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Dormancy in Breast Cancer

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Abstract

The pattern of delayed recurrence in a subset of breast cancer patients has long been explained by a model that incorporates a variable period of cellular or tumor mass dormancy prior to disease relapse. In this review, we critically evaluate existing data to develop a framework for inferring the existence of dormancy in clinical contexts of breast cancer. We integrate these clinical data with rapidly evolving mechanistic insights into breast cancer dormancy derived from a broad array of genetically engineered mouse models as well as experimental models of metastasis. Finally, we propose actionable interventions and discuss ongoing clinical trials that translate the wealth of knowledge gained in the laboratory to the long-term clinical management of patients at a high risk of developing recurrence.

CLINICAL EVIDENCE FOR BREAST CANCER DORMANCY

In 1934, Rupert Willis postulated that “tumor dormancy” might explain the long-standing observation that patients whose primary tumors had been successfully treated, and who had no evidence of local recurrence, could nevertheless experience the delayed occurrence of metastases many years later. He proposed that “neoplastic cells must have lain dormant in the tissues in which they were arrested, and their resumption of growth must be attributed

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to some alteration in the qualities of these tissues, or to some release of growth restraints exercised by them on tumor cells" (Willis 1934).

Two decades later, Geoffrey Hadfield further advanced this insight by speculating that a "temporary mitotic arrest" of tumor cells could explain the protracted latency window between the definitive treatment of a primary tumor and subsequent reemergence of a secondary tumor that had been observed in a variety of human cancers, including breast cancer (Hadfield 1954). Historically, a latency window of >5 yr between primary tumor resection and recurrence was considered unusually long (Hadfield 1954), and this threshold continues to be used as a clinical reference for distinguishing "early" from "late" tumor recurrence in dormancy-related research (Klein 2020).

An implicit assumption underlying the above concepts is that minimal residual disease (MRD) constitutes the source from which recurrent tumors arise. MRD is defined as the microscopic tumor cell burden that persists at primary or metastatic sites following definitive surgery, radiation therapy, and/or systemic (neo)adjuvant therapy. MRD is challenging to observe directly in patients, primarily due to the ultrarare frequency of these cells coupled with clinical limitations associated with sampling tumor cells from particular anatomical sites. Nevertheless, several clinical observations support the possibility that MRD can exist in a latent state. The most striking example of this phenomenon derives from reported cases of cancer transmission from organ donors thought to be cancer-free at the time of donation to recipients (Au et al. 2018; Matser et al. 2018; Greenhall et al. 2022). Cancer transmission via the transplantation of organs that are themselves uncommon sites of metastases, such as the heart or kidney, suggests a model in which disease recurrence from otherwise undetectable residual tumor cells may occur when the growth restraints that they experience, including those that may be imposed by an intact immune system, are compromised following transplantation into the recipient.

Multiple additional lines of evidence support a model in which recurrent tumors are seeded by MRD. For example, the presence of cytokeratin-positive disseminated tumor cells (DTCs) in the bone marrow of patients is strongly associated with shorter time-to-recurrence at both local and at distant sites, as well as poorer disease-specific survival and overall survival (Wiedswang et al. 2003; Braun et al. 2005; Bidard et al. 2008; Hartkopf et al. 2014, 2021). In particular, a pooled analysis of over 10,000 early-stage breast cancer patients detected DTCs in ~30% of patients, and the presence of detectable DTCs was confirmed as a prognostic marker that is independent of breast cancer subtype (Hartkopf et al. 2021). Further, a study designed to deplete the DTC pool that persists following adjuvant chemotherapy with additional chemotherapeutic agents reported improved metastasis-free survival in responders who converted to a DTC-negative status, compared to nonresponders who remained DTC-positive (Naume et al. 2014). Although a control group was not included in this study, these provocative data suggest a link between DTC presence and the development of recurrent metastases. The persistence of DTCs following neoadjuvant chemotherapy is also strongly associated with poor prognosis in breast cancer patients (Mathiesen et al. 2012).

Disease latency preceding the clinical detection of recurrent tumors could formally be explained by models in which residual tumor cells (1) are reversibly arrested in a dormant, G_0-like state, which could occur as a consequence of cell-intrinsic or extrinsic (i.e., micro-environmental) signals; (2) proliferate at an extremely low rate; or (3) maintain a constant tumor cell mass through balanced proliferation and cell death, such as might occur due to ongoing immune-mediated elimination of tumor cells or a restrictive micro-environment lacking a vascular supply sufficient to support sustained proliferative outgrowth (Aguirre-Ghiso 2007; Klein 2011). Consistent with the notion of a form of cellular dormancy or immune-mediated control of residual cancer cells, DTCs exist most commonly as single cells or small clusters of cells (Woelfle et al. 2005), are nonproliferative as evidenced by the absence of staining for the cell-cycle marker Ki67 (Pantel et al. 1993), and may express the dormancy marker NR2F1 (Sosa et al. 2015; Borgen et al. 2018; Khalil et al. 2022). Each of these observations supports a model in which residual tumor cells can persist in a resting, G_0-like state. However, functionally demonstrating in patients that even a majority of DTCs exist in a state of reversible quiescence is difficult with existing methods, thus making it challenging to definitively rule out that tumor cells proliferate very slowly and/ or are otherwise restrained by their vascular and/ or immune microenvironment. Nevertheless, in light of proof-of-principle studies targeting angiogenesis or residual tumor cell survival in dormancy models (Carlson et al. 2019; Calvo et al. 2021), these data support the possibility that therapeutic targeting of MRD during this latent phase could constitute a tractable approach to prevent lethal recurrences in patients.

The latency interval between initial tumor diagnosis and recurrent disease is especially pronounced in breast cancer patients in whom excess mortality is observed more than 30 yr after primary tumor diagnosis (Pedersen et al. 2022). At first glance, this phenomenon appears to be breast cancer–subtype dependent, wherein early-stage estrogen receptor (ER)-negative breast cancer patients demonstrate the highest risk of recurrence and death within the first 5 yr following surgery, whereas ER^+ breast cancer patients demonstrate a relatively constant annual rate of recurrence of ~1%–2% from 5 to 20 yr following initial therapy (Pan et al. 2017). Consequently, a crossover is observed whereby a greater fraction of recurrences after 5 yr are attributable to ER^+ tumors.

However, closer inspection reveals some evidence that the distinction between the risk of late recurrence for ER^+ and ER-negative disease is not absolute. For example, a recent study stratified breast cancer patient samples from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) into 11 genomic subgroups to define populations of patients at high risk of late relapse and poor prognosis (Rueda et al. 2019). Beyond identifying highly variable probabilities of relapse from 5 to 20 yr among ER^+ genomic subsets, this study also identified ER-negative genomic subsets that displayed an increasing probability of late recurrence within this same time frame. Consistent with these findings, long-term follow-up of breast cancer patients who were disease-free 10 yr after diagnosis revealed that ~8% of ER-negative patients developed recurrent tumors over the ensuing 15 yr, demonstrating unequivocally that late recurrence can occur in patients with ER-negative primary tumors (Pedersen et al. 2022). These data suggest that the propensity of breast cancers to pass through a dormant phase prior to undergoing stochastic reactivation to give

rise to late recurrences is unlikely to be explained by ER status and anti-estrogen therapies alone.

In this regard, while delayed recurrence for some ER^+ breast cancers may typify an extreme form of latency, it is important to note that its existence does not preclude the possibility of clinically relevant shorter periods of dormancy in patients harboring ER-negative breast cancers. For example, pancreatic cancer is widely considered to be a tumor type that does not display clinical dormancy, based in part upon the observation that ~75% of patients develop metastases within 2 yr of primary tumor removal. Nevertheless, Ki67-negative DTCs have been identified in the livers of such patients (Pommier et al. 2018). While it is unknown whether such Ki67-negative cells are indeed reversibly quiescent and capable of giving rise to "recurrent" pancreatic tumors, this finding shows that the kinetics of clinical recurrence alone may not be sufficient to distinguish cancers that do or do not pass through a dormant state at the cellular level. This conclusion is further underscored by laboratory and clinical evidence for early dissemination of breast and pancreatic cancers insofar as even those metastases that become clinically manifest soon after primary tumor diagnosis may have been seeded years earlier during primary tumor development (Schardt et al. 2005; Rhim et al. 2012; Harper et al. 2016; Hosseini et al. 2016; Linde et al. 2018; Hu et al. 2020). Consequently, therapies targeting the window of dormancy in MRD may have clinical utility for both ER-negative and ER^+ disease, as well as different modes of disease progression.

In addition to the above considerations, it is important to note that from a biological perspective the designation of 5 yr postdiagnosis as the distinction between "early" and "late" recurrence is arbitrary, particularly given the lack of knowledge about the cellular state of DTCs in patients as a function of time. Moreover, the presumption that a clinically defined recurrence threshold of >5 yr implies a dormant phase, whereas a shorter period rules out such a phase, is even less tenable when considering changes in diagnostic and treatment modalities that have occurred over time (Klein 2011). Indeed, large-scale meta-analyses of breast cancer patient responses to targeted therapies clearly show historical changes in the kinetics of disease recurrence. For example, data from randomized trials reporting long-term outcomes in ER^+ breast cancer patients demonstrate that adjuvant treatment with 5 yr of tamoxifen suppressed recurrence more strongly at 5 yr, compared to 10 yr, following diagnosis (Early Breast Cancer Trialists' Collaborative Group [EBCTCG] et al. 2011). This resulted in an increased proportion of patients that recurred at 10 yr versus 5 yr in the tamoxifen-treated arm compared to controls (37.9% vs. 30.7%). That is, among patients who recur, adjuvant treatment with tamoxifen for 5 yr increases the relative fraction of patients who recur after 5 yr compared to those who recur within 5 yr of diagnosis. Further, continuing adjuvant endocrine therapy in ER^+ patients for 10 yr versus 5 yr further delays disease recurrence. Thus, endocrine therapy itself, as well as changes in its duration, alter the temporal distribution of recurrent tumors. These observations suggest that anti-estrogen therapy might facilitate disease latency, perhaps via some of the dormancy mechanisms proposed above, and clearly show how changes in therapy over time result in changes in the distribution of recurrent tumors relative to the 5-yr threshold that is used to classify "early" versus "late" recurrence (Davies et al. 2013; Pan et al. 2017).

Analogous to therapeutic targeting of ER, meta-analyses of adjuvant trials assessing the impact of adding trastuzumab to chemotherapy in early-stage $HER2^+$ breast cancers revealed that the annual rate of recurrence was suppressed by about a third within the first 5 yr following diagnosis. However, as observed for tamoxifen, although the addition of trastuzumab suppressed the overall percentage of patients who recurred, trastuzumab treatment increased the relative proportion of patients that recurred at 10 yr versus 5 yr compared to controls (25.8% vs. 19.8%) (Early Breast Cancer Trialists' Collaborative Group [EBCTCG] 2021). This provides further evidence that targeted therapy changes the temporal distribution of recurrent tumors, thereby altering the balance between "early" and "late" recurrence. Notably, these therapy-induced changes in the fraction of tumors that recur "early" versus "late" may be independent of any intrinsic biological tendency of a breast cancer subtype to pass through a dormant state.

The observation that the annual hazard rate for ER-negative breast cancer patients peaks within 2 yr after diagnosis could reflect a scenario in which recurrent tumors detected within this time frame arise from a proliferating pool of residual tumor cells that had escaped treatment. However, it is also possible thatshorter durations of therapy for ER-negative compared to ER^+ patients, as well as the dearth of targeted therapies for ER-negative, HER2-negative disease, may contribute to the rapid kinetics of recurrence. For these reasons, it is intriguing to speculate that improvements in targeted therapies for ER-negative disease might increase the relative proportion of tumors that recur beyond the "early" threshold of 5 yr. Thus, it is likely that both the intrinsic biological properties of breast cancer subtypes, as well as the therapies used to treat them, impact on the relative proportion of tumors that recur after 5 yr and the propensity of residual tumor cells to pass through a dormant state. Further, there may be drug–tumor interactions such that different breast cancer subtypes may respond differently to the same therapy with respect to either the frequency or phenotype of dormancy.

In sum, an accumulating body of clinical evidence suggests that uncoupling historical definitions of clinical tumor latency from the histopathologic observation of quiescent residual tumor cells would provide greater clarity and advance mechanistic discussions regarding tumor dormancy. While a clinical definition predicated solely on temporal patterns of disease recurrence has been interpreted by many as suggesting that breast cancer dormancy is restricted to ER^+ disease, both clinical and histopathologic evidence are consistent with the possibility that reversible cellular quiescence may be a stage of cancer progression through which both ER^+ and ER-negative breast cancer may pass, as well as other human cancers not generally considered to undergo dormancy. Accordingly, the ability to identify tumors with a propensity to undergo dormancy in a more nuanced manner that does not rely on ER status alone would constitute an important advance in the field. Furthermore, the molecular characterization of DTCs in concert with their matched primary tumor may also refine the identification of patients with MRD who are more or less likely to undergo intrinsic or therapy-related dormancy.

WHAT IS DORMANCY?

Cancer dormancy has been proposed to present in two distinct modes (Aguirre-Ghiso 2007): (1) tumor mass dormancy, characterized by an equilibrium state where a small tumor mass or masses are kept constant and asymptomatic by either a lack of angiogenic support or immune surveillance (Holmgren et al. 1995; Farrar et al. 1999; Mahnke et al. 2005; Naumov et al. 2006a; Koebel et al. 2007), and (2) cellular dormancy, in which single or small clusters of DTCs predominantly exist in a growth-arrested state (Aguirre-Ghiso 2007, 2018; Giancotti 2013; Sosa et al. 2014). This does not imply that residual DTCs never divide, but rather that their divisions are separated by long periods of growth arrest, which defines their phenotype. Since once cells commit to mitosis, this process is not paused and progresses actively unless damage is encountered, still the timing of cellular dormancy would mainly be controlled by G_0/G_1 phases. These possibilities for cellular dormancy can occur in residual cancer cells at the site of the primary tumor (also called “local recurrence”) and at the distant metastatic site (e.g., lungs, bone, liver). Tumor mass dormancy is an intriguing phenomenon that has been difficult to characterize and track in patients as recently described (Wiecek et al. 2021). In this study, overt tumors (~9000) available via public databases were profiled for the presence of dormancy signatures derived from angiogenic dormancy or immune-equilibrium models. This study did not really profile dormant tumor masses; nevertheless, the overt tumor masses revealed variable enrichment for tumor mass dormancy gene-expression signatures. While in some tumor subypes the signatures were represented in 26%–30% of samples (including breast), the rest showed low enrichment of these dormancy signatures (2%–7%). These data argue that in overt tumors, tumor mass dormancy gene profiles can be found, likely due to varying levels of hypoxic niches that can also influence immune infiltration (Baldominos et al. 2022). Another space where angiogenic tumor mass dormancy may be proven to exist in patients is with the use of metronomic chemotherapy or anti-angiogenic therapy (Montagna et al. 2014; Natale and Bocci 2018). Metronomic chemotherapy in breast cancer may have some advantage as it may target the endothelium and angiogenesis and this may be improved by the use of anti-VEGF- or VEGFR-targeted therapies (Montagna et al. 2014). However, whether metronomic use of anti-angiogenic therapies alone induces tumor mass dormancy was not reported (Montagna et al. 2014; Natale and Bocci 2018). Further, whether the above approaches recapitulate gene signatures from experimental models of tumor mass dormancy has also, to our knowledge, not been reported (Montagna et al. 2014; Natale and Bocci 2018). While open questions on tumor mass dormancy persist in breast cancer metastasis, the data above suggest that these could be explored by further modeling and focused clinical trials with molecular readouts.

In describing cellular dormancy in cancer, the field has employed different definitions based on potential states for the growth arrest: (1) quiescence, (2) senescence, (3) differentiation, or (4) embryonic diapause. A recent review addressed in detail the overlap and differences between cancer cell dormancy and the four potential states indicated above (Risson et al. 2020). The common denominator is the obvious acquisition of a G_0-G_1 arrest. The literature tends to favor the notion that cancer cells adopt a quiescent state with programs carrying characteristics of quiescent adult stem cells, interactions with their niches, and embryonic pluripotency programs found during diapause (Aguirre-Ghiso and Sosa 2018; Dhimolea et

al. 2021; Lim and Ghajar 2022). Embryonic diapause is a unique developmental state in mammals where the blastocyst as a whole enters a reversible growth arrest in response to reduced maternal hormonal input signals (Scognamiglio et al. 2016). Interestingly, blastocysts in diapause display reduced Myc activity but maintain pluripotency, which is also found in models of cancer cell dormancy (Shachaf and Felsher 2005). These data further support that cellular dormancy is therefore distinct from senescence, a cell-cycle arrest activated by oncogene and replicative stress, and a first barrier to transformation (Gorgoulis et al. 2019). However, some stress-adaptive pathways, such as the unfolded protein response, and specific secretory programs may be shared between quiescent and senescent states. Differentiation programs, in particular lineage commitment transcriptional programs, can be partially activated in dormant cells that are quiescent (Sosa et al. 2015; Laughney et al. 2020). However, the spontaneously reversible nature of dormancy does not conform to the specialized functional state of fully differentiated cells. Finally, therapy-associated dormancy has been explored in the context of “persister cancer cells,” a population of drug-tolerant cells that evades cell death induced by therapy, including in genetically engineered mouse (GEM) models for breast cancer dormancy and recurrence following targeted therapy (Moody et al. 2002, 2005; Gunther et al. 2003; Abravanel et al. 2015; Ruth et al. 2021). These cells may represent epigenetic variants, rather than selected genetic clones, partially regulated by niche-driven cues and damage signals that can also trigger senescence (Shen et al. 2020). As the microenvironment surrounding dormant cancer cells significantly impacts cellular dormancy, understanding the niche signals regulating the DTC state is of utmost importance.

EXPERIMENTAL MODELS OF BREAST CANCER DORMANCY

Clinical observations in patients strongly suggest that recurrent breast cancers are seeded by MRD, which in turn may exist in a state of reversible quiescence or balanced proliferation and cell death. Considering the limitations of what can reliably be inferred from clinical data, experimental modeling of disease progression is critical for probing the biological underpinnings of dormancy initiation, maintenance, and exit to identify novel treatment strategies for preventing tumor recurrence (Fig. 1A).

How a state of reversible quiescence is acquired and maintained remains an area of active research. GEM models of breast cancer dormancy rely on simulating disease progression as it is observed in patients. Importantly, given that the great majority of early-stage breast cancer patients receive some form of systemic therapy, breast cancer dormancy in patients typically occurs in the setting of therapy, either ongoing or completed. Accordingly, mouse models for breast cancer dormancy and recurrence have been developed that incorporate this critical feature (Fig. 1B). These include models for oncogene-dependent primary tumor formation, cell survival following targeted therapy, persistence of tumor cells in a dormant state, and subsequent spontaneous recurrence (e.g., *Myc* [D’Cruz et al. 2001], *Her2* [Moody et al. 2002, 2005; Abravanel et al. 2015; Ruth et al. 2021], *Wnt1* [Guntheret al. 2003; Ruth et al. 2021], and *Fgfr1* [Janghorban et al. 2021]). In such models, targeted therapy imposes a bottleneck that results in either the de novo acquisition, or adaptive selection, of tumor cells with the ability to undergo dormancy. Notably, employing quiescence-associated gene-expression signatures (Janghorban et al. 2021) derived from GEM models

to gene-expression data sets of patients treated with neoadjuvant chemotherapy reveals the acquisition of a dormancy-associated transcriptional phenotype following treatment.

The application of dormancy-related gene-expression signatures derived from in vivo GEM models of breast cancer to patient data sets demonstrates their potential utility in identifying the subset of patients who are at greatest risk for tumor recurrence. For example, the interrogation of large patient data sets using gene-expression signatures derived from dormant MRD in two different GEM models that mimic targeted therapy of local disease found that patients whose primary tumors exhibited a dormancy signature displayed decreased rates of recurrence, potentially reflecting an increased frequency or ability of tumor cells to persist in a latent state (Ruth et al. 2021). Further, this study's demonstration that a gene-expression signature derived from local dormant residual disease in mouse models predicts recurrence-free survival in patient data sets composed predominantly of metastatic recurrences suggests that the properties of dormant tumor cells at local and distant sites may be related (Ruth et al. 2021). Defining the overlap between such cellular dormancy-associated gene-expression signatures and other models of persistent cancer cells that have not been confirmed to exhibit features of dormancy, such as local residual disease in patient samples following neoadjuvant chemotherapy (Creighton et al. 2009; Balko et al. 2014; Kim et al. 2018) or xenograft models (Echeverria et al. 2019), may help clarify the contribution of quiescent residual tumor cells to breast cancer recurrence.

A commonly cited limitation of existing GEM models that mimic human disease progression through a latent phase is the relative lack of models for estrogen-dependent breast cancers expressing ER (Bushnell et al. 2021). The development of such models would no doubt hold great value for teasing apart whether particular subtypes of breast cancer, such as ER^+ tumors, have a unique intrinsic propensity or ability to enter or persist in a dormant state, and whether this capability is amplified by the use of targeted therapies. Nevertheless, the recent demonstration that the gene-expression state of dormant tumor cells derived from ER-negative GEM models predicts the probability of recurrence in patient data sets composed primarily of ER^+ tumors indicates that ER-negative models can provide clinically relevant insights into dormancy mechanisms, while providing further evidence that cellular dormancy is unlikely to be restricted to ER^+ disease (Ruth et al. 2021).

Another paradigm for dormancy modeling involves mimicking the adaptation of tumor cells to foreign microenvironments encountered during metastasis, which is important given that breast cancer most commonly recurs at distant sites (Fig. 1B). Frequently used models include related sets of breast cancer cell lines derived from common origins that exhibit indolent (e.g., D2.OR, 4TO7) or aggressive growth (e.g., D2A1, 4T1) at metastatic sites including the lung, liver, and bone (Mahoney et al. 1985; Aslakson and Miller 1992; Morris et al. 1993, 1994). Additional strategies include the in vivo selection of stable clones that display dormant phenotypes (e.g., HCC1954-LCC1 [Malladi et al. 2016], MDA-MB-231-SCP6 [Lu et al. 2011]), or the use of established but unrelated human breast cancer cell lines with indolent (e.g., MCF7, T47D) or aggressive (e.g., MDA-MB-231) growth properties (Montagner and Sahai 2020). In aggregate, these models encompass a variety of breast cancer subtypes and enable the study of organ-specific barriers to macrometastatic outgrowth.

One recent study derived dormancy-associated gene-expression signatures from noncycling tumor cells in the lungs and bones of tumor-bearing *MMTV-PyMT* mice, as well as quiescent D2.OR tumor cells in the lung, and found the striking conservation of a core set of dormancy-related genes (Ren et al. 2022). Furthermore, dormancy-associated gene-expression signatures derived from indolent ER^+ D2.OR cells and from *MMTV-PyMT* noncycling tumor cells isolated from lung and bone were associated with better prognosis in breast cancer patients (Montagner et al. 2020; Ren et al. 2022). Together, findings from gene-expression profiling of multiple clinically relevant models of dormancy support a model in which a conserved set of genes is enriched in dormant tumor cells, irrespective of the subtype of breast cancer or the site at which the tumor cells exist in a dormant state (Fig. 1B). Parsing these gene-expression signatures should help identify both novel markers of dormant MRD and functional regulators of disease progression through a dormant state. Additionally, developing dormancy models that mimic the high degree of heterogeneity and clonal evolution observed in patients as a consequence of therapy, metastatic dissemination, and cross talk with immune and stromal cell components in different microenvironments is likely to refine our understanding of mechanisms promoting tumor cell persistence in a dormant state.

MECHANISMS UNDERLYING DORMANCY ENTRY AND PERSISTENCE

Therapy-Associated Cancer Cell Dormancy

Cancer cell dormancy can be manifested following treatment directed toward the primary or secondary tumor. Therapies suppressing oncogene signaling or DNA-damaging, antiproliferative therapies invoke changes in signaling pathways and epigenetic programs, enabling persister cells, as one potential evasionmechanism, toenter dormancy and escape therapy (Sharma et al. 2010). Alternatively, preexisting spontaneously slow-cycling or deep dormant cells could survive treatment (Shaffer et al. 2017). While some cells may remain unable to reactivate posttreatment, a fraction of therapy-resistant or therapy-tolerant persister cells can eventually reactivate and proliferate, fueling cancer relapse.

In the context of breast cancer, chemotherapy has been shown to leave persister cancer cells behind. Echeverria and colleagues found that triple-negative breast cancer (TNBC) patient-derived xenograft tumors and biopsies treated with standard chemotherapy regrew from residual drug-tolerant cancer cells. Using clonal tracking and sequencing via barcodes, they showed that recurrent tumors maintained the architecture and heterogeneity of initial tumors and were thus not clonally selected upon chemotherapy (Echeverria et al. 2019). Further, a preclinical study using the *MMTV-PyMT* mouse model found that a small subset of primary tumor cells underwent an epithelial-to-mesenchymal transition (EMT), but these cells were not the predominant population in lung metastasis (Fischer et al. 2015). However, cells that underwent EMT resisted treatment with the chemotherapeutic agent cyclophosphamide and were thus responsible for metastatic recurrence. These cells displayed reduced proliferation, increased resistance to apoptosis, and elevated expression of drug-metabolizing genes. Therefore, cells that underwent EMT that either preexisted in the primary tumor or were forced into this state by therapy may represent the persister cell population in the context of conventional chemotherapy.

Therapies targeting specific oncogene dependencies also fail to completely eradicate all cancer cells (Gu et al. 2016). Initial modeling of oncogene addiction and targeting was performed using doxycycline-inducible GEM models activating breast cancer–relevant pathways, including *Myc*, *Wnt1*, and *Her2/neu*. In these models, de-induction of oncogenes resulted in residual breast cancer cells that eventually reprogrammed and could fuel recurrences (Moody et al. 2002; Gunther et al. 2003; Boxer et al. 2004). These and other studies of residual disease and recurrence in *Her2/neu* and *Wnt1* doxycycline-inducible GEM models implicated dormant residual tumor cells as an intermediate in the stochastic spontaneous recurrence of mammary tumors following treatment, in some cases in the context of EMT, and revealed striking similarities between populations of dormant residual tumor cells isolated from *Her2/neu* and *Wnt1* GEM models (Moody et al. 2005; Ruth et al. 2021). In particular, Ruth et al. showed that, after oncogenic HER2 inhibition in mouse cells, or a combination of anti-HER2 and anti-ER therapy in human breast cancer cells, residual dormant cancer cells were found both at the primary site and in the lungs (Ruth et al. 2021). Furthermore, these cells were able to reenter the cell cycle and proliferate to form recurrent tumors after long intervals. Intriguingly, analysis of gene programs revealed that oncogene inhibition also differentially regulated genes associated with microenvironment-induced dormancy (see next section), such as TGF-β2 (Fluegen et al. 2017; Nobre et al. 2021b), supporting the idea that these persister cells might also generate niches that support their dormant state (Fig. 1B).

In previous studies of doxycycline-inducible GEM models, subsets of residual tumor cells and recurrences have been shown to activate Met or Notch signaling following HER2 inhibition (Feng et al. 2014; Abravanel et al. 2015). Activation of both the Met and Notch pathways was demonstrated to be associated with tumor recurrence in mice as well as breast cancer patients (Feng et al. 2014; Abravanel et al. 2015). Fox et al. (2020) further found that HER2 inhibition promoted changes in cellular metabolism, leading to oxidative stress and up-regulation of the antioxidant transcription factor NRF2. Similar to the Notch study, activation of NRF2 accelerated tumor recurrence and up-regulated de novo nucleotide synthesis. These data reveal that residual cancer cells that enter dormancy in response to targeting of oncogenes use a multiplicity of adaptive pathways to enter a reversible growth arrest resembling quiescence and that they may also produce signals that mimic niches supporting dormancy (Fig. 1B).

More recent work further investigated the response of HER2-driven breast cancer cell lines and $HER2^+$ patients' tumors to lapatinib (a HER2 tyrosine kinase inhibitor). Using a barcode lentiviral tracing system, it was shown that drug-tolerant persister cells could be found as cycling or noncycling populations (Oren et al. 2021). Cycling persister cells maintained their proliferative capacity and up-regulated a reactive oxygen species (ROS) signature and antioxidant mechanisms, shifting their metabolic state.

Few studies in breast cancer focus on additional oncogenic pathways besides *Her2* and their correlation with cancer dormancy. This is mostly likely due to the limited number of driver-mutated kinases found in breast cancer. Use of a constitutive *Wnt1*-driven, inducible *Fgfr1* mouse mammary tumor model has been used to explore residual dormant tumor cell biology after treatment with a fibroblast growth factor receptor (FGFR) inhibitor (Holdman

et al. 2015; Janghorban et al. 2021). These dormant cells gave rise to recurrent tumors that displayed activation of EGFR signaling and surrounding dense stroma (Holdman et al. 2015). Notably, single-cell RNA sequencing of residual lesions in this model uncovered significant remodeling of the tumor microenvironment, including the stromal and immune compartments, which is associated with the dormant state (Janghorban et al. 2021).

Focusing on ER^+ breast cancer and its response to hormonal therapy, a 2019 study by Hong et al. used single-cell RNA sequencing to characterize the phenotypic plasticity and adaptation of this subset of breast cancer after endocrine therapy (Hong et al. 2019). Here, they identified a population of cells (called preadapted) that expressed a dormancy transcriptional signature and preferentially survived treatment.

Another therapeutic intervention linked to dormancy in breast cancer is treatment with the histone deacetylase (HDAC) inhibitor entinostat. HDAC inhibitors were shown to epigenetically stimulate expression of leukemia inhibitory factor receptor (LIFR) in breast cancer cells, independent of ER status (Clements et al. 2021). They induced a pro-dormancy gene program and reduced tumor growth in mice. From clinical data, treatment of advanced breast cancer patients with entinostat and azacitidine (DNA methyltransferase inhibitor) showed some promise in a subset of patients (Connolly et al. 2017). The use of azacytidine combined with retinoic acid was also shown to induce a program of dormancy across various epithelial cancers, including a TNBC model, suggesting that such “reprogramming” approaches may prove fruitful in suppressing metastasis via dormancy induction (Sosa et al. 2015; Khalil et al. 2022).

In summary, the different types of therapy described herein leave behind cancer cells that survive treatment in a dormant state. Further work into understanding the mechanisms of therapy-induced DTC dormancy and subsequent reactivation is needed to optimize intervention strategies.

Niche-Derived Signals Instruct Cancer Cells to Activate Dormancy Programs

Signals from the microenvironmental niche, both at the primary site and at the site of metastatic colonization, appear to be integrated to induce or maintain DTC dormancy (Fig. 2).

Dormancy Signals from the Primary Site and Target Organ Niches—The earliest evolved lesions, such as ductal carcinoma in situ (DCIS), which are considered localized and noninvasive, were classically thought to be incapable of disseminating cancer cells as this would require invasion. However, early work revealed that DTCs can be detected in the bone marrow of patients with DCIS (Hüsemann et al. 2008), and that genetic alterations linked to malignancy in invasive cancer were already present at this DCIS stage (Schmidt-Kittler et al. 2003). These findings supported the idea that in situ lesions may be capable of disseminating cancer cells. However, this does not rule out that as lesions progress dissemination continues through late stages, albeit in some instances, but not all, with reduced impetus (Hosseini et al. 2016). A long-standing hypothesis was that, by virtue of being evolved early, these lesions would produce DTCs prone to enter dormancy. In three papers (Harper et al. 2016; Hosseini et al. 2016; Linde et al. 2018), the mechanisms driving

early dissemination were identified in various mouse models (*MMTV-Her2* and *MMTV-PyMT*) and human samples across various breast cancer subtypes. In the two mouse models tested, a population of *Her2*$^{+}$ or *PyMT*$^{+}$ (a TNBC-like model) DTCs in *MMTV*-transgenic models of breast cancer activated a WNT-dependent EMT-like Twist1^{hi}E-cad^{lo} program to travel to and colonize distant sites, such as the lungs (Harper et al. 2016). Of note, during early stages of progression, these HER2 lesions express both ERα and progesterone receptor (Hosseini et al. 2016), suggesting that hormonal regulation could foster dissemination. In fact, Hosseini et al. (2016), elegantly showed how progesterone-induced signaling was key for the regulation of dissemination of the HER2^{+} DTCs. These pioneering findings may also shed light into how pregnancy-associated breast cancer may foster dissemination seeding and metastasis (Goddard et al. 2019; Lefrère et al. 2021). However, further studies are needed to understand how ER^{+}/PR^{+}/ HER2^{-} lesions accomplish the process of early dissemination and how early DTCs are affected by anti-estrogen therapies.

Supporting that early DTCs may enter dormancy, upon arrival to the lung, the Twist1^{hi}E-cad^{lo} DTCs remained in a dormant, nonproliferative state for long periods, supporting that early DTCs can remain dormant and give rise to metastases. A recent expansion of this work has shown that a primed pluripotency regulator ZFP281 regulates early dissemination and subsequent dormancy of early DTCs in the MMTV-HER2 and -PyMT models also via regulation of TWIST1 and a class II cadherin, CDH11 (Nobre et al. 2022). It was also shown that *Her2*$^{+}$ and *PyMT*$^{+}$ DTCs were aided by CD206^{+}/Tie2^{+} macrophages in the mammary gland for efficient early dissemination (Linde et al. 2018). Impressively, eliminating these macrophages exclusively during the early lesion stage decreased metastasis by more than 50% months later, arguing for the strong contribution of early DTCs to metastasis after a protracted dormancy phase. The rest of the metastases were presumably contributed by later disseminating DTCs as they were not affected by the macrophage depletion (Linde et al. 2018). These data do not indicate that late DTCs are not fit to initiate metastasis, but rather reveal an unexpected and understudied role for early DTCs in the breast cancer metastatic process. Another unexplored aspect that may connect early and late DTCs is whether they cooperate in metastasis formation as proposed previously (Sosa et al. 2014; Aguirre-Ghiso and Sosa 2018).

The above results suggest that early DTCs, those that disseminate from early evolutionary stages of breast cancer, like DCIS (as defined by pathology), may be prone to enter dormancy. This could be due to signals from the microenvironment at the primary site that, while promoting dissemination, may also promote entry into dormancy in the target organ. When examining late-stage primary tumors (late stage means overt invasive breast cancer as defined by a pathologist) in patient-derived xenografts and transgenic mouse models, hypoxic microenvironments in breast primary tumors were found to induce the up-regulation of dormancy genes, such as NR2F1 and p27 (Fluegen et al. 2017). The DTCs leaving the tumor carried this signature and a TGF-β2^{hi} dormant phenotype to secondary sites.

Multiple studies have investigated the effects of signals coming from the target organ microenvironment surrounding DTCs on their phenotype and fate. Common sites of metastatic colonization from breast cancer cells derived for example from ductal carcinomas are bone, lungs, and brain (Kennecke et al. 2010).

While hypoxia in specific areas of the primary tumor initially imprints a dormancy program in disseminating cancer cells, high hypoxic conditions in the colonized organ have been shown to eventually reactivate DTCs. In breast cancer cells disseminated to the bone marrow, hypoxia down-regulated LIFR, leading to down-regulation of dormancy and cancer stem cell–associated genes (Johnson et al. 2016). Thus, LIFR is a positive regulator of dormancy in the bone and hypoxia can cause reactivation. In this same tissue, studying the function of bone-resident $NG2^+$/$Nestin^+$ mesenchymal stem cells (MSCs), MSCs in homeostasis were found to maintain DTC dormancy (Nobre et al. 2021b). In intracardiac injection models of metastasis to the bone, genetic depletion of MSCs awakened dormant DTCs, which formed metastases. MSC-mediated dormancy was driven by TGF-β2 and the induction of p27 in DTCs. Other studies on bone marrow components have found that the vascular endothelium and the perivascular niche protect DTCs from chemotherapy (Carlson et al. 2019). Targeting the niche, specifically the endothelial von Willebrand factor (vWF) and vascular cell adhesion molecule 1 (VCAM1), rendered dormant cancer cells sensitive to chemotherapy, without having them dangerously reenter the cell cycle.

The lung niche plays a relevant role in DTC fate. Dormant D2.OR breast cancer cells disseminated to the lung interact with alveolar epithelial type I (AT1) cells, favoring integrin-dependent survival pathways (Montagner et al. 2020). In vivo screening for genes required for cross talk identified the molecule SFRP2 on breast cancer cells. Further, targeting of SFRP2 by shRNA-mediated silencing was shown to eradicate dormant cells.

The microenvironment surrounding DTCs is not only composed of resident host cells, but also of the abundant ECM. Dormant cancer cells were found to produce a type III collagen–enriched ECM, which was essential to maintain DTC dormancy both at the primary site and in the lung (Di Martino et al. 2022). When type III collagen was not present, DDR1-mediated signaling was inactive and cells began proliferating.

A less studied organ that harbors dormant breast DTCs is the brain. A recent study identified signals from the brain niche that could drive DTC dormancy (Dai et al. 2022). Here, they showed that dormant DTCs in the brain resided close to astrocytes. Astrocytes produced laminin-211, inducing DTC dormancy via dystroglycan receptor signaling in cancer cells. It was also shown that a bone morphogenetic protein (BMP) signaling antagonist DAND5/Coco can awaken dormant breast cancer cells in the lung (Gao et al. 2012; Giancotti 2013), and BMP4-SMAD7 signaling is also a mediator of metastasis suppression in other breast cancer models (Eckhardt et al. 2020). The ECM protein thrombospondin-1 has also been implicated in both angiogenic dormancy (Naumov et al. 2006b; Aguirre-Ghiso 2007) and cellular dormancy (Ghajar et al. 2013), possibly by its ability to directly affect endothelial cell proliferation but also via direct effects on DTCs in the perivascular niche. These data support how a multiplicity of factors create a redundancy network to suppress metastasis initiation via dormancy induction and maintenance.

Immune-Mediated Tumor Cell Dormancy—Immune cells constitute a major component of the tissue microenvironment. Only recently has the dormancy community focused on examining the role of innate and adaptive immune cells in regulation of DTC dormancy (Fig. 2).

In a mouse model of chemotherapy-induced dormancy of triple-negative 4T1 cells, $CD4^+$ and $CD8^+$ T cells were required for dormancy (Lan et al. 2019). 4T1 cells treated with chemotherapeutic agents (methotrexate or doxorubicin) and dormant D2.OR cells were enriched for type 1 interferon (IFN) response genes. Specifically, they up-regulated IRF7, IRF9, STAT1, and STAT2. RNAi-mediated silencing of IRF7 allowed escape from dormancy, increasing local tumor growth and metastatic burden. Further, in clinical samples of serum of breast cancer patients collected during neoadjuvant chemotherapy, the presence of IFN-β was associated with longer metastasis-free survival. Another study found that 4TO7 primary tumor-derived $CD8^+$ T cells induced protective immunity and dormancy at distant sites (Tallón de Lara et al. 2021). A subpopulation of $CD39^+$ $PD\text{-}1^+$ $CD8^+$ T cells was proposed to be responsible for this phenotype, but functional validation was lacking.

Aside from T cells, natural killer (NK) cells have also been linked to breast cancer dormancy. Dormant $HER2^+$ DTCs evaded immune surveillance by NK cells via autocrine DKK1-mediated inhibition of WNT signaling (Malladi et al. 2016). After dormancy escape, DTCs could be detected and cleared by NK cells. In a more recent study, NK cells sustained triple-negative MDA-MB-231 dormancy in the liver through IFN-γ (Correia et al. 2021). Activation of hepatic stellate cells, as seen during liver injury, prevented the expansion of NK cells through CXCL12 and induced NK cell quiescence. Inhibited NK cells were unable to maintain IFN-γ DTC dormancy, leading to proliferation and metastatic growth.

Autophagy and Novel Pathways Regulating Dormant Cell Survival—Multiple studies have reported the role of autophagy in the survival and dormancy of DTCs (Fig. 2; Sosa et al. 2013). Autophagy is a process of degradation and recycling of cytoplasmic components that occurs intracellularly.

One of the models used to study the role of autophagy in spontaneously arising breast cancer dormancy is represented by the dormant D2.OR and proliferative D2A1 paired cell lines. D2.OR cells were found to activate autophagy, and autophagy inhibition with hydroxychloroquine (HCQ) or other drugs reduced viability of dormant cells injected intravenously to reach the lung (Vera-Ramirez et al. 2018). A genetic model of *Atg7* silencing corroborated the findings that autophagy is important for survival of dormant breast cancer cells. Autophagy may also be involved in the induction and or maintenance of the dormant state of DTCs. Autophagy inactivation by depletion of *Atg3*, *Atg7*, or *p62/sequestosome-1* led to exit from dormancy and metastatic outgrowth of D2.OR cells (La Belle Flynn et al. 2019). Metastatic recurrence was associated with increased 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*Pfkfb3*) expression, which is an autophagy substrate normally degraded in dormant cells. Accordingly, autophagic degradation of NBR1 also restricts metastasis and its loss favored metastatic reactivation from DTCs (Marsh et al. 2020). However, whether the pro-dormancy effects of autophagy via NBR1 regulation were due to a regulation of the timing of DTC dormancy was not formally explored (Marsh et al. 2020).

An additional intracellular recycling-related pathway shown to be important for DTC survival is the TFEB-lysosomal biogenesis pathway. An in vivo shRNA screen identified genes required for breast DTC survival upon dissemination to the lungs, specifically

the transmembrane protein EphB6, a regulator of the TFEB axis (Zangrossi et al. 2021). Interestingly, EphB6 expression was stimulated by the lung microenvironment and influenced the response of lung AT1 cells, pointing to the cross-regulation of DTCs and their surrounding milieu.

The discovery of novel pathways and genes involved in cancer dormancy has been accelerated by the use of unbiased genome-wide screens in recent years. A study by Gawrzak et al. reported the discovery of mitogen- and stress-activated kinase 1 (MSK1) as a regulator of bone micrometastatic dormancy in a model of ER^+ breast cancer (Gawrzak et al. 2018). MSK1 loss was associated with early relapse in ER^+ breast cancer patients and enhanced bone metastasis in mice.

Another novel regulator of breast cancer metastatic dormancy was found to be the long noncoding RNA NR2F1-AS1 (Liu et al. 2021). NR2F1 was previously implicated as a potent dormancy factor in breast and non-breast cancer cells (Sosa et al. 2015; Borgen et al. 2018). In a recent study, NR2F1-AS1 was up-regulated in latent mesenchymal cancer cells and promoted metastatic seeding. However, it inhibited their proliferation and forced them into dormancy via up-regulation of NR2F1.

MECHANISMS UNDERLYING DORMANCY EXIT AND RECURRENCE

After long periods of dormancy (in both spontaneous and therapy-associated conditions), DTCs can awaken and reenter the cell cycle by the combination of both intrinsic and extrinsic mechanisms. The former include accumulation of genetic mutations and epigenetic alterations, while the latter involve signals from the organ microenvironment related to inflammation (see below for breast cancer examples) and changes of target organs related to aging as shown in melanoma (Fane et al. 2022) and as seen for entry into dormancy (Fig. 2).

An example of genetic alteration-mediated escape from dormancy was described by Bui, Gu, and colleagues, who used a GEM model of *PyMT* oncogene activation with specific deletion of integrin β1 (ITGB1) in the epithelium upon doxycycline administration (Bui et al. 2022). Mammary epithelial deletion of ITGB1 impaired tumor initiation and lung metastasis. ITGB1-deficient tumors exhibited tumor mass dormancy, explained by a combination of proliferation, apoptosis, and senescence, and they were able to escape dormancy after p53 mutation and accumulation of a protumorigenic stromal compartment.

Genetic (loss of heterozygosity) loss of receptors linked to dormancy induction suggests how such changes could lead to the awakening of dormant cells in different cancers (Sharifi et al. 2007; Kobayashi et al. 2011; Bragado et al. 2013) but also support that DTCs are in constant communication with the surrounding environment and cannot be analyzed without taking into consideration physiological events happening around them. For example, loss of homeostatic niches for hematopoietic stem cell quiescence and coordinated by $Nestin^+$ MSCs led to rapid awakening of dormant breast cancer cells across luminal B and TNBC subtype models (Nobre et al. 2021b). Profibrotic environments in the lung have also been shown to cause awakening of dormant breast cancer DTCs by altering cytoskeletal and MAPK signaling (Barkan et al. 2010; Barkan and Green 2011; Weidenfeld et al. 2016). The

ability of residual breast cancer cells to reactivate after blocking expression of the HER2 oncogene was linked to their ability to control redox homeostasis. Loss-of-function of the antioxidant transcription factor NRF2 blocked recurrence (Fox et al. 2020), suggesting that antioxidant responses are able to provide residual cancer cells with adaptive fitness during recurrence.

Inflammation, in both systemic and local contexts, has been shown to trigger escape from dormancy. Specifically, lipopolysaccharide treatment in the lung awakened dormant D2A1 cancer cells, resulting in proliferation and metastasis (De Cock et al. 2016). The effect appeared to be mediated by neutrophils, but this analysis lacked mechanistic insights, warranting further studies. Albrengues et al. found that during lung inflammation (as after exposure to cigarette smoke), lung neutrophils form neutrophil extracellular traps (NETs) (Albrengues et al. 2018). In mouse models of breast cancer metastasis (D2.OR/D2A1, 4TO7/4T1), the proteases neutrophil elastase (NE) and matrix metalloproteinase 9 (MMP9) on NETs cleaved laminin, generating a version that activated integrin-mediated signaling and proliferation of previous dormant cancer cells. Whether these mechanisms occur or constitute rate-limiting events in breast cancer recurrence in patients is unknown.

Another study investigated broader systemic inflammation and its effect on DTC dormancy (Krall et al. 2018). This study used mammary fat pad injection of D2A1 cells and subsequent sham surgery. After surgery, a systemic inflammatory response was induced, and specific T-cell-mediated tumor restriction was overrun. However, primary tumor removal in place of sham surgery would be a best-suited model to test the systemic response to surgery, as primary tumors are removed from patients.

Along with influencing DTC state at secondary sites, inflammation promotes breast cancer recurrence locally. HER2 down-regulation in a conditional mouse model of HER2-driven breast cancer caused an inflammatory program through TNF-α signaling (Walens et al. 2019). The cytokine CCL5 was found high in residual tumors and attracted $CCR5^{+}$ macrophages. Further, macrophage infiltration promoted collagen deposition and tumor regrowth.

OPPORTUNITIES FOR CLINICAL INTERVENTION

Advances in breast cancer diagnosis and treatment in recent decades have resulted in markedly improved outcome for patients. Nevertheless, in up to 30% of patients, residual tumor cells reawaken following extended periods of dormancy and resume growth, ultimately resulting in incurable recurrent cancers. At present, there are no clinically approved treatments that exploit the mechanisms underlying dormant MRD in breast cancer. New trails in other hormone receptor–driven cancers (Aguirre-Ghiso 2021) aimed at inducing dormancy in biochemically recurrent prostate cancer (NCT03572387) may offer new ideas on how to manage MRD in the breast cancer setting. However, it is possible that strategies in prostate cancer may not play out in breast cancer or may be limited to some subtypes. Insights into the mechanisms underlying breast cancer dormancy gained from patient-relevant experimental models have far-reaching consequences in the clinic. Careful application of the knowledge gained from such studies could revolutionize breast

cancer management such that the remission period (i.e., no evidence of disease [NED]) following definitive treatment of the primary cancer becomes one of active surveillance and preemptive intervention, rather than passive monitoring as is currently the standard-of-care for breast cancer patients.

A framework for exploiting residual tumor cell dormancy to prevent tumor recurrence can be envisaged to (1) eliminate or deplete residual disease burden; (2) maintain residual tumor cells in a dormant state; or (3) induce a controlled exit from dormancy in residual tumor cells coupled with therapies that are highly effective in targeting proliferating cells (Fig. 3). Of these approaches, eradication of residual disease burden would ostensibly be associated with the least risk of recurrence and the greatest facility for monitoring treatment efficacy over time. The induction of dormancy as a maintenance therapy, along with proper biomarkers to monitor patients, may also constitute a safe approach. In contrast, intentionally reactivating dormant cells to then target them with antiproliferative therapies represents the most risky approach, as replication may be associated with clonal evolution and therapy resistance. Thus, even the most effective therapies for targeting proliferating cancer cells are unlikely to achieve complete eradication and may thereby leave a small, but potentially lethal, proliferating population of tumor cells.

The observation that breast cancer patients with detectable DTCs in their bone marrow are at a considerably higher risk of developing recurrences is the guiding principle driving the idea of rationally targeting MRD to prevent recurrence. Several benefits exist for such an approach. First, the massive debulking of primary tumors that accompanies (neo)adjuvant therapy, radiation, and/or surgery may render the more limited burden of residual disease a more tractable target for therapy. Second, some studies have detected a decrease in clonal heterogeneity following therapy in experimental models (Walens et al. 2020), as well as in a subset of patient samples (Almendro et al. 2014; Kim et al. 2018). Limited heterogeneity would theoretically limit the possibilities for developing resistance following residual disease-targeted therapy. Third, the presence of most residual tumor cells in a nonproliferative state may limit the rapid propagation of any recurrence-promoting inherited genotype that drives treatment refractory recurrent disease outgrowth. Fourth, dormancy-inducing therapies may provide additional means to mitigate acquired resistance while providing more time to employ therapies that eradicate MRD (Aguirre-Ghiso 2021; Khalil et al. 2022).

Conversely, some conceptual limitations exist for targeting MRD to prevent recurrences. An important consideration is that only about 40% of patients with detectable DTCs in their bone marrow develop recurrences over a 10-yr follow-up period (Braun et al. 2005). Thus, treating MRD should be approached with care to avoid overtreating patients who otherwise might not develop recurrent disease. Refining existing latency-associated genomic and transcriptomic signatures to more accurately identify those survivors at greatest risk for late recurrence would help mitigate this concern, although it is unlikely to eliminate it. Additionally, if the majority of residual tumor cells in patients exist in a nonproliferative state, it would be anticipated that these cells might be less conducive to elimination by chemotherapies whose efficacy is influenced by cell-cycle status. However, this idea has been challenged by a recent study that found that dormant DTCs can be sensitized to

chemotherapy by disrupting their interactions with endothelial cells in their niche without inducing cell-cycle reentry. However, the mechanism by which antiproliferative therapies targeting topoisomerase II (TOPOII) can kill quiescent cells was not elucidated (Carlson et al. 2019). Taken together, clinical trials designed to target MRD must thoughtfully maximize the benefits of an interventional approach while mitigating potential risks.

An ideal clinical trial in the residual disease surveillance period would combine the identification of patients at highest risk of recurrence with knowledge of signaling pathways that maintain residual tumor cell survival and dormancy to target MRD (Fig. 3; Cescon et al. 2022). Identifying breast cancer patients who are most likely to benefit from such an approach could involve considering pathological features such as tumor grade and lymph node status (Pan et al. 2017), in combination with the application of predictive dormancy-associated gene-expression signatures to primary tumor samples harvested from patients. More refined risk classification might be achieved by direct sampling of bone marrow or blood of patients to quantify their residual tumor cell burden. The ability to isolate these ultrarare cells combined with improvements in single-cell technologies could additionally help characterize the dormancy status of residual tumor cells in patients. A complementary approach would be to determine whether host-derived niche signals known to maintain dormancy are altered, informing the potential risk of relapse. Such an approach revealed that breast cancer patients with low levels of the pro-dormancy factor BMP7 (Kobayashi et al. 2011) were at a higher risk of developing lethal bone metastasis (Nobre et al. 2021a). These types of information could be invaluable insofar as they might reveal the presence of potential targets and inform choices of therapeutic regimens. Moreover, MRD sampling could also be incorporated into the trial itself to measure the efficacy of the intervention at defined intervals as a potential surrogate end point for recurrence-free and overall survival in patients.

A proof-of-principle DTC-guided clinical trial (NCT00248703) enrolled patients with persistent DTCs in their bone marrow following standard-of-care chemotherapy and administered docetaxel in a postadjuvant therapy setting to deplete DTCs. Of the patients who remained DTC-positive after the secondary postadjuvant therapy, 46.7% experienced recurrence compared to 8.8% in the patients who were DTC-negative (Naume et al. 2014). Of note, this trial lacked a control arm consisting of DTC-positive patients who did not receive additional therapy. Thus, other biological differences may exist between patients who “clear” their bone marrow DTCs following additional chemotherapy as compared to those who do not.

Another trial (NCT00172068) enrolled patients who were DTC-positive and randomized them into a control arm that received adjuvant therapy alone or adjuvant therapy in addition to zoledronic acid (Banys et al. 2013). After 24 mo, 16% of patients in the control arm still had detectable DTCs, whereas none of the zoledronic acid–treated patients had detectable DTCs. A trending decrease in overall and disease-free survival was reported for patients in the control arm versus the treatment arm; however, the analysis was limited by the small sample size. These data support the important possibility that eliminating MRD might constitute a promising approach to prevent recurrence.

More recent trials have employed rational drug combinations designed to leverage dormancy biology to reduce or eliminate DTCs. Patients at elevated risk for recurrence enrolled in the Penn-SURMOUNT screening study (NCT02732171) undergo bone marrow sampling for detection of DTCs, and patients in whom DTCs are detected are offered enrollment in DTC-targeting phase II clinical trials (Bayne et al. 2018, 2021). The CLEVER trial (NCT03032406), which was first among this cohort of trials, uses the autophagy inhibitor HCQ and the mTOR inhibitor everolimus, alone or in combination, in patients with detectable bone marrow DTCs who are at elevated risk of recurrence (Bayne et al. 2018). The ABBY trial (NCT04523857) tests the efficacy of a CDK4/6 inhibitor (abemaciclib) either alone or in combination with the autophagy inhibitor (HCQ) to eliminate DTCs. The PALAVY trial (NCT04841148) assesses the efficacy of inhibiting DTC persistence in ER^+ breast cancer patients by inhibiting autophagy (HCQ) or CDK4/6 with a different inhibitor (palbociclib), in combination with a checkpoint inhibitor (avelumab) to circumvent immune evasion. Data from this cohort of controlled clinical trials in breast cancer patients harboring DTCs will provide critical insights into the feasibility and efficacy of a DTC-targeting approach to mitigate recurrences.

In conclusion, our evolving understanding of dormancy presents a unique therapeutic opportunity with the potential to radically redefine the clinical management of breast cancer by preventing recurrent disease and reducing its associated mortality.

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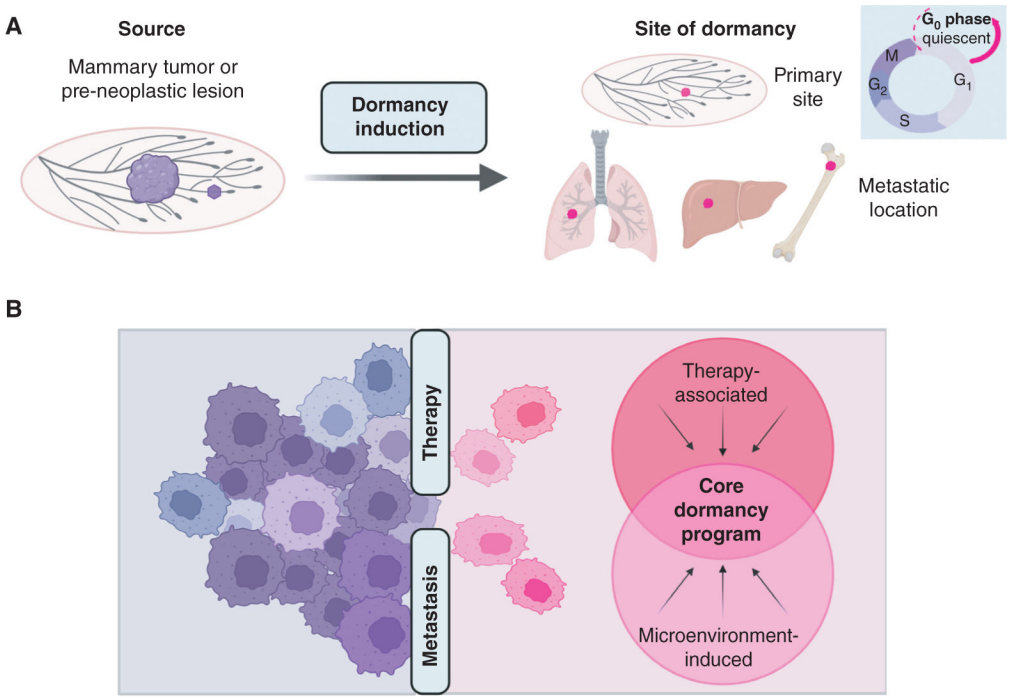

Figure 1.

Sites and mechanisms of dormancy induction. (*A*) Cells from pre-neoplastic lesions or primary tumors can undergo dormancy at the primary site or at secondary metastatic locations. (*B*) Dormancy can be induced following therapy directed at the primary tumor and/or by the microenvironment in the process of dissemination. Several core regulators are likely to be conserved independently of the mechanism of dormancy induction.

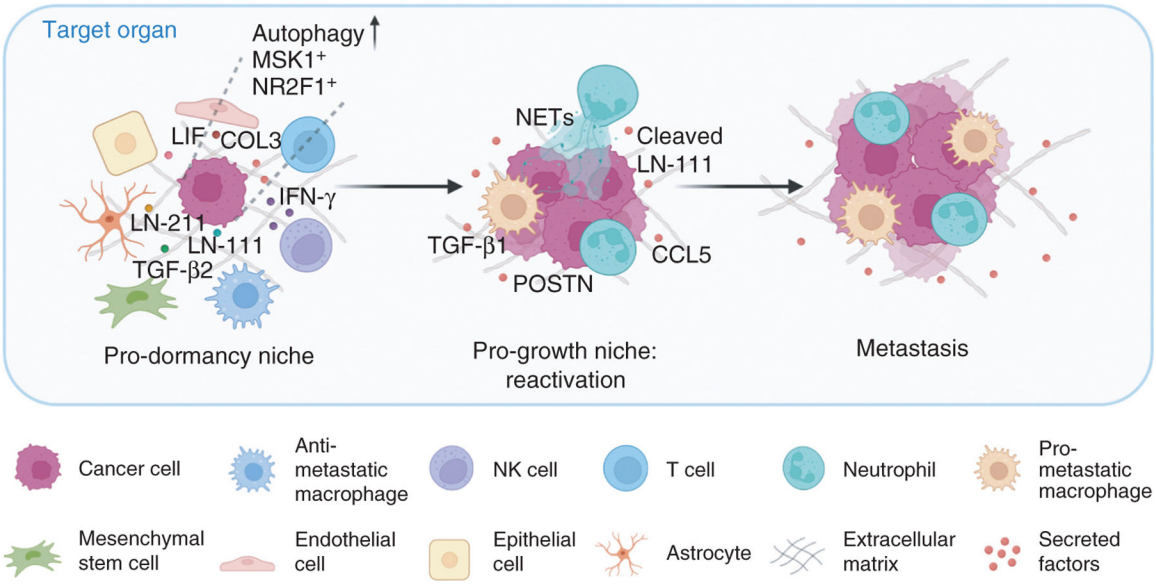

Figure 2.

Cross talk between tumor cells and the tumor microenvironment regulates dormancy. In addition to epithelial cells present in the target organ, stromal and immune cells, as well as the extracellular matrix (ECM), can regulate whether the tumor cells remain dormant or reactivate to give rise to recurrences. (NK) Natural killer.

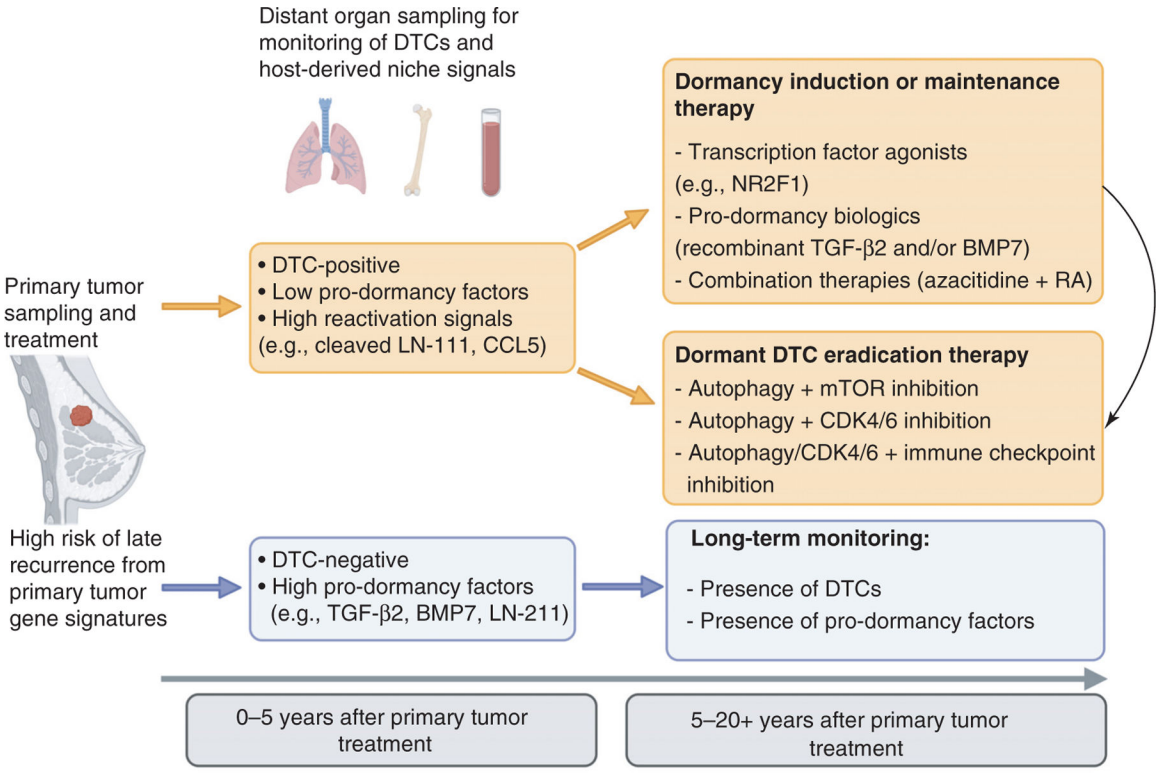

Figure 3.

Proposed scheme for the application of dormancy-oriented therapies in e clinic. Breast cancer patients who undergo primary tumor-oriented therapy can be screened for their risk of developing recurrences by gene expression profiling of their primary tumor samples and/or monitoring for the presence of minimal residual disease (MRD) (e.g., disseminated tumor cells [DTCs]). High-risk patients who are DTC-positive can be enrolled into trials that either induce/maintain dormancy, or those that eradicate dormant DTCs. Patients classified as low risk based on their DTC status can be monitored long term for DTC presence as well as levels of niche-derived pro-dormancy cues. (RA) Retinoic acid.