metaplastic carcinomas	Gazdar et al.[31]
PMC	Primary Malignant Culture	PMC-42	Invasive ductal carcinoma, forms organoids *in vitro*	Morphogenesis; polarization; extracellular matrix role in tumor progression	Whitehead et al.[32]
UACC	University of Arizona Cancer Center	UACC-812	metastatic site (likely lymph node)	Drug resistance; gene signatures and drug response	Barretina et al.[33]
		UACC-893	HER2-positive ductal carcinoma		
EMG	Epidermal Malignant Growth	EM-G3	scirrhous carcinoma, desmoplastic subtype	Desmoplasia; tumor microenvironment crosstalk	Mladkova et al.[34]

(*Continued on next page*)

Table 1. ***Continued***

Cell line family	Acronym origin	Representative cell lines		Key research applications of the cell line family	Reference
		Name	*Source*		
HDQ	*Unknown*	HDQ-P1	Primary ductal carcinoma with BRCA-2 mutations	Synthetic lethality strategies; resistance mechanisms	Holstege et al.[35]
EFM	European Foundation for Medicine	EFM-19	Malignant pleural effusion	epigenomic modifications; alternative survival pathways	Glont et al.[36]
IBEP	Instituto de Biomedicina, Estudio de Proliferación	IBEP-1	Invasive ductal carcinoma, luminal-like	intratumoral heterogeneity; clonal evolution	Dai et al.[37]
		IBEP-2	Invasive ductal carcinoma, basal-like		
KPL	Kurebayashi Pleural Line	KPL-1	malignant ascites of a HER2-positive patient	antibody-drug conjugate mechanisms	Kurebayashi et al.[38]
LY	Dr. Anne Lykkesfeldt	LY-2	tamoxifen-resistant subline of MCF-7	HR and growth factor pathways; role of autophagy in acquired resistance	Brunner et al.[39]
T	Tissue culture	T-47D	Pleural effusion	progesterone receptor (PR) signaling; CDK4/6 inhibitor responses	Keydar et al.[40]
BSMZ	Bützow, Sager, Müller, Zurich	BSMZ	Mucinous carcinoma	glycoprotein-mediated immune evasion and matrix adhesion	Holliday and Speirs[18], Watanabe et al.[41]
AU	Auburn University	AU565	Metastatic site	antibody-drug conjugates	Bacus et al.[42]
21	Age of patient (21 years old)	21-MT-1	Metastatic breast tumor	PARP inhibitor responses; metastasis-initiating cells	Ince et al.[43]
		21-PT	Primary breast tumor		
HMT	Hanyang Medical Team	HMT-3902S1	Primary breast tumor	TGF-β-driven EMT and metastasis in xenograft models	Petersen et al.[44]
MA	Metastatic Adenocarcinoma	MA-11	Bone metastasis	bisphosphonate efficacy; tumor-osteoclast crosstalk in metastases	Micci et al.[45]

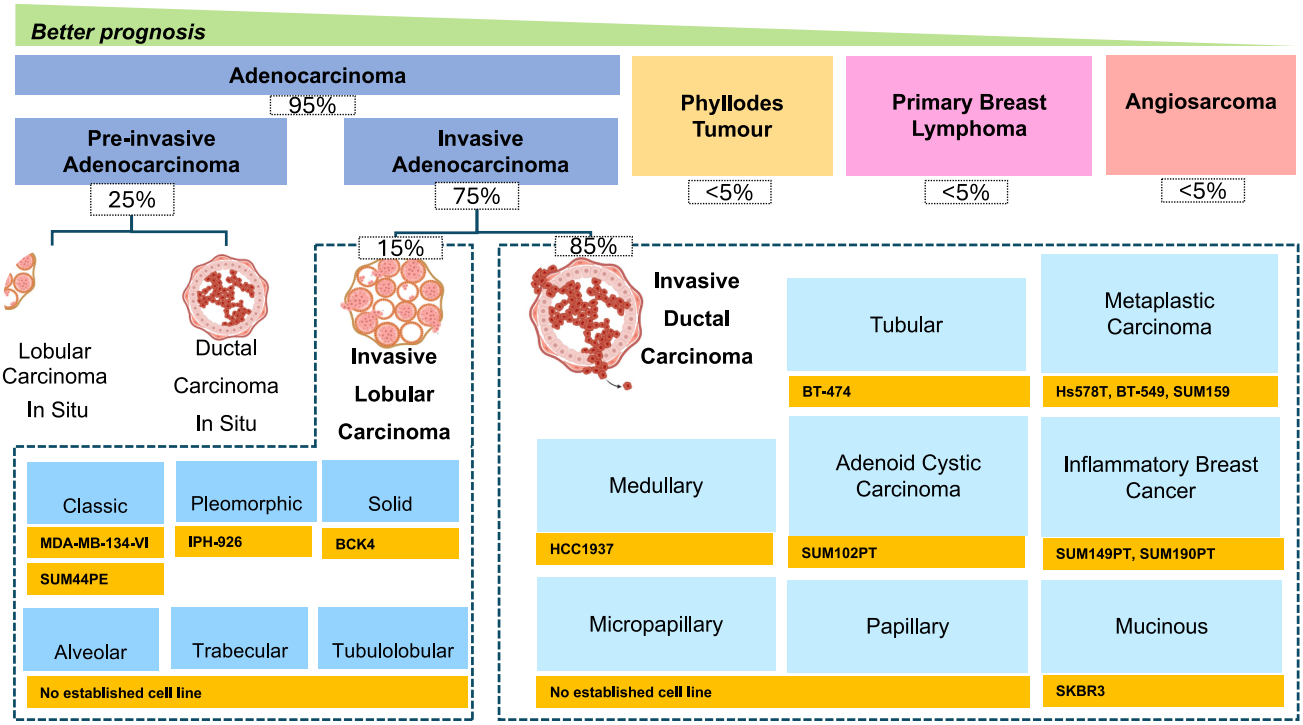

Figure 1. Histological subtypes of breast cancer

Breast cancer cell lines are grouped by the histological subtype of the deriving tumors and their sub-classifications, alongside examples of widely used cell lines for each cancer type. For example, SUM149PT and SUM190PT were established from inflammatory breast cancer, while MDA-MB-134-VI and SUM44PE originated from classic ILC. However, not all subtypes are well-represented by directly derived models. In such cases, some widely employed cell lines (e.g., MCF-7 and MDA-MB-231), though not derived from rare subtypes like papillary or metaplastic carcinoma, may still serve as functional models due to their phenotypic behavior.

Invasive lobular carcinomas (ILCs) account for 10–15% of breast cancer cases, arise from the terminal duct-lobular units, and are marked by the loss of E-cadherin, typically due to *CDH1* mutations.[49] They are commonly harder to diagnose from mammograms due to not forming calcifications. ILC is usually ER-positive and HER2-negative, with distinct genomic alterations.[50] The majority of ILCs consist of low-nuclear-grade malignant cells[50] (i.e., classic ILCs). In a minority of ILCs, the tumor consists of high-nuclear-grade malignant cells (i.e., pleomorphic ILC). ILC can exhibit a range of histological growth patterns, including solid, alveolar, trabecular, and tubulolobular variants.[49] These patterns reflect the morphological diversity of ILC and occasionally pose diagnostic challenges. Regardless of pattern, these tumors typically retain the hallmark feature of E-cadherin loss, confirming their lobular origin.[29,50] Invasive Ductal Carcinoma (IDC), which constitutes 70–80% of invasive breast cancer cases, invades the stroma and causes desmoplastic reactions.[48] IDC is molecularly heterogeneous, with luminal subtypes expressing hormone receptors, HER2-enriched tumors exhibiting *ERBB2* amplification, and basal-like tumors being triple-negative.[7,37] The majority IDCs are classified as no special type (IDC-NST), yet several distinct histological and clinical variants exist.[48]

Metaplastic carcinoma (MpC) is an aggressive form of invasive breast cancer, often classified as triple-negative.[7] It is highly heterogeneous, typified by epithelial-to-mesenchymal transition, which produces variable differentiation, including squamous, spindle, or chondroid elements.[51] This subtype of breast cancer is noted for its resistance to chemotherapy.[52] Tubular carcinoma (TC) is a rare form of breast cancer defined by the proliferation of angulated, oval, or elongated tubules reminiscent of normal breast ducts. Its invasive nature, coupled with the absence of myoepithelial cells, distinguishes it from benign lesions.[53] Micropapillary carcinoma (MiC) is another aggressive subtype seen in 1–2% of breast cancer cases, characterized by clusters of tumor cells arranged in an inside-out pattern without fibrovascular cores.[54] Despite often being ER-positive, this cancer type displays high rates of lymph node involvement and commonly exhibits HER2 amplification or *PIK3CA* mutations.[54] Adenoid cystic carcinoma (ACC) is an extremely rare subtype (<0.1% incidence), featuring biphasic cell populations, luminal and basaloid, that form tubular, cribriform, or solid patterns surrounded by mucinous material.[7,55] As opposed to its salivary gland counterpart, breast ACC rarely metastasizes, with the surgical excision often proving curative.[56]

Hormone-receptor status

Receptors are proteins typically found in the cell membrane that can be bound by matching extracellular molecules to elicit intracellular signaling or to enable inter-cellular communication.[57,58]

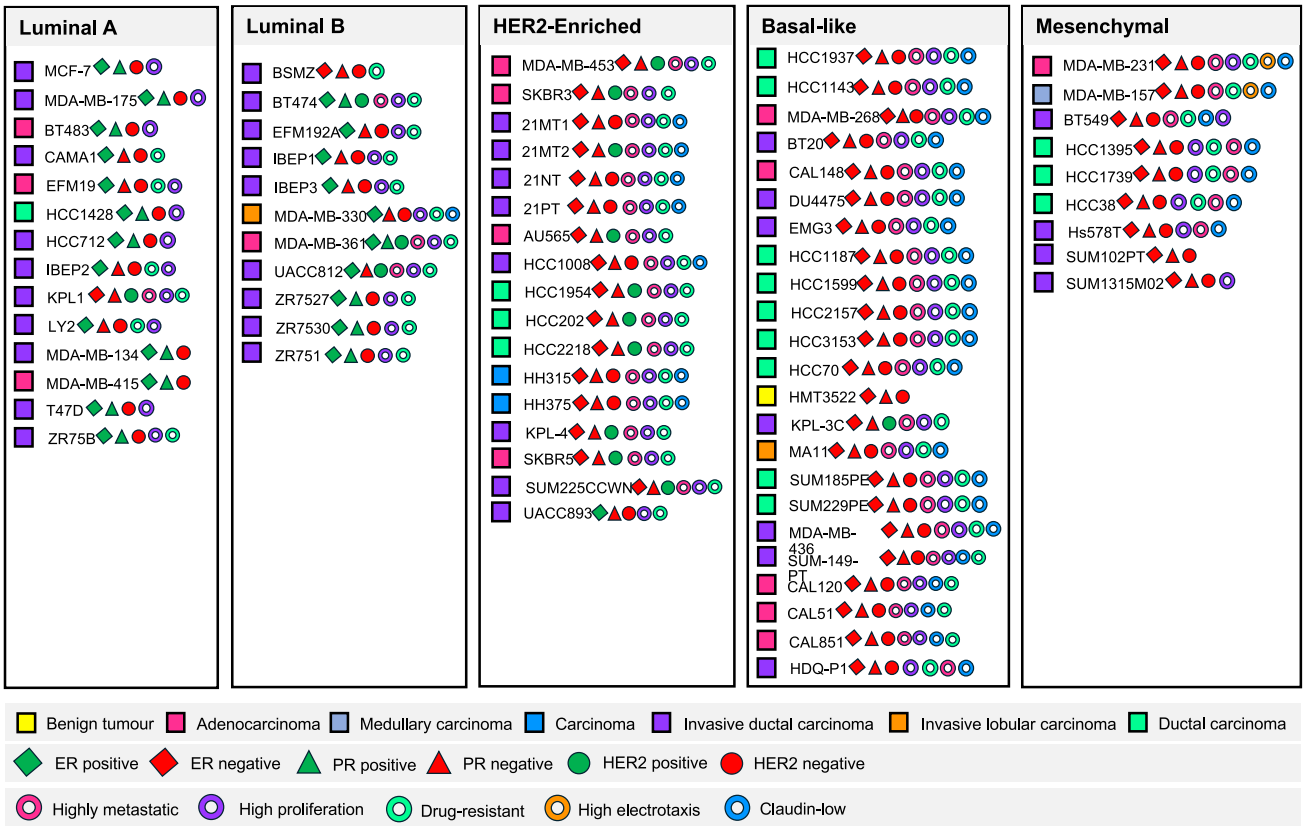

Figure 2. Classification of selected commercially available human breast cancer cell lines

The schematic organizes cell lines into five intrinsic subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like, and Mesenchymal) displayed in columns. Color-coded symbols represent key attributes used in absolute and relative classifications previously discussed hereby to map their molecular and functional characteristics.

Some breast cancer cells possess certain receptors to hormones (HRs) that contribute to cellular behaviors, including growth, proliferation, and motility.[59,60] HR status has been widely used to classify breast cancer cell lines (Figure 2). HRs include the estrogen receptor (ER) and the progesterone receptor (PR). Another important receptor is the human epidermal growth factor receptor 2 (HER2). HR/HER2 expression, among other variables, is one of the most important factors in estimating the prognosis and therapeutic responses of breast cancer.[61] Estrogen receptor-positive (ER+) breast cancer is the most frequently diagnosed subtype. However, only about 30% of the commercially available breast cancer cell lines are ER+, and these models frequently derive from advanced disease states.[62] From those, very few can be grown in mice, such as MCF7, T47D, and ZR-75-1, requiring high levels of exogenous estrogen (E2).[8] This does not reflect the low levels of estrogen found in postmenopausal women, where most cases of ER+ breast cancer develop, making these models limited in scope. The majority of ER+ breast cancer is also PR-positive (PR+).[57] Elevated PR levels are predominantly observed in luminal A tumors, which yield better outcomes compared to luminal B tumors, where PR expression is lower.[63] Approximately 15% of breast cancers are human epidermal growth factor receptor 2 positive (HER2+), a subtype that typically affects younger patients and is diagnosed at advanced stages.[64] HER2 overexpression, an independent predictor of poor survival, often occurs irrespective of ER and PR expression.[16] Triple-positive breast cancer (TPBC) is a luminal B subtype co-expressing ER, PR, and HER2, accounting for roughly 10–15% of cases.[65] It often demonstrates suboptimal responses to standard chemotherapy and hormone therapy due to intricate crosstalk between the ER and HER2 pathways.[66] Triple-Negative Breast Cancer (TNBC) lacks the expression of ER, PR, and HER2, and accounts for approximately 15% of cases.[67] TNBC is predominantly basal-like, is more common in younger women, and exhibits an increased risk of early recurrence and distant metastasis. It is strongly associated with *BRCA1* mutations.[68] The ER+/HER2– subtype represents the most common breast cancer phenotype (approximately 75% of cases) and is typically classified as luminal A-like,[69] while the ER+/PR+/HER2+ pattern is classified as luminal B-like.[14] Luminal B cancers with an ER+/PR–/HER2+ profile generally portend a worse prognosis than their ER+/PR+ counterparts, while HER2-positive cancers that are ER–/PR– are managed predominantly with HER2-targeted.[70]

Genetic mutations

Breast cancer is primarily driven by genetic factors, with age and family history being the most significant risk factors.

Approximately 5–10% of breast cancer cases are associated with inherited gene mutations.[10,71] Germline alterations in *BRCA1* and *BRCA2*, among the most widely known mutations, compromise DNA repair and confer a markedly increased lifetime risk, predisposing tumors to either triple-negative or predominantly ER-positive phenotypes, respectively.[72] Mutations in *TP53*, present in nearly 30% of cases, disrupt critical cell cycle checkpoints and promote aggressive tumor behavior with poorer outcomes.[9] *PTEN* mutations are strongly correlated with HER2+ breast cancers,[73] and inversely associated with luminal type breast cancers[74] affecting cell growth, proliferation, and inhibiting cancer stem cell activity.[75] Other important mutations include defects in *CHEK2* and *ATM,* weakening cell cycle control and apoptotic responses.[76] Alterations in *PALB2*, *CDH1*, *STK11*, and *NF1* contribute to genomic instability, drive invasive characteristics, and influence therapeutic resistance.[77] Genetic aberrations define distinct molecular subtypes in breast cancer and are essential for guiding targeted treatments in precision oncology. Genetic drift represents a different problem, further addressed in this review.

Molecular subtype

Gene expression profiling and hierarchical clustering have delineated five principal molecular subtypes, each with distinct biological behavior, risk factors, and therapeutic responsiveness, namely luminal A, luminal B, HER2-enriched, basal-like, and claudin-low.[78] Luminal A is the most common subtype of breast cancer, accounting for around 40% of all breast cancer cases.[78] It is characterized by an expression of luminal (low molecular weight) cytokeratins, ER, and PR, with a HER2 negative profile accounting for the low expression of cell proliferation marker Ki-67 (less than 20%).[79] Luminal B subtype represents 20–30% of cases, expressing ER (with often reduced PR) and displaying high proliferation indices (Ki67 above 20%).[79] These are generally of higher histologic grade, more aggressive, and have a higher recurrence rate compared to Luminal A subtypes, necessitating combined endocrine and chemotherapeutic approaches.[80] HER2-enriched comprise approximately 15% of breast cancer cases.[81] These are HER2-positive tumors, while often exhibiting low or absent ER and PR levels.[82] This category is subdivided into luminal HER2 (E+, PR+, HER2+ with intermediate Ki67, 15–30%) and HER2-enriched (E−, PR–, HER2+ with high Ki67, >30%), both marked by high-grade invasive ductal carcinomas with nodal positivity and aggressive clinical behavior.[78] Basal-like is often used as a synonym of triple-negative breast cancer (TNBC), lacking ER, PR, and HER2 expression, while expressing basal cytokeratins.[83] Basal-like breast cancers are typically high grade, occurring in patients with *BRCA1* mutations, and have limited treatment options outside chemotherapy. Claudin-low tumors are characterized by the low expression of cell adhesion molecules and a stem cell–like phenotype. This rare and aggressive subtype is often considered a subclass of basal-like but has gained interest as an *in vitro* model to reproduce highly aggressive cancers.[52]

Patient age, gender, and ethnicity

Additional absolute criteria include patient age, gender, and ethnicity. Age critically influences breast cancer risk, tumor morphology, and treatment response.[78] Tumors in patients under 40 typically exhibit reduced levels of estrogen receptor, progesterone receptor, and luminal cytokeratin, alongside elevated Ki67, HER2, and p53 expression, indicative of aggressive behavior.[84] In contrast, tumors in individuals over 70 generally display indolent features. Yet, most commercially available cell lines were derived from older patients, potentially limiting experimental relevance.[84] Ethnicity further modulates breast cancer biology, as disparities in healthcare result in later diagnoses in Hispanic and Asian populations, while non-Hispanic black patients exhibit a tumor microenvironment enriched with protumorigenic immune cells, enhanced microvasculature, and elevated mitotic kinases and transcription factors that promote aneuploidy.[85] More in general, marketed cell lines are predominantly caucasian.[80] With regards to gender, although breast cancer is most commonly viewed as a female disease, it can also occur in men, where it accounts for less than 1% of all cancers in men and breast cancer cases overall.[86] However, male breast cancer incidence has risen over the past 30 years, with inherited pathogenic variants being the most significant risk factors.[85] Transgender individuals may also face breast cancer risks, particularly if receiving hormone treatment. Studies have shown an increased risk of breast cancer in transexual women compared with cisgender men, and a lower risk in trans men compared with cisgender women.[87]

RELATIVE CLASSIFICATION CRITERIA

This section details key classification parameters specific to breast cancer to emphasize their mechanistic underpinnings and clinical utility. Such features are defined as relative due to providing a qualitative measure of cancer cell behavior. Important relative criteria include metastatic ability, proliferation rate, often measured by Ki-67 expression or mitotic indices,[88] response to radiation reflecting the tumor sensitivity to DNA damage-induced cell death,[89] and drug resistance encompassing mechanisms by which tumors evade therapeutic agents (such as *ESR1* mutations in hormone-resistant HR+ disease).[90] Inflammatory status reflects, among all, immune microenvironment composition and stem cell-like properties.[91] A summary of key relative criteria, their working mechanisms, and implications is summarized in Table 2, alongside absolute criteria to provide a complete overview.

Metastatic ability

Metastatic ability refers to the capacity of tumor cells to colonize distant organs such as bone, brain, and liver, and it is driven by EMT.[11] In metastatic behavior, transcription factors downregulate E-cadherin, enhancing motility and favoring migration to colonize new sites. Bone metastasis, one of the hardest to treat, involves osteolytic factors that activate osteoclasts via RANKL signaling.[11] Circulating tumor cells expressing HER2 or EpCAM have been associated with increased metastatic risk.[102] Metastatic behavior also differs based on subtype-specific organotropism: luminal tumors often metastasize to bone, HER2+ to liver and lungs, and basal-like tumors to brain and lung.[12] These preferences reflect intrinsic properties of the tumor cells, including receptor expression, secreted factors, and ability

Table 2. Absolute and relative classification criteria, key features, and clinical relevance

	–	Breast cancer subcategory	Key features and molecular details	Clinical relevance	Reference
Absolute criteria	Histological subtype	Adenocarcinoma	>95% of cases; arises from glandular epithelium; glandular differentiation and mucin production	Subtyped as ductal vs. lobular; informs targeted therapy	Fogh et al.[24], Rakha and Ellis[47]
		Ductal carcinoma	Divided into DCIS and IDC; IDC is molecularly heterogeneous (luminal, HER2, basal-like)	Provides prognostic stratification based on grade and subtype	Makki[7], Allred[48]
		Lobular carcinoma	Arises from TDLUs; loss of E-cadherin (*CDH1* mutations); usually ER+ and HER2–; associated with *FOXA1*, *TBX3* mutations	Complicates detection; influences therapeutic strategies	Christgen et al.,[49], Cristofanilli et al.[50]
		Inflammatory breast cancer	Rare (1–5%); typically, triple-negative or HER2+; overexpresses EGFR, ANXA1, and COX-2; activation of WNT/β-catenin and NF-κB pathways	Highly aggressive with rapid progression	Robertson et al.[92]
		Medullary carcinoma	Syncytial growth (>75%), absence of glandular/tubular structures; frequent mitoses	Rare IDC variant with distinct histological features	Makki[7]
		Mucinous carcinoma	Extracellular mucin; clusters; typically, ER+, HER2–; low *TP53* mutation; *AKT1* E17K mutations	Generally lower grade and favorable prognosis	Marrazzo et al.[93]
		Papillary carcinoma	Papillary with fibrovascular cores; subtypes include intraductal, encapsulated, solid, and invasive forms	Crucial to differentiate benign from malignant lesions	Pal et al.[94]
		Metaplastic carcinoma	Aggressive TNBC subtype; heterogeneous with evidence of EMT transition; chemoresistant	High resistance profiles; therapeutic challenges	Yan et al.[51], Hennessy et al.[52]
		Tubular carcinoma	Well-differentiated; small cell tubules arranged radially; invasive	Rare, low-grade, and excellent prognosis	Peters et al.[53]
		Micropapillary carcinoma	Often ER+ with high lymph node metastasis; MUC1 overexpression; may have HER2 amplification or *PIK3CA* mutations	Poorer prognosis necessitating adjuvant chemotherapy	Cheng et al.[95]
		Adenoid cystic carcinoma	Rare (<0.1%); TN yet indolent; *MYB-NFIB* fusions triggering NOTCH pathway activation	Surgical excision is often curative	Persson et al.[56]
	Hormone Receptor Status	ER+	Most prevalent; includes MCF7, T47D, ZR-75-1; require high exogenous estrogen; responsive to endocrine therapy	Cell lines may not mimic low estrogen conditions of postmenopausal patients	Putti et al.[57]

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Table 2. *Continued*

–	Breast cancer subcategory	Key features and molecular details	Clinical relevance	Reference
	PR+	Expressed in response to ER activation; higher levels common in luminal A; prognostic marker	PR positivity generally correlates with better outcomes	Clark et al.[58]
	HER2+	15% of cases; overexpression of HER2; adverse prognostic indicator independent of ER/PR	Managed with HER2-targeted agents (e.g., trastuzumab)	Jerusalem et al.[64]
	Triple positive (TPBC)	Co-expression of ER, PR, and HER2; luminal B subtype (~10–15%); pathway crosstalk	Requires combinatorial therapeutic approaches	Vici et al.[66]
	Triple negative (TNBC)	Lacks ER, PR, and HER2; predominantly basal-like; distant metastasis; linked with *BRCA1* mutations	Limited targeted therapies	Dai et al.[59]
	ER+/HER2–	Most common phenotype (~75% of cases); classified as luminal A	Endocrine treatments	Stravodimou and Voutsadakis[69]
	ER+/PR–/HER2+	Luminal B variant with altered receptor signaling; poorer prognosis compared to ER+/PR+HER2+	Potential endocrine therapy resistance	Ding et al.[70]
	ER–/PR–/HER2+	HER2-overexpressing cancers lacking hormone receptors	Treated with HER2-targeted drugs	Mukai[81]
Genetic Mutations	*BRCA1*	Germline mutations (16% of hereditary BC); crucial for DNA repair and cell cycle regulation; TNBC	Key target for PARP inhibitor strategies	van der Groep et al.[96]
	BRCA2	Functions in DNA repair and genomic stability; 70–80% of *BRCA2*-mutated cancers are ER+	Influences endocrine therapy in mutation carriers	Andreassen et al.[72]
	TP53	Mutated in ~30% of BC; mediates cell-cycle arrest, apoptosis, or senescence upon DNA damage	Determines tumor aggressiveness	Cancer Genome Atlas Network[9]
	PTEN	Regulates the PI3K/Akt pathway; linked with HER2+ cancers, inversely with luminal types	Its loss may direct targeted treatment strategies	Lebok et al.[74]
	PALB2	Works in tandem with *BRCA1/2* in DNA repair; often associated with aggressive TNBC phenotypes	Emerging target in personalized therapeutic approaches	Toss et al.[97]
	CHEK2	Checkpoint kinase mutation; majority are luminal A with some lobular features	Reflects cell cycle checkpoint dysfunction	Toss et al.[98]
	ATM	Involved in cell cycle regulation and apoptosis; loss-of-heterozygosity in	May influence chemotherapeutic decisions.	Stucci et al.[99]

(*Continued on next page*)

Table 2. *Continued*

	–	Breast cancer subcategory	Key features and molecular details	Clinical relevance	Reference
			~40% of sporadic BC; mainly in luminal B/HER2-		
		CDH1	Encodes E-cadherin; loss leads to cell dissociation; mutations predispose to invasive lobular carcinoma	Essential for hereditary lobular BC surveillance	Corso et al.[100]
		STK11	Associated with Peutz-Jeghers syndrome; predisposes to other tumor types (ovarian, lung, GI)	Important for multi-tumor screening	van Lier et al.[101]
		NF1	Mutations in NF1 occur in ~27% of BC; endocrine resistance and metastasis, especially in ER+ cases	May predict targeted endocrine resistance management	Dischinger et al.[82]
	Molecular Subtype	Luminal A	~40% of cases; ER/PR positive; low Ki67 (<20%); typically, IDC; high endocrine therapy response	Represents the best prognostic subgroup	Eliyatkin et al.[79]
		Luminal B	20–30% of cases; ER positive (with lower PR expression); high Ki67 (>20%); variable HER2	More aggressive; requires combined therapy approaches	Marrazzo et al.[93]
		HER2-enriched	~15% of cases; overexpresses HER2; subdivided into luminal HER2 and HER2-enriched	Aggressive; outcomes improve with HER2-targeted therapies	Fragomeni et al.[78]
		Basal-like (TNBC)	15–20% of cases; lacks ER, PR, and HER2; expresses basal cytokeratins; linked with *BRCA1*	Limited treatment options	Fragomeni et al.[78]
		Mesenchymal (claudin-low)	Rare; low expression of cell adhesion molecules and stem-cell-like phenotype	High tumor plasticity and potential resistance	Dischinger et al.[82]
Relative Criteria	Metastatic ability		EMT transition; downregulation of adhesion molecules; organ tropism. SNAIL, TWIST, ZEB1; downregulation of E-cadherin; PTHrP, IL-11; RANKL; HER2, EpCAM; overexpression of COX2, HB-EGF, *ST6GALNAC5* in brain-tropic cancers	Enhanced motility; colonizes distant sites	Nguyen et al.[11], Bos et al.[102]
	Tumor, migration and invasiveness		Cell detachment; ECM degradation; cytoskeletal reorganization; invadopodia formation; integrin-mediated motility. MMP-9, MMP-14; RhoA/ROCK; TGF-β–driven integrin αvβ6; proliferation markers like Ki-67; Cyclin D1–CDK4/6	Invasive capacity; differential proliferation in luminal A vs. B subtypes; targeted therapeutic strategies	Goldhirsch et al.[88], Sossey-Alaoui et al.[103]

(*Continued on next page*)

Table 2. *Continued*

–	Breast cancer subcategory	Key features and molecular details	Clinical relevance	Reference
		complexes; *MYC* amplification; PI3K/AKT hyperactivity		
Apoptotic resistance		Upregulation of anti-apoptotic proteins; Overexpression of Bcl-2 (luminal tumors), *TP53* mutations (basal-like), FLIP upregulation in TNBC; *BRCA1/2*; HIF-1α and carbonic anhydrase IX in hypoxic conditions	Chemo/radio resistance; combined treatments such as PARP inhibition with radiotherapy	–
Gene expression and drug resistance		Resistance through specific mutations. PAM50, Oncotype DX, MammaPrint; *ESR1* mutations (Y537S, D538G); *PTEN* loss; *PIK3CA* mutations; βIII-tubulin overexpression; sensitivity to HER2-targeted agents, platinum salts, and AR inhibitors	Recurrence risk assessment; endocrine and targeted therapy resistance	Kleer[104]
Epigenomic modifications		*BRCA1* hypermethylation reversible by HDAC inhibitors; *EZH2*	Influences chemoresistance, recurrence, and therapeutic response	Neve et al.[30]
Tumor -immune system interactions		Tumour-infiltrating lymphocytes; IL-6/STAT3-mediated PD-L1 upregulation; ALDH1, CD44/CD24; Notch, Hedgehog, WNT/β-catenin; HR deficiency scores; *APOBEC3B*	Immunotherapeutic resistance and increased invasive capacity	Walker[16], Mackenzie et al.[105]

to modify the pre-metastatic niche. Some cell lines, such as MDA-MB-231, consistently metastasize to lung and bone in murine models, while others such as MCF-7 require estrogen supplementation and genetic manipulation to become metastatic.[106] Additional drivers include specific signaling cascades, for example, CXCR4-CXCL12, involved in bone homing, and MMPs, which degrade the extracellular matrix to facilitate invasion.[11] Exosomes from metastatic cells can precondition distant niches to favor colonization.[12] Moreover, stem-like subpopulations with $CD44^{high}/CD24^{low}$ profiles exhibit heightened metastatic ability and are linked to relapse.[19,52]

Migration and invasiveness

Migration and invasiveness describe the ability of cancer cells to detach from the primary tumor site, degrade the extracellular matrix, and infiltrate surrounding tissues.[12] Invasive breast cancer, particularly TNBC and HER2-positive subtypes, exhibits heightened migration via RhoA/ROCK-mediated cytoskeletal reorganization and MMP-9/MMP-14-dependent extracellular matrix degradation.[103] *In vitro* models, for instance, MDA-MB-231 cell invasion assays, reveal that TGF-β signaling enhances motility by upregulating integrin αvβ6.[83] These behaviors are often initiated during partial EMT, which increases cellular plasticity while maintaining some epithelial traits, allowing dynamic adaptation to microenvironmental cues. Migratory activity can be random or directional (chemotaxis), with the directional migration often guided by gradients of stromal-derived factors.[107] Tumor-associated fibroblasts and macrophages also promote invasion by remodeling the ECM and secreting pro-migratory cytokines.[108] Additionally, invadopodia (actin-rich protrusions) formation facilitates local matrix degradation and is prominent in highly invasive cell lines.[69]

Apoptotic resistance

Apoptotic resistance denotes the tumor evasion of programmed cell death, enabling survival despite genomic damage or therapy.[13,90,109] Apoptotic evasion in breast cancer is linked to Bcl-2 overexpression in luminal subtypes and *TP53* mutations in basal-like tumors.[35,83] TNBCs frequently exhibit FLIP upregulation, which inhibits caspase-8 activation.[107] PARP inhibitors (e.g., olaparib) exploit synthetic lethality in *BRCA1/2*-mutated tumors by impairing DNA repair and forcing apoptosis.[110] Some breast cancer cells bypass apoptosis entirely by entering a senescent-like state or activating autophagy as an adaptive survival strategy under therapeutic stress.[15] Apoptotic resistance is commonly assessed *in vitro* using Annexin V/PI staining, TUNEL assays, and caspase-3/7 activity measurements.[5,13] Cell lines such as MDA-MB-231 and BT-549 are often used to model high apoptotic resistance, particularly in response to chemotherapy or radiation.[15]

Gene expression profile

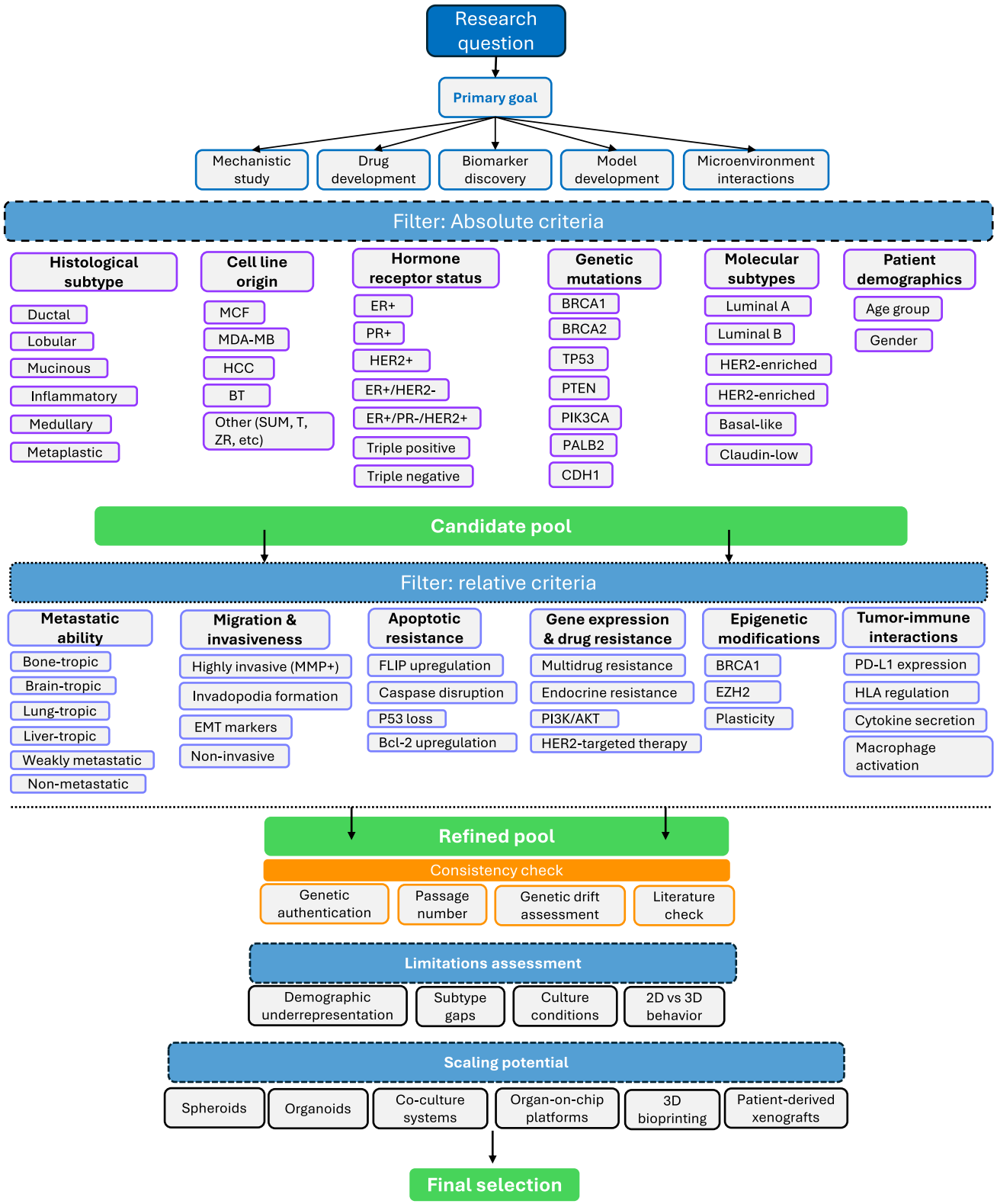
Gene expression profiles represent the transcriptomic signatures that classify breast cancer into intrinsic subtypes. Intrinsic subtypes luminal A, luminal B, HER2-enriched, and basal-like, originated from breast cancer transcriptomics.[111] The PAM50 assay further refines classification, identifying a claudin-low subgroup with stem-like features.[83] Oncotype DX and MammaPrint quantify recurrence risk using proliferation (e.g., Ki-67) and invasion-related genes (e.g., *MMP11*).[112] As a relative criterion, gene expression profiling enables comparison of cell lines beyond subtype labels, revealing functional differences in pathway activity, hormone signaling, immune evasion, or stemness. For instance, two basal-like cell lines may diverge significantly in EMT gene signatures or interferon response genes, making one of them more suitable for metastasis or immunotherapy studies. Expression levels of DNA repair genes (e.g., *BRCA1*, *RAD51*), growth factors (e.g., *TGFB1*), or apoptotic regulators (e.g., *BCL2*, *CASP9*) offer insights into therapeutic vulnerabilities.[113] Additionally, cell lines cultured in 2D versus 3D conditions can exhibit shifts in gene expression,[114] highlighting the need to interpret transcriptomic data in context and to develop *in vivo*-like models to improve accuracy.

Epigenomic modifications

Epigenomic modifications, for example, DNA hypermethylation of *BRCA1* occurring in a significant fraction of sporadic tumors, are reversible with HDAC inhibitors that restore ERα expression, while *EZH2* overexpression in TNBC correlates with stemness and metastasis.[115] In a model selection context, epigenomic features help distinguish cell lines by their regulatory landscape rather than transcriptional output alone. DNA methylation at tumor suppressor *loci* or enhancer regions can create long-term silencing that persists across treatments, as well as histone modifications that can influence chromatin accessibility and lineage identity.[109] Functional assays using 3D organoids and patient-derived xenografts provide dynamic insights into treatment response and resistance.[109] Cell lines differ in the stability of these states, some retaining locked epigenomes, while others are more prone to reprogramming, particularly in 3D culture or co-culture with stromal cells.[114]

Tumor-immune interactions

Tumor-immune interactions are one of the most important features of cancer cells when fighting against therapies and initiating invasion. Cancer cells can evade immune cells and recruit them as tumor-associated macrophages, regulatory T cells or myeloid-derived suppressor cells, further exacerbating the malignancy of the tumor.[108] Even typically anti-tumor cells (e.g., T cells or NK cells) can become exhausted or suppressed in the TME due to interaction with specific factors, hypoxia, or the absence of stimulatory signals.[108] Some breast cancer cell lines, for example, MDA-MB-231, HCC38, HCC70, and BT-549, can recapitulate this interaction, namely, cells with high immunogenicity.[116] These are often highly modulated when co-cultured with macrophages or T cells and express high levels of cytokines, chemokines, and high PD-L1 under IFN-γ stimulation.[108] Low immunogenic cell lines, such as MCF-7, T47D, and ZR-75-1, lack the expression of PD-L1 and have a low response to IFN- γ or immune co-cultures, making them less suitable for immune checkpoint or immunotherapies studies.[16] However, even high-interaction lines are still imperfect models, as standard cell lines lack the full complexity observed in immune microenvironments *in vivo*.[105]



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TOWARD INFORMED CELL MODEL SELECTION

To consolidate the criteria discussed throughout this review and translate them into actionable steps, this section proposes a structured workflow for breast cancer cell line selection (Figure 3). Starting from a defined research question and primary goal, whether mechanistic exploration, drug development, biomarker discovery, or modeling tumor–microenvironment interactions, the approach can guide users through a series of filters based on absolute and relative criteria. The layers include fundamental features followed by dynamic functional attributes relevant to experimental aims. Across levels, the candidate pool progressively narrows down to lines that are, however possible, biologically appropriate, experimentally feasible in the given context, and clinically relevant to their conceived application. The workflow further integrates downstream considerations, for example, validation needs (e.g., passage number, genetic drift), with suggestions for additional experimental steps before proceeding, as well as representation gaps (e.g., age range of applicability). Crucially, it also introduces the concept of scaling potential, i.e., the likelihood that a given cell line will perform well in advanced disease models such as organoids, co-culture systems, organ-on-chip platforms or patient-derived xenografts. Pre-selecting based on functional and phenotypic compatibility to more advanced platforms can reduce resource misuse and improve translational fidelity, possibly aiding in avoiding the use of unscalable *in vitro* models in studies originally conceived for *in vivo* applications. This structure might lay the foundation for building adaptive and AI-supported tools that link cell line metadata with model performance to optimize preclinical design.

The practical value of this approach is presented in two research scenarios requiring thoughtful cell line selection (Figure 4). In the first case, investigating bone-specific metastasis mechanisms in ER+ breast cancer through a standard literature search or an AI chat assistant might yield conflicting results, with studies using MCF-7 cells despite their limited metastatic capacity or MDA-MB-231 cells despite being ER-negative.[117] By applying the presented model, one would first filter by absolute criteria (ER+ status) and then by relative criteria (bone-metastatic potential), leading to the selection of MCF-7-derived bone-seeking variants or ZR-75-1 cells, which express PTHrP and other bone-metastasis mediators while maintaining ER positivity.[90]

INTEGRATION WITH ADVANCED AND EMERGING DISEASE MODELS

The development and deployment of disease models aiming to recapitulate breast cancer complexity benefit from the integration of the defined criteria into the set of advanced and emerging technologies, for example, multi-omics profiling to enhance model fidelity, machine learning to forecast treatment outcomes, and microsystems mimicking tissue microenvironments (Figure 5).[33,36,105,118] Integrated analyses show that many breast cancer cell lines cluster into familiar intrinsic subtypes (e.g., luminal vs. basal) based on gene expression, mirroring patient tumor classes, and multi-omics approaches have even *revised* prior cell line classifications.[36,47] The combination of genomics (mutations, copy-number variation), transcriptomics (mRNA, miRNA), epigenomics, and proteomics allows us to obtain a holistic molecular portrait that improves subtype matching. This approach also enables the spotting of discrepancies, as breast cell lines often carry more numerous mutations than tumors, and several key metastatic drivers (e.g., *ESR1* mutations) found in patient tumors are absent in commonly used lines.[90,117] Bringing together multi-omics data from patients (e.g., The Cancer Genome Atlas) with cell line and organoid data helps identify which models best recapitulate the molecular wiring of a given subtype, potentially enabling the discovery of hybrid molecular subgroups and biomarkers that are overlooked in single-omics analysis.[67] Multi-omics integration enables us to match absolute features (e.g., *BRCA1* mutation, ER status, luminal B signature) with its functional behaviors (e.g., apoptotic resistance, metastatic tropism), as captured by the relative criteria, simulating clinically relevant contexts *in vitro*. For example, a drug targeting *CDK4/6* should not solely be tested in a luminal-type cell line, but also in one with high Cyclin D1 expression and low apoptotic priming, integrating a profile that combines static and dynamic features. On top of this, machine learning is increasingly able to predict drug responses and classify breast cancer subtypes with accuracy through algorithms trained to recognize complex patterns that correlate with clinically relevant features. The NCI-DREAM Challenge assembled genomic, transcriptomic, proteomic, DNA methylation, and mutation data for 53 breast cancer cell lines to predict relative sensitivity to various drugs.[119] A recent study built a deep neural network that integrates gene expression, DNA copy number, mutations, and phospho-proteomic (RPPA) data, including a graph-embedded layer of protein–protein interactions.[9] Other groups have developed multi-omics machine learning frameworks to classify breast tumors into subtypes and predict patient therapy outcomes.[120] As a real-world example, ensemble models trained on cell line screens have been applied to patient tumor data to distinguish responder vs. non-responder profiles.[119]

Traditional 2D monolayer cultures often fail to recapitulate the complex architecture and microenvironment of breast tumors, potentially distorting cellular behavior with cells spreading unnaturally, losing their apico-basal polarity, lacking contact with a native ECM, and altering gene expression and drug responses.[105,114] For example, 2D-cultured cells have continuous access to oxygen and nutrients, as opposed to cells in a tumor mass, potentially making them more sensitive to drugs than real tumors. Advanced culture systems such as 3D spheroids,

Figure 3. Decision-making workflow for breast cancer cell line selection

The diagram outlines the proposed stepwise filtering process, beginning with the research objective. It then guides selection through sequential filtering criteria: absolute filters (e.g., receptor status, molecular subtype), and relative filters (e.g., metastatic tropism, resistance traits). Additional layers incorporate experimental feasibility, validation requirements (e.g., passage history, genetic drift), and representation gaps. The final step considers the scalability of selected lines toward advanced disease models such as organoids, co-cultures, and xenografts.

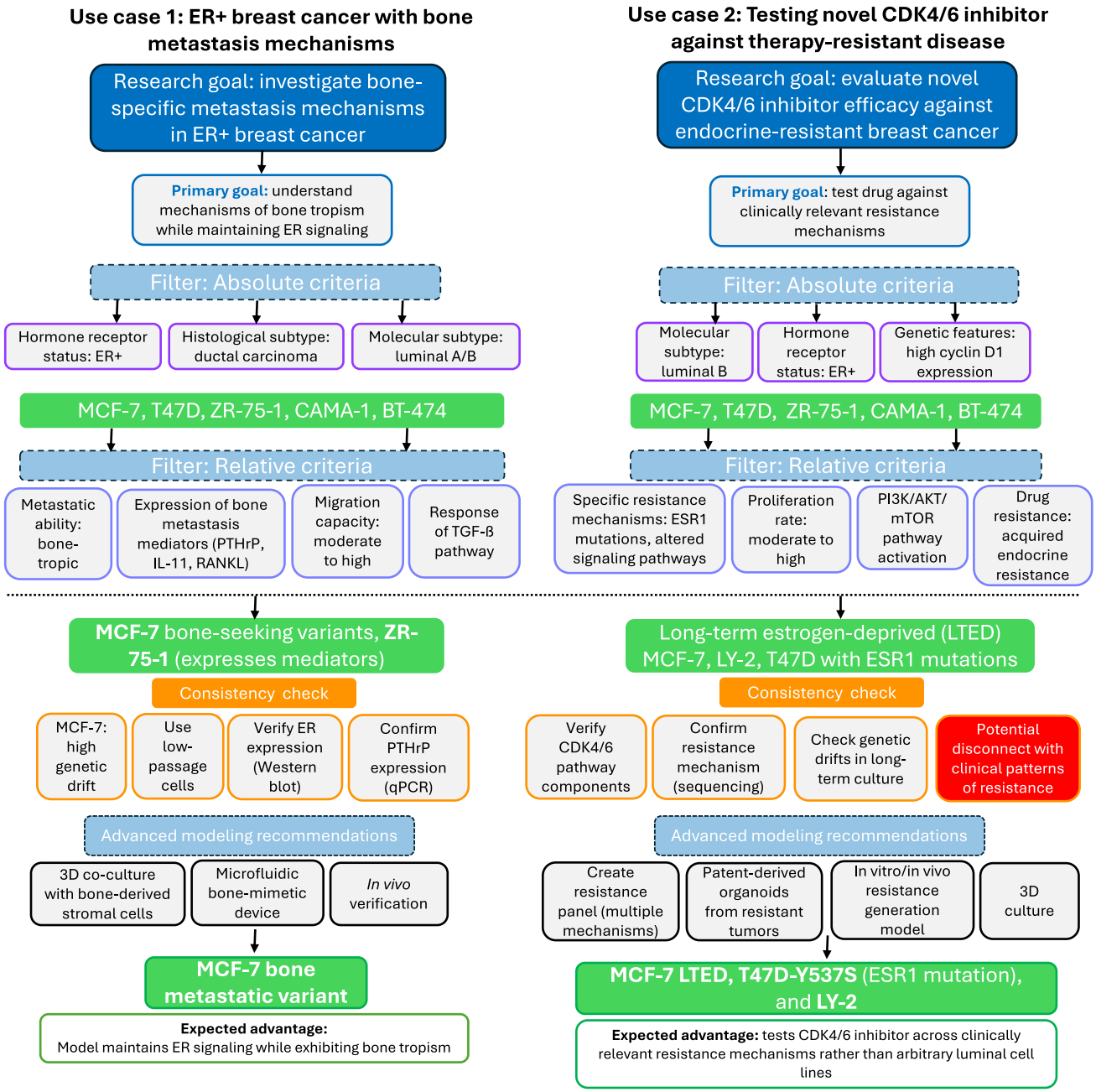

Figure 4. Use cases of the proposed workflow

The first use case (left panel) addresses bone metastasis in ER+ disease, identifying optimal cell models by filtering for both receptor status and metastatic competence. The second (right panel) illustrates drug testing for therapy resistance, combining genomic features with acquired phenotypes to select resistant luminal B models. In both cases, this approach could outperform standard selection methods by working with contextual fidelity. It also integrates considerations of genetic drift and validation needs across strains to potentially add experimental validation steps or pivot.

The second sample use case presented here involves a novel CDK4/6 inhibitor being tested against therapy-resistant disease. Instead of arbitrarily selecting a panel of luminal cell lines, the framework guides the combination of absolute criteria (luminal B classification, high Cyclin D1 expression) with relative criteria (acquired endocrine resistance). This would lead to selection of long-term estrogen-deprived (LTED) MCF-7 derivatives, LY-2 cells with acquired tamoxifen resistance, or T47D cells with *ESR1* mutations, providing a physiologically relevant resistance context.[90] The consideration of genetic drift would alert potential inconsistencies between different laboratory strains and encourage authentication and early-passage usage. In both cases, this approach might deliver contextually relevant models that mirror specific disease states over generic categorizations, to enable translational outcomes compared with a simple database consultation or literature search.

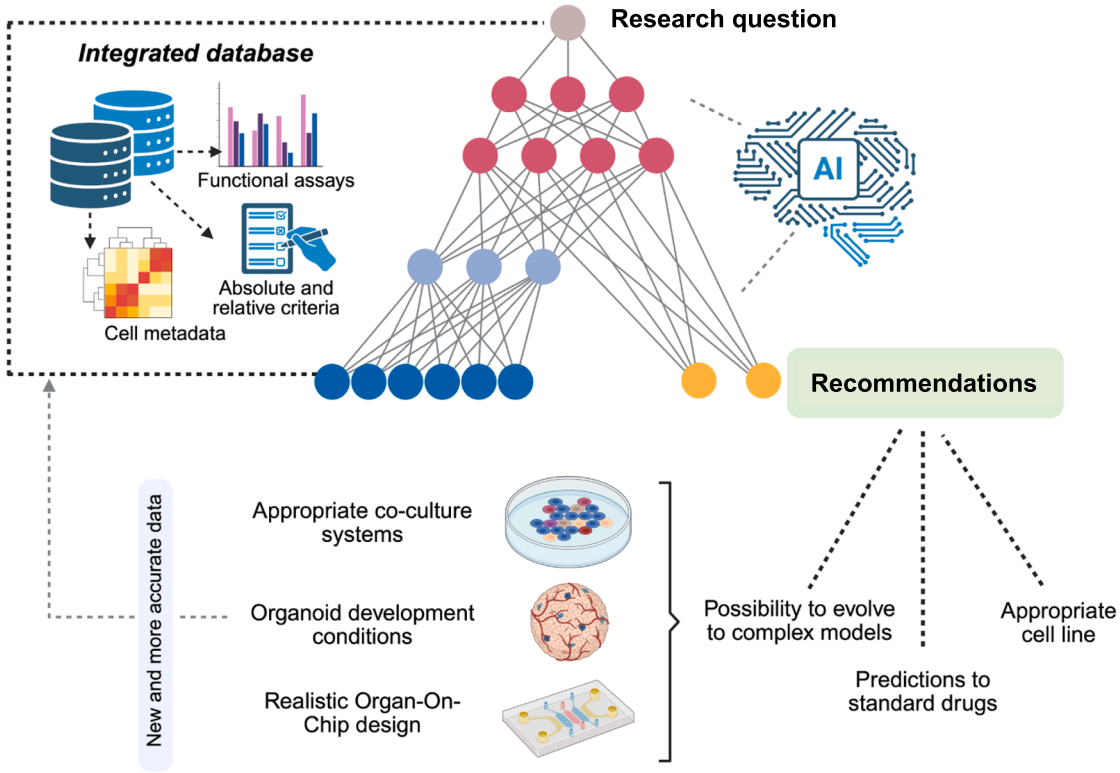

Figure 5. Sample workflow in AI-assisted optimization of cell line selection for 2D culture and advanced disease models

Cell line metadata and experimental data are compiled into a structured database, from which data is fed as inputs into a machine learning architecture that integrates absolute and relative criteria to generate predictive recommendations tailored to research goals. Suggested cell lines could be deployed in increasingly complex *in vitro* platforms to enhance biological fidelity and clinical relevance. The resulting experimental outputs, once validated, could feedback into the system, allowing continuous model refinement and improved prediction accuracy over time.

patient-derived organoids, co-culture models, and 3D bioprinting are now improving the physiological relevance of preclinical studies. 3D cultures allow cells to aggregate or grow in a matrix, restoring more *in vivo*-like morphology and cell–cell interactions. A core benefit of 3D tumor spheroids is the emergence of oxygen, nutrients, and drug gradients across the spheroid, leading to heterogeneous zones of proliferating vs. quiescent cells, mirroring solid tumors.[121] Studies comparing *in vitro* models have shown that breast cancer cells in 3D can become significantly more drug-resistant than in 2D monolayers.[105,118,121] For instance, when MCF-7 spheroids were treated with common chemotherapeutics (doxorubicin, paclitaxel, tamoxifen), the 3D cultures were markedly less responsive than 2D cultures.[121] This *in vitro* drug resistance in 3D is attributed to the restoration of tumor-like cell–cell/ECM interactions and diffusion barriers that limit drug penetration. *Organoids* are often derived from patient tumor samples or stem cells, that self-organize into mini-structures ideally containing multiple cell types, maintain the DNA copy number aberrations and sequence mutations of the parental tumor, as well as estrogen receptor (ER), HER2, and other subtype-defining features. Organoid biobanks and consortia (e.g., the HUB/Hubrecht Institute and Human Cancer Models Initiative) are now cataloging large collections of breast cancer organoids for research.[122]

Another approach to breast cancer modeling revolves around the development of co-culture systems, recalling that tumors are ecosystems of cancer cells interacting with stromal cells, immune cells, neurons, and extracellular matrix. Breast cancer spheroids have been co-cultured with cancer-associated fibroblasts or immune cells to reconstitute aspects of the tumor microenvironment.[105] These methods can reveal emergent behaviors, such as how fibroblasts induce drug resistance or how immune cells infiltrate tumor spheroids. A parallel method around the development of advanced systems is organ-on-chip technology, which uses microfabrication techniques to recreate environments that allow blood flow or gradients of nutrients across a biological sample. A study reports the use of a 3D *bioprinted* breast tumor model in a microfluidic chip, arranging breast cancer cells (MCF7, MDA-MB-231) and healthy mammary cells (MCF10A) within a hydrogel scaffold.[114] This method demonstrated realistic cell migration and invasion patterns in response to gradients. 3D bioprinting, in turn, represents a popular frontier for building custom tumor models that include patient-derived cancer cells, supporting fibroblasts, endothelial cells to mimic blood vessels, and immune components, all in one 3D construct. In this context, bringing together cell lines that collectively recapitulate a desired environment becomes more complex to do by hand, as well as crucial for ensuring the validity of experimental outcomes. Then, *in vivo* validation

Table 3. The three biggest and most widely used breast cancer databases in research

Feature	CCLE	TCGA-BRCA	METABRIC
Sample type	Breast cancer cell lines	Primary tumor	Primary tumor
Sample size	76	1098	1992
Expression platform	RNA-seq	RNA-seq	Microarray
Subtype distribution	Skewed toward TNBC, HER2+	Balanced; ER+ enriched	ER+ enriched
Drug response	Yes	No	No
Passage information	Limited	NA	NA
Tissue source	*In vitro* cultured cells (varied origins)	Fresh frozen tissue	Fresh frozen tissue
Ancestry diversity	Limited/not annotated	Mostly European ancestry	UK/Canadian, white
Population metadata	Limited	Rich	Moderate

CCLE, cancer cell line encyclopedia; TCGA-BRCA, Cancer Genome Atlas Breast Invasive Carcinoma; METABRIC, EGA European Genome-Phenome Archive.

remains a crucial step. A gold standard approach is the use of patient-derived xenografts (PDX), where fragments of a breast tumor are implanted into immunocompromised mice.[123] Studies have shown that if a drug causes regression in a cohort of breast cancer PDX models, there is a good chance it will show efficacy in patients with similar tumor profiles.[118] PDXs are also instrumental in co-clinical trials, i.e., parallel studies where patients receive therapy while their tumor xenografts in mice are treated similarly, acting as a great standard to comprehensively evaluate the given technology. All the approaches introduced in this section present intrinsic limitations that are out of scope for this review and are extensively addressed elsewhere.

CORE CHALLENGES

Genetic drift

Genetic drift is one of the most influential factors affecting the repeatability of *in vitro* experiments. Instability is associated with the clonal dynamics and appearance of new genetic variants. A sample study shows that in 106 cell lines compared across two labs, up to 90% of non-silent mutations were discordant between datasets.[124] MCF-7, specifically, had 27 strains with high genomic diversity, including differential mutations, copy number alterations, and gene expression.[124] These variations are triggered by variations in growth medium, inducing clonal shifts, and even single-cell-derived clones are genomically unstable after long-term culture. This has tremendous consequences for drug discovery research. Out of 321 anti-cancer drugs tested across the 27 MCF-7 strains, almost 75% of effective compounds in some strains were completely inactive in others.[124] In a different study, while researching LUCA-15 function in breast cancer, conflicting reports were found regarding its presence in MCF-7 cells, prompting them to characterize their available sublines. It was reported that the chromosomal region where LUCA-15 maps is unstable in MCF-7 cells, and one subline entirely lacked the LUCA-15 gene.[13] This loss correlated with reduced sensitivity to TNF-α–induced apoptosis, which is predominant in MCF-7, since this cell line does not have caspase activity, while overexpression of LUCA-15 restored apoptotic responsiveness. These findings suggest LUCA-15 is highly susceptible to genetic drift, leading to different apoptotic behavior in the same cell line. Although most of the studies refer to MCF-7 as the most unstable breast cancer cell line, it has been demonstrated that HCC1143, HCC38, HCC1937, T47D, BT-549, and MDA-MB-361 are also highly unstable.[17] MDA-MB-231 and HCC1806 are also moderately unstable, while control cell lines such as MCF710A are usually stable. Approaches to minimize these drawbacks include utilizing cell lines at low passages and standardizing culture conditions for all experimental settings. Genetic drift poses challenges in unifying genomic databases and topic-trained AI assistants, while at the same time making their potential impact even more consistent.

Conflicting characterizations

Key information such as age, grade, tumor size, ER/HER2 status, and race is also missing or inconsistently reported across many studies, limiting the utility of the data for epidemiological or prognostic modeling. A good example of these challenges in harmonizing data from different breast cancer datasets is observed in Table 3, which compares three of the most commonly used breast cancer databases.[120] The first issue revolves around data collection: CCLE and TCGA-BRCA contain RNA-Seq data, METABRIC contains microarray data. RNA-Seq is much more sensitive than microarrays, leading to technical bias when bringing data together.[125] Furthermore, as discussed earlier, the drug response and characteristics of cell lines are often non-comparable with those of primary tumors due to genetic drift and the absence of a relevant microenvironment. Because of this, it is challenging to associate cell-line data from CCLE to equivalent tumors from TCGA-BRCA or METABRIC and vice versa. While each database provides valuable insights, we cannot directly link cancer cell line features to population-based outcomes without accounting for the underlying biases that affect their representativeness. A multi-omic comparison of 57 breast cancer cell lines to 1019 metastatic breast cancer patient samples from METABRIC and TCGA revealed a mismatch between commonly used cell lines and the genomic landscape of metastatic tumors.[126] One of the most critical findings was that many of the breast cancer cell lines used to model metastatic disease, such as MDA-MB-231, showed poor genomic similarity to actual metastatic breast cancer tumors, particularly within the basal-like subtype. Through a large-scale integrative analysis comparing 57 cell lines with over 1000 metastatic tumor samples, the authors demonstrate that

historical model selection often lacks molecular justification. In contrast, less frequently used lines such as HCC38, HCC1395, and BT-549 were found to more closely resemble the genomic and transcriptomic profiles of metastatic tumors.[126]

Underrepresentation

The underrepresentation of cell lines from younger patients and non-Caucasian ethnicities is another limitation of most breast cancer models, affecting the translational relevance, particularly for studies aimed at investigating racial disparities in TNBC outcomes.[118] Healthcare access barriers lead to late-stage diagnoses in Hispanic and Asian women, contributing to fewer available data and lower reported incidence. In developed countries, earlier detection is supported by better screening access, while socioeconomic disparities, poor cancer literacy, unhealthy diets, and obesity contribute to higher breast cancer mortality in certain ethnic groups. This disparity continues to bias clinical trials and treatments toward Caucasian patients, further exacerbating healthcare inequalities and limiting the effectiveness of treatments for other ethnic groups. Moreover, most available breast cancer cell lines are derived from older patients, with relatively few from those under 40. Breast cancer in a 23-year-old can be substantially different from the same type of cancer in a 73-year-old. These differences impact how cancer cells behave and respond to treatment, making age another crucial factor to consider. This is becoming increasingly critical because many studies highlight the rising incidence of breast cancer in younger individuals, yet very few of them employ cell lines derived from younger patients (e.g., MCF-7 is from a 69-year-old woman). Integrating all features of every commercial cell line in a common interactive platform would help identify gaps, misclassifications, and inform a more reliable and relevant experimental design.

CONCLUSION

This work highlights the need for multilevel and AI-assisted comparative models for commercially available cell lines, associated 3D scaling, with the aim of improving clinical relevance for *in vitro* breast cancer research. The same schema could be translated to any other cancers, as well as to a set of other diseases commonly studied in bioengineering. The proposed framework suggests structuring cell line selection around absolute (e.g., origin, receptor status, mutations) and relative (e.g., metastatic potential, drug resistance, immune interaction) characteristics to enable a more rational and grounded use of existing resources. The approach, despite being statically presented, carries an intrinsically dynamic nature given by the possibility of integrating the proposed blocks into AI-assisted bioinformatics resources. Interfacing the latter with emerging disease models such as 3D organoids and co-culture systems would enable a bidirectional information flow between offices, laboratories, and clinics. In parallel, new ideas worth exploring in the biotechnology front for further integration include cancer virtual modeling using digital avatars trained on multi-omics and functional assay data, automated scoring systems for culture scalability and model fitness, and real-time feedback platforms that dynamically adjust cell line recommendations based on evolving biological parameters. These paradigms point to a future where cell model selection turns into a critical process aimed at clinical translation.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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