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Review

Dormant Tumor Cells: Current Opportunities and Challenges in Clinical Practice

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Simple Summary: It is still not clear today why some patients experience relapse after definitive treatment for localized cancer. A potential explanation for this is the presence of cancer cells in distant sites, which disseminate very early in the disease process. These cells can stay in a state of inactivity, called dormancy, for years and can be responsible for cancer recurrence. Here, we provide an overview of the current data on the mechanisms of tumor dormancy and the clinical research that has been performed.

Abstract: Tumor dormancy plays a pivotal role in cancer relapse. Dormant tumor cells have been identified in distant sites, even in early-stage tumors, and are associated with worse outcomes. This review explores the current understanding of the molecular and cellular mechanisms behind tumor dormancy, including the role of the immune system and the microenvironment. Targeting dormant tumor cells could be a therapeutic strategy to offer long-term remission and potentially cure cancer. Unfortunately, the translation of this knowledge in clinical practice is lacking. We assess the feasibility of detecting and measuring dormant tumor cells in clinical practice, and give an overview of potential therapeutic targets, both in terms of maintaining tumor cells in a dormant state, and in terms of eradicating this tumor population.

Keywords: disseminated tumor cells; dormancy; minimal residual disease; breast cancer

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1. Introduction

The majority of cancer deaths are related to the presence of metastasis [1]. In several cancers, metastasis can occur long after the treatment of the primary tumor, following a prolonged period with no apparent disease. Strong evidence suggests that the dissemination of cancer cells can be an early event in tumor evolution, with disseminated tumor cells (DTC) frequently found even in localized tumors [2–4]. The phenomenon of late metastatic recurrences, such as in breast cancer [5], therefore support that disseminated cancer cells probably follow an interrupted rather than an exponential growth pattern. The capacity of some tumor cells to remain quiescent in a dormant state during a prolonged period of time, before regaining their proliferative capability, plays a critical role in this phenomenon of late recurrence [1].

Tumor cell dormancy represents a state in which tumor cells remain viable without proliferating, with the capacity to remain undetectable for prolonged periods of time [6]. Tumor dormant cells can be found in the primary tissue, or in distant sites. Within micrometastatic niches, dormant cancer cells can evade the immune system and resume growth and division at some point, leading to late cancer progression or relapse. While cancer

cells face several challenges in the colonization of distant tissue (such as entering the bloodstream and penetrating and surviving in hostile foreign environments), it has now become apparent that the development of macro-metastasis also involves the critical process of entering, surviving, and escaping dormancy [7].

Dormancy is thought to be a dynamic, reversible process that is controlled by both intrinsic and extrinsic factors, related to the tumor microenvironment [6]. Two types of dormancies can in fact be distinguished from a clinical and tumor evolutionary perspective. Cancer cells themselves can enter a state of dormancy, leading to a mitotic arrest, with low or no proliferation. This low proliferative state promotes resistance to chemotherapy and radiotherapy, which target rapidly dividing cells [8]. On the other hand, small clusters of proliferative cancer cells can form clinically "dormant" tumor micro-metastases, whose growth is regulated by a balance between proliferation and apoptosis through regulated angiogenic response [9], or under the influence of the immune system [10,11]. Such micro-metastases continue to actively proliferate and are biologically different from truly dormant cells. Although crucial in tumorigenesis and in the metastatic process, the concept of a "population dormancy" as found in micro-metastases is outside the scope of this current review, which will focus on tumor cell dormancy.

In the following review, we provide a clinician's perspective on the relevance of tumor dormancy for patient management. We begin by presenting an overview of the biological mechanisms behind tumor dormancy. We then discuss the impact of tumor dormancy in terms of prognosis and the practical challenges of detecting dormant tumor cells in clinical practice. Finally, we explore the potential therapeutic opportunities that tumor dormancy provides.

2. Defining Tumor Cell Dormancy

The characteristics of disseminated tumor cell dormancy overlap with other cellular states, such as cancer cell senescent cells and drug-tolerant persister cells (DTP), which are commonly found in cancer treatment resistance [12]. Indeed, tumors at different stages often contain a variety of cell states, with some populations of cells characterized by slow or absent proliferation.

Dormant cells are characterized by very slow or halted proliferation. They can be present in both untreated and therapy-treated tumors, as well as in disseminated tumor cells in micrometastatic sites. They are characterized by an arrest in the cell cycle, increased expression of genes linked to stemness and epithelial–mesenchymal transition. Drug-tolerant persister cells encompass a broader definition, representing a cell population characterized by their ability to resist treatment. Drug-tolerant persister cells can exhibit slow growth, although not necessarily in all instances [12]. Various mechanisms are involved in drug-tolerant persister cells, mainly driven by epigenetic reprogramming. Cancer stem cells represent a rare cellular population within tumors, with a high capacity for self-renewal and proliferation, which sustains the cancer cell population and its heterogeneity. Cancer stem cells play a pivotal role in tumorigenesis, resistance to therapy, and in the process of metastasis formation [13].

Even if dormant cells, cancer stem cells and drug-tolerant persister cells share common features such as a quiescent phenotype enabling resistance to treatment and promoting tumor relapse or progression, they present key differences [6]. While dormant cells exhibit a cellular cycle arrest, drug-tolerant and cancer stem cells represent slow-cycling cells, with an active albeit low division rate. Additionally, cancer stem cells present a de-differentiated state, with self-renewal capacity and markers of stemness, such as CD34, NANOG, POU5F1, and SOX2 [13]. On the contrary, dormant cells usually exhibit a similar state of differentiation as their active counterpart and an inconsistent expression in stemness markers [6].

Senescence, in contrast, represents an irreversible form of proliferative arrest, usually arising as a protective mechanism against various cellular aggression or damage [14–16]. This stable form of cell cycle arrest generally does not involve stemness activation. Unlike quiescence, which occurs in the G0 phase, senescence primarily arrests the cell cycle in G1. Although previously considered a permanent state, senescence is now recognized as a possibly transient and reversible state, where senescent cells can, under specific circumstances, re-enter the cell cycle and regain their proliferative characteristics [14].

The intersected nature of dormant, senescent, and cancer stem cell populations and their overlapping characteristics suggest that these cellular states should be viewed as dynamic interconnected states, rather than strictly distinct and separate entities.

3. Mechanisms of Tumor Cell Dormancy

Cancer cell dormancy represents a particular cellular state, in which cells harbor distinctive characteristics or hallmarks [6]: cell cycle arrest, drug resistance, tumor niche dependency, immune evasion, and reversibility. These characteristics result from the interplay between intrinsic mechanisms and the interaction with the local tumor microenvironment. In the following section, we first dissect these intrinsic molecular mechanisms responsible for tumor dormancy. We then discuss the crucial role of the microenvironmental niches in which they reside. Mechanisms of tumor cell dormancy are illustrated in Figure 1.

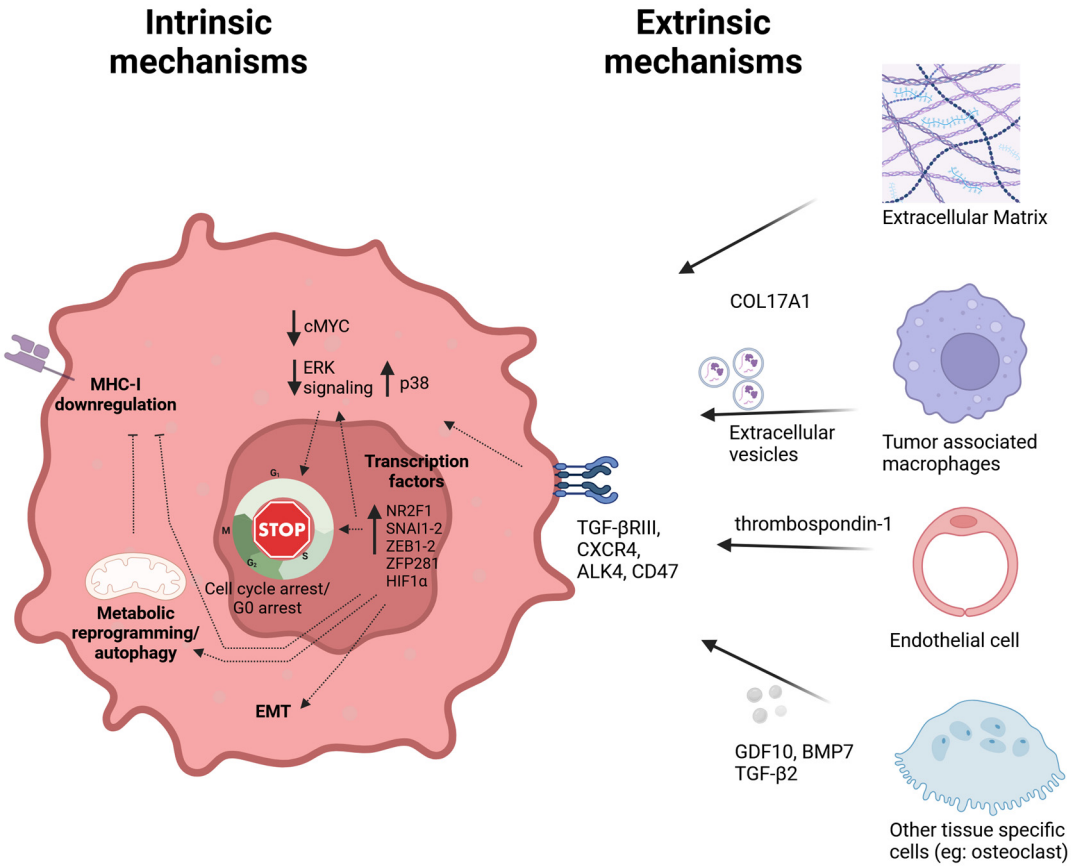

Figure 1. Mechanisms of tumor cell dormancy. Created in BioRender. Borgeaud, M. (accessed on 4 December 2024) https://BioRender.com/l71u799.

3.1. Intrinsic Mechanisms

As dormancy is a reversible state, the intrinsic driving mechanisms at play consist primarily of epigenetic reprogramming and translational modifications, rather than genomic alterations [17]. The molecular features of dormant cancer cells comprise var-

ious alterations in protein kinase activity, cell cycles proteins and regulation, metabolic changes, anti-apoptotic and autophagy-related features, and epithelial–mesenchymal transition [18,19]. These characteristics of dormancy have been observed across models of various cancer types. As an example, a similar dormancy gene expression signature was found in xenografts of both lung and colorectal cancers. Upregulated genes consisted of genes involved in stemness/pluripotency such as KLF4, TGF-β signaling, epithelial–mesenchymal transition, and cell adhesion [20]. A non-exhaustive list of potential markers of dormancy is shown in Table 1.

Table 1. Non-exhaustive list of potential markers of dormancy.

Markers	Implicated Pathways	Tumor Type	Potential Detection Method
$P38^{high}/ERK^{low}$	Upstream regulator: TGFβ2/TGFBR3, fibronectin, uPAR, BMP7 Downstream pathway: BHLHB3, P53, c-Jun, FoxM1, p21, p27, CDK4	Breast, HNSCC, Prostate, Melanoma, squamous cell carcinoma	IHC
NR2F1	Upstream regulator: p38, H3K4me3 & H3K27ac, HER2, WNT4, Downstream pathways: SOX9, RARβ, p27, p16 CXCL12/CXCR4.	Breast, Prostate, HNSCC	IHC
$Pfkfb3^{low}$ (and increased autophagy)	Upstream regulator: TGF-β1, Atg3, Atg7 Downstream pathways: p62	Breast	Transcriptomic Analysis, IHC
BMP4/BPM7	Upstream regulator: BMPR2 Downstream pathways: p38, p21, NDRG1	Prostate	IHC

The MAPkinase-ERK pathways, which play a fundamental role in cellular proliferation, have been consistently shown to be downregulated in dormant states [18]. Cellular proliferation is halted notably through p38 MAPK inhibition of ERK signaling, with a cell-cycle arrest in the G0/G1 phase [21]. Nuclear receptor subfamily 2 group F member 1 (NR2F1) is a transcriptional factor playing the role of a master regulator of tumor cell dormancy [22]. NR2F1 can activate or repress transcription of effector genes and it exerts an epigenetic regulation function through an interaction with chromatin-remodeling enzymes [23]. While the expression of NR2F1 is silenced in proliferating cancer cells, its upregulation correlates with a dormancy gene expression signature. Such signatures imply other transcription factors such as SOX9 and retinoic acid (RA) receptor β (RARβ), and the up-expression of cyclin-dependent kinase inhibitors p27 and p16, which promote cell cycle stop in the G0/G1 phase [22]. Interestingly, NR2F1 positivity, in disseminated breast cancer cells in the bone marrow, correlated with less metastatic relapse compared with lower expression [24].

Epithelial–mesenchymal transition in dormancy is driven by several transcription factors such as SNAI1-2, ZEB1-2, and ZFP281 [25]. For instance, ZEB2 has been shown to be implicated in colorectal and lung cancer dormant cells. The transcription factor ZFP281 was shown to activate mesenchymal transcriptional programs associated with dormancy [26]. Interestingly, Nobre and colleagues demonstrated that the downregulation of ZFP281 led to the loss of a dormancy phenotype and to a shift toward lung metastatic growth, supporting that such transcriptional programs can maintain cancer cells in a dormant state by preventing the activation of an epithelial-like proliferative program [26].

Metabolic changes also occur with tumor dormancy [27]. For instance, the transcription factor HIF1α, which is activated under conditions of hypoxia, plays a crucial role in cellular metabolism reprogramming [28]. Through metabolic reprogramming, HIF1α signaling has been shown to be involved in the promotion of cancer stem cells, by enabling these cancer cells to survive hostile hypoxic microenvironment [28]. This metabolic plastic-

ity and stress adaptation could also contribute to resistance to chemotherapy or targeted therapy [29], by sustaining energy production through glycolysis. Autophagy, which refers to the process of degrading and recycling cellular components in response to stress to maintain cellular homeostasis, is a cellular process commonly involved in tumor cell dormancy [30]. This was illustrated by the fact that inhibiting autophagy impaired the survival of dormant cells in the lungs in preclinical models [31]. On the other hand, a possible mechanistic relation between autophagy and dormancy was highlighted in another study, which showed that the enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (Pfkfb3) was downregulated as autophagy was induced, coinciding with the onset of dormancy [32]. Inhibiting autophagy restored Pfkfb3 expression and triggered reactivation from dormancy. Therefore, the precise role of autophagy in the induction and maintenance of dormancy remains to be elucidated.

3.2. Extrinsic Mechanisms of Tumor Cell Dormancy

The influence of the tumor microenvironment, within the so-called cancer niches, is of paramount importance in initiating and sustaining dormancy [33]. The cancer niche refers to the specific micro-environment, consisting of cancer cells, extracellular matrix, and stromal and immune cells, which can serve as a protective and supporting environment for dormant cancer cells. The role of the tumor microenvironment in regulating the cancer cell phenotype is complex, and occurs through several mechanisms, such as hypoxia and nutrient availability; the presence of soluble factors, which may be cytokines or tissue-specific factors; extracellular matrix composition and remodeling; direct cell–cell interaction; and the promotion of an immunosuppressive microenvironment.

The tumor microenvironment can act as a regulator of quiescence through the secretion of diverse soluble factors [33]. Various examples of soluble factors found in diverse tissues have been shown to promote a dormant phenotype in cancer cells by downregulating the MAPK-ERK pathways and promoting cell cycle arrest [34,35]. Such examples of soluble factors are growth arrest-specific protein 6 (GAS6), osteoclast-derived factors like growth differentiation factor 10 (GDF10) and bone morphogenetic protein 7 (BMP7) [36] in the bone marrow, or thrombospondin-1, which is produced by endothelial cells, in the lung [37]. Extracellular vesicles may play a role in regulating cancer cell dormancy, although evidence is still scarce [38]. For instance, some studies suggested that extravesicular vesicles released by pro-tumorigenic M2 macrophages could promote cell cycle arrest in breast cancer models [39].

The extracellular matrix also plays a role in the regulation of cancer progression and, on the contrary, in dormancy, as well as cell–cell interactions. Diverse components and characteristics of the extracellular matrix have been associated with either the promotion of dormancy, or on the contrary, in the awakening of dormant cells. For example, Ohta and colleagues showed that dormant colorectal cancer cells could increase in number after chemotherapy by exiting dormancy through the disruption (by chemotherapy) of the interactions with the extracellular matrix component COL17A1, a hemidesmosome protein [40]. Dormancy has also been shown to be influenced by extracellular matrix stiffness, regulated by lysyl oxidase (LOX) activity and collagen deposition [41].

The direct interaction between cancer cells and other cells from the microenvironment is critical for the induction and maintenance of cancer cell dormancy. One example is illustrated by the role of tissue-resident alveolar macrophages in the lung, which suppress breast cancer metastases in lung alveoli by inducing dormancy, notably through the expression of TGF-β2 and macrophage–cancer-cell interactions via the TGF-βRIII receptor [42,43]. Indeed, in experimental models, the depletion of alveolar macrophages, or the inhibition of the TGF-β2 receptor in cancer cells, re-initiates cancer cell growth and promotes the

development of new metastasis. Breast cancer cells can escape this state of dormancy to retrieve proliferative capacities, through the downregulation of the TGF-β2 receptor [42]. The role of the interaction with tissue-specific cellular components has also been described, such as in the lung metastatic niche, where breast cancer cells and host lung AT1 alveolar cells activated the expression of dormancy-related genes, through SFRP2 [44]. Similarly, in the bone metastatic niche, osteogenic cells and breast cancer cell interactions promoted an epithelial–mesenchymal transition state in preclinical models [45].

To survive long periods of time, one of the characteristics of cancer cell dormancy is to persist in its capacity to evade immune recognition [46]. Interestingly, preclinical models suggest that the dormant phenotype of disseminated cancer cells might be selected by the anticancer immune response during the metastatic process. For example, Pommier et al. showed that only disseminated cancer with a dormant phenotype and lacking class-I major histocompatibility molecules were capable of seeding in immunocompetent mice liver after intraportal injection [47]. As such, the postulated mechanisms of immune evasion are thought to reside in the downregulation of tumor-specific antigens and major histocompatibility molecules [47,48]. The link between dormancy and the downregulation of the antigen presentation machinery can be mediated by several factors, such as transactivator NLRC5 or the endoplasmic reticulum stress response [47,48]. Interestingly, the downregulation of major histocompatibility molecules seems to be reversed in cells regaining their proliferative activity. Another important mechanism of immune evasion is the hiding in immune-privileged niches [49]. For instance, Baldominos et al. demonstrated that quiescent cancer cells can manipulate immune cells, by establishing protective niches where T cells are less effective [49]. These niches were favored by hypoxia-induced transcriptional programs, and feature compromised dendritic cells, suppressive fibroblasts, and an increased proportion of exhausted T cells.

4. Clinical Implications of Tumor Dormancy

4.1. Correlation with Survival

Micro-metastases in the bone marrow are detected in approximately 30% of patients with early breast cancer. Detection of disseminated tumor cells (DTC) has been clearly associated with an overall worse prognosis, both in terms of overall survival and distant-free survival [50–53]. In colorectal cancer, the presence of DTCs in the bone marrow is also correlated with worse metastasis-free, disease-specific, and overall survival [54,55]. The detection of distant tumor cells, in the absence of macro-metastases, has been reported across several other tumor types. In uveal melanoma, which is known for a bad prognosis, with 30% of patients dying of metastatic disease within 10 years after initial diagnosis, autopsy studies have detected distant tumor cells in the liver, kidney, lungs, bone marrow, and myocardium [56]. In particular, the persistence of DTCs after systemic therapy (in the adjuvant and neoadjuvant setting) is a negative prognostic factor [57–59].

4.2. Detection Methods

Unlike in hematological malignancies where minimal residual disease can inform therapeutic management, in solid tumors, DTC detection is not part of standard procedures. DTCs have been reported in distant sites such as the bone marrow, the liver, the lung, and lymph nodes.

The most accessible site for dormant tumor cell detection is the bone marrow, and this site has been used the most in clinical trials. Despite an effort to standardize DTC detection, studies using DTCs have used different detection methods (immunohistochemistry, immunomagnetic selection, RT-PCR, etc.), which have been detailed in Table 2. In breast cancer, recommendations have been emitted by the European Disseminated Malig-

nancies (DISMAL) project consortium [60]. Histology and immunohistochemistry remain the mainstay for detecting disseminated tumor cells. The recommended markers are cytokeratins (cytokeratin 7, 8, 18, and/or 19 by antibodies A45-B/B3, AE1/AE3, or 2E11) or sialomucin (E29) [61]. This technique is still used today, for example, in one of the larger current trials investigating dormancy, the PENN-SURMOUNT trial (NCT02732171). This is a longitudinal screening study examining bone marrow and blood biomarkers associated with recurrence in patients with early breast cancer, with a particular focus on dormant DTCs. DTC positivity is supposedly defined as a positive pancytokeratin cell (AE1/AE3) by immunohistochemistry in bone marrow aspirate, as defined by Naume et al. [62–64]. We await published data for more clarification on detection methods and DTC characterization, particularly in terms of dormancy. However, this technique has limitations. Heterogeneity has been reported in DTC cytokeratin expression patterns in the bone marrow. A study compared the performance of the A45-B/B3 (A45) and the AE1/AE3 (AE) antibodies for detecting DTCs in the bone marrow. High concordance was reported between the two antibodies (84.4%); however, the overlap among positive cases was low (3.2%), suggesting that different subpopulations could be detected with different markers. It was also reported that patients with hormone receptor-positive breast cancer had higher positivity for DTCs stained with A45, whereas patients with hormone receptor-negative breast cancer had higher positivity for DTCs stained with AE. AE detected CK5, CK7, CK8, and CK19, whereas A45 recognized CK7 and CK18. The difference in detection patterns could in part be explained by the upregulation of CK8, 18, and 19, and down-regulation of CK5–7 and CK14–17 in hormone receptor-positive tumors compared with hormone receptor-negative tumors [65]. EpCAM has been established as the gold standard for circulating tumor cells (CTC), and is the approach used by CellSearch® system, the only US Food and Drug Administration (FDA) approved system for CTC detection. It is a cell adhesion molecule expressed in epithelial tissues and has a role in cell proliferation, stemness, and epithelial–mesenchymal transition. EpCAM is however not a standard biomarker in DTCs. Compared to pancytokeratin, which solely detects epithelial markers, EpCAM can potentially identify DTCs with stem-like properties, or undergoing epithelial-to-mesenchymal transition (EMT). In colorectal cancer, a study tested immunomagnetic selection using an anti-EpCAM antibody and immunocytochemistry using AE1/AE3 to detect bone marrow DTCs, with a detection rate of 17% (41/235) versus 12% (28/235). Among the 64 positive bone marrow samples for DTCs, 5 samples (7.8%) were positive by both methods [54]. This suggests that immunohistochemistry may fail to capture the full population of DTCs.

RT-PCR is another technique that has been tested to detect bone marrow DTCs and has been compared to immunohistochemistry. This approach has the advantage of speed and reproducibility. One method used the CK19 transcript and reported comparable positivity rates for DTC detection compared to immunohistochemistry (A45B-B3 antibody) [66], including more markers and increased detection rates. A multimarker assay based on mRNA markers keratin 19 (KRT19), mammaglobin A (hMAM), and TWIST1 was compared to immunohistochemistry using AE1-AE3 anti-cytokeratin monoclonal antibodies.

Table 2. Overview of studies analyzing DTCs in common tumor types.

Study Name	Tumor Type	Intervention	Detection Method	Timing of DTC Detection	Detection Rate	Prior Treatment	Prognostic Factor for Progression/Death
Studies testing treatment effect in DTC-positive population							
SATT-trial [63,67] NCT00248703	Breast	Adj docetaxel after FEC (single arm)	IHC: AE1/AE3 qRT-qPCR: KRT19, hMAM, TWIST1	8–9 months after FEC	IHC: 7% qRT-PCR: 40%	Yes	DTC persistence after docetaxel
CLEVER trial [64] NCT03032406	Breast	HCQ vs. EVE vs. HCQ + EVE vs. 3-month observation then HCQ + EVE	Not reported	Up to 5 years after adj CT	30%	Yes	Not reported
MRD-1 trial [68] NCT0017206	Breast	Adj CT + ZOL vs. Adj CT	IHC: A45B/B3	At surgery	Not reported	No	DTC persistence after CT ± ZOL
Hoffmann et al. [69]	Breast	Adj ibandronate after SOC (single arm)	IHC: A45-B/B3	2–10 years after adj CT	33%	Yes	Not reported
NCT00295867 [70]	Breast	ZOL after SOC	IMS: EPCAM+/CD45−	At surgery—up to 1 year after surgery	Not applicable: reported as cells/mL	Yes	Baseline DTC
Studies testing DTCs as a predictive factor							
Aft et al. [71] NCT00242203	Breast	Neoadj CT vs. neoadj CT + ZOL	IHC: AE1/AE3	At diagnosis	45.7%	No	Not reported
NCT02682693 GEPARX study [72,73]	Breast	Neoadj CT ± denosumab	IHC: A45-B/B3	At diagnosis	25%	No	Not reported
Studies testing DTCs as a prognostic factor							
Domschke et al. [51,74]	Breast	SOC	IHC: 2E11	At surgery	49%	No	Baseline DTC
Benoy et al. [52]	Breast	SOC	IHC: A45-B/B3 qRT-PCR: CK19, MAM	At diagnosis	IHC: 28% qRTPCR CK19: 29% qRT-PCR MAM 21%	No	Baseline DTC (by IHC and by qRT-PCR)
Pooled Analysis of DTC Detection in Early Breast Cancer (PADDY) [53]	Breast	SOC	IHC: pan-CK (not specified)	At diagnosis	27.3%	No	Baseline DTC
Kollerman et al. [75]	Prostate	SOC	IHC: A45-B/B3	At diagnosis	44%	No	Baseline DTC
Berg et al., Berg et al., Lilleby et al. [76–78]	Prostate	SOC	IHC: AE1/AE3	At diagnosis	18%	No	Baseline DTCs
Vogelaar et al. [79] Leiden MRD trial	CRC	SOC	IHC: A45-B/B3	At surgery (primary)	18%	No	No association with baseline DTC
Brudvik et al. [80]	CRC	SOC	IHC: AE1/AE3	At surgery (liver metastasis and/or primary)	32%	Yes	No association with baseline DTC
Hinz et al. [81]	CRC	SOC	RT-PCR: CK20	At surgery (liver metastasis)	25%	Possible	Baseline DTCs
NCT03640572 Pach et al. [55]	CRC	SOC	IHC: A45-B/B3	At surgery (primary)	46%	Possible	Not reported
Flatmark et al. [54]	CRC	SOC	IHC: AE1/AE3 IMS: anti-EpCAM	At surgery (primary)	IMS: 17% IHC: 12%	No	DTC persistence

Adj: adjuvant, CRC: colorectal cancer, CT: chemotherapy DTC: disseminated tumor cells, EVE: everolimus, HCQ: hydroxychloroquine, IHC: immunohistochemistry, IMS: immunomagnetic selection, Neoadj: neoadjuvant, RT-PCR: real-time polymerase chain reaction, SOC: standard of care, ZOL: zoledronic acid.

The RT-PCR-based method was able to identify DTCs in 124 (40%) bone marrow samples, compared to 23 (7%) with immunohistochemistry [67]. The inclusion of TWIST1, an EMT-marker in the multimarker assay, therefore enabled the detection of further DTC-positive bone marrow samples. This approach could potentially increase sensitivity by detecting DTCs that lose epithelial markers. Compared to immunohistochemistry, one of the limitations of RT-PCR is the rapid degradation of RNA after bone marrow sampling, which can lead to lower sensitivity of this method. This becomes particularly problematic if a pre-enrichment strategy is used before tissue analysis (density gradient centrifugation, membrane filtration, immunomagnetic beads).

Beyond detecting the presence of disseminated tumor cells in distant organs, assessing their functional state -proliferating versus dormant- is not yet standardized. Ki67 or Mib1 are routinely used in oncology as markers of cellular proliferation. However, Ki67 levels are heterogeneous in cells undergoing G0/G1 phase [82]. This implies that dormant tumor cells in G0/G1 arrest could be falsely considered proliferating cells. As previously described, NR2F1 is a transcription factor that limits pluripotency and proliferation, and that is upregulated in dormant DTCs [22]. In breast cancer, correlation has been described between NR2F1 levels in bone marrow DTCs and relapse rate and overall survival. Detection of NR2F1 high DTCs was associated with improved disease-free survival compared to NR2F1 low DTCs [24]. In prostate cancer, NR2F1 levels are higher in DTCs detected in the early versus advanced disease setting [22]. Compared to Ki67, NR2F1 seems to be a more durable and stable marker of a dormant phenotype [24]. This has been recently integrated into clinical trials. The phase I trial by Patel et al. is one of the only trials reporting dormancy biomarkers; dormancy was estimated using BMP4/BMP7 levels and dormant circulating tumor cells were detected with NR2F1 [83].

Blood-based biomarkers like circulating tumor cells have also been analyzed. These have the advantage of being easily accessible for analysis, and potentially detecting tumor cells before they seed in non-bone marrow, i.e., in a non-accessible niche. However, circulating tumor cells have a short half-life (approximately 2 h) before ongoing apoptosis, making this a less reliable marker over time compared to DTCs [84]. In breast cancer patients, these cells can be detected up to 22 years after treatment and while being considered cancer-free. This begs the question of the origin of these CTCs, from the primary tumor or distant sites [84].

Few data are available on the correlation between circulating tumor cells and disseminated tumor cells. The largest correlation study was performed on breast cancer patients. In 341 patients with early breast cancer who were screened for DTCs in the bone marrow or CTCs in peripheral blood, 10% were positive for CTCs versus 14% for DTCs. Among CTC-positive patients, 26% of patients experienced relapse versus 29% in DTC-positive patients; 8 patients were both positive for CTCs and DTCs. The author's conclusion was that so far CTC sensitivity was insufficient to replace bone marrow DTC as a detection method for minimal residual disease [73]. Across different tumor types (colorectal, prostate, breast cancer), detection rates of CTCs have been reported up to 30% [85]. Like DTCs, detection methods for CTCs are heterogeneous. Cytokeratin 20 and carcinoembryonic antigen are often used for colorectal cancer, cytokeratin 19 and carcinoembryonic antigen in non-small-cell lung cancer, and cytokeratins and prostate-specific antigen in prostate cancer [85].

Current radiology techniques are not precise enough to detect single cells. However, exploratory radiomics analyses from the SURMOUNT study have suggested a correlation between MRI features and the presence of DTCs in bone marrow aspirate. This suggests that certain radiomic phenotypes in breast cancer may be associated with a certain risk of

presenting with DTCs [86]. Further research is needed to explore the role of radiomics in predicting long-term outcomes, and in selecting patients at high risk of disease relapse.

5. Therapeutic Strategies

Despite a relatively high rate of DTC detection in distant sites in patients with localized cancer, not all patients presenting with DTCs experience disease progression. In breast cancer, 30% of patients can present with positive bone marrow for DTCs, and among these patients, more than half of patients remain cancer-free after 10 years [50]. Different strategies to target DTCs have been proposed: leveraging dormancy to maintain a non-proliferative state, targeting DTCs specifically to eliminate residual disease, and modifying the host microenvironment in order to indirectly exert the previously mentioned effects. We have reviewed the latest research on these strategies.

5.1. Maintaining the Dormant State

5-azacytidine (AZA) and all-trans retinoic acid (atRA) are agents used for the treatment of hematological malignancies. Preclinical data have shown that the combination of AZA and atRA could induce quiescence in a head and neck squamous carcinoma in a patient-derived xenograft, via activation of the SMAD2/3/4 transcription pathway, which upregulates the anti-proliferative TGF-ß signaling. With this combination, disseminated cancer cells were maintained in a SMAD4+/NR2F1+ state, and were non-proliferative [87]. The combination of AZA and atRA was subsequently tested in a phase I clinical trial by Patel et al. in patients presenting with biochemical recurrence after local therapy for prostate cancer. In addition to androgen deprivation, patients also received AZA and atRA. The pro-dormancy factors BMP 4 and BMP 7 were increased upon treatment, suggesting that dormancy could be effectively induced. PSA doubling time was prolonged in 46% of patients [83]. Compared to previous trials analyzing dormancy biomarkers, this group did not analyze DTCs for example in the bone marrow, and introduced BMP4 and BMP7 as potential markers to measure the state of dormancy induced by the host. Final data are awaited from this trial.

CXCR4 is a chemokine receptor that is implicated, along with its ligand CXCL12, in metastasis, tumor growth, and cell cycle progression. In mouse xenograft breast cancer models, findings have shown that CXCR4 increases in response to hypoxic and oxidative stress induced by antitumoral drugs, and subsequently triggers a transition from a dormant to a proliferative state [88,89]. The CXCL12/CXCR4 pathway is therefore an attractive target to induce or maintain dormancy. CXCR4 inhibitors such as AMD3100 have been tested in phase 2 clinical trials in the advanced setting (NCT04058145, NCT00395967). Data regarding dormancy in in vivo settings have yet to be reported.

5.2. Eradicating Dormant Tumor Cells

To our knowledge, the largest adjuvant chemotherapy trial testing treatment escalation in case of residual disease is a trial conducted by Naume et al. in early breast cancer [63]. Patients who received 6 cycles of adjuvant FEC (fluorouracil, epirubicin, and cyclophosphamide) had serial bone marrow analyses, with the first performed at 2–3 months and the second performed at 8–9 months after the end of FEC. If the bone marrow was positive for DTCs at 8–9 months after the end of FEC, patients received 6 cycles of docetaxel. Out of 1066 patients, 7.2% (77 patients) were positive for DTCs at 8–9 months and received docetaxel. Among these patients, 20% (15 patients) remained DTC-positive after the end of docetaxel treatment. Disease-free interval in this population was worse, with 46% experiencing relapse, versus 8.8% in the population with DTC-negative bone marrow after docetaxel. Interestingly, of the 77 patients with DTC-positive bone marrow at 8–9 months after FEC,

83% (64 patients) were considered DTC-negative at 2–3 months after FEC. Seventy-eight patients considered initially DTC-positive at 2–3 months were then DTC-negative at 8–9 months. This trial was very informative in confirming the worse prognosis associated with minimal residual disease. It showed the efficacy of chemotherapy to reduce minimal residual disease. However, the lack of randomization in the DTC-positive population implies that DTC negativity with chemotherapy cannot be used as a surrogate marker for survival. Furthermore, the change in DTC positivity at 2–3 months, and at 8–9 months begs the question of a spontaneous decrease in DTCs or detection limitations. This trial could not report dormancy markers in DTCs (Ki67 analyses were performed on a small number of cases) and therefore cannot inform on the impact of chemotherapy on dormant DTCs.

Autophagy has been shown to be activated by cancer cells during dormancy [31]. This has provided the rationale to test the combination of hydroxychloroquine and mTOR inhibitors. Hydroxychloroquine is an autophagy inhibitor and can exert specific toxicity in dormant breast cancer tumor cells in preclinical models [31]. mTOR inhibitors induce autophagy and lead to cytostatic activity (as opposed to cytotoxic activity) in animal models [90]. When combined, it has been hypothesized that hydroxychloroquine blocks the cell's rescue mechanism (i.e., autophagy) and renders tumor cells sensitive to the cytotoxic effect of mTOR inhibition [91]. Such a strategy was tested in the phase II CLEVER trial, which randomized patients with early triple-negative breast cancer, with bone marrow-positive DTCs to receive hydroxychloroquine, everolimus, or the combination. After 42 months, 2 patients (3.6%) experienced disease recurrence overall. The probability of DTC reduction > 80% after 3 months of therapy was approximately 99% across all treatment arms [64]. Translational data from the CLEVER trial will also give more insight into the resistance mechanisms to everolimus and hydroxychloroquine in relapsing patients. Such resistance pathways could be the upregulation of MAPK and AKT, as reported in preclinical models [92,93]. Dual mTORC1/2 inhibitors such as vistusertib may overcome this resistance pathway. They have not yet been tested in the early-disease setting, so far only being tested in the advanced-disease setting [94].

The CLEVER trial group is also conducting two other phase 2 trials in patients with early breast cancer and DTC-positive bone marrow. The ABBY trial (NCT04523857) is testing the combination of an anti-CDK4/6 agent (abemaciclib) and hydroxychloroquine. The PALAVY trial (NCT04841148) is testing another anti-CDK4/6 agent (palbociclib) in combination with avelumab and hydroxychloroquine.

Polyploid giant cancer cells (PGCCs) are a subset of dormant tumor cells that have become of particular interest recently. PGCCs are a formation of multiple fused cancer cells, with multiple nuclei and/or a giant nucleus with multiple sets of chromosomes. These cells display dormancy features as they are arrested in the G2/M phase, and have a role in treatment resistance and disease progression. These cells express stemness markers (such as NANOG, Sox-2, OCT4) [95]. A group has shown that in nasopharyngeal carcinoma mouse models, PGCCs are induced by chemotherapy, and that PGCCs express high autophagy. They subsequently showed that adding hydroxychloroquine to chemotherapy reduced PGCC formation and ultimately reduced tumor recurrence in mice [96]. The same group has started a clinical trial wherein patients with early nasopharyngeal carcinoma are randomized to receive hydroxychloroquine or a placebo, in combination with chemoradiotherapy (NCT06389201).

In dormant breast cancer tumor cells, the Notch 1 signaling pathway was shown to be conserved. This pathway is activated in cases of tumor recurrence in mouse models. Microarray data from patients with early breast cancer have shown that Notch1 was independently associated with higher recurrence rates [97]. Interestingly, the role of Notch1 in tumorigenesis seems to be restricted to the dormant stage, since Notch promotes tumor

recurrence but does not play a dominant role thereafter. Notch pathway inhibitors have been tested in early-phase trials in advanced cancers, with limited clinical activity and significant toxicity. They have not been tested in the adjuvant setting [98].

PERK is a kinase involved in the cellular stress response related to the endoplasmic reticulum, particularly in the Unfolded Protein Response pathway. This is a survival strategy in cancer cells, including dormant cancer cells, that can be targeted in mouse and patient-derived xenograft breast cancer models [99]. Subsequently, a PERK inhibitor (HC-5404) has been tested in a phase 1a trial in patients with advanced solid tumors. Data on clinical response are awaited. It is not clear whether dormancy markers will be analyzed [100].

5.3. Role of Immunotherapy

As described previously, the role of the immune system in maintaining tumor cell and tumor mass dormancy is known but little understood. The role of immunotherapy on tumor mass dormancy is beyond the scope of this review; we will focus on the effect of immunotherapy on dormant tumor cells.

One mechanism used by dormant cells to avoid immunosurveillance is downregulating MHC class I, as described earlier. However, a clinical study showed that autologous HER2-specific T lymphocytes were able to target disseminated T cells, somehow contradicting the previous statement. In this study, a patient with advanced HER2-positive breast cancer with liver metastasis received autologous HER2-specific T-cell clones in association with IL2. Interestingly, the T cells were not able to penetrate the solid tumor masses due to the tumor stroma, but were able to penetrate the bone marrow. DTC levels were high prior to T-cell infusion, and no DTCs could be detected after autologous lymphocyte infusion [101]. This potentially identifies a clinical opportunity for T-cell-based therapies in the early setting, where isolated DTCs can be more efficiently targeted, as opposed to the advanced setting, where tumor stroma inhibits the penetration of T cells. Chimeric antigen receptor (CAR)-T cells act in an MHC-1-independent fashion. In acute lymphoblastic leukemia, CAR-T-cell therapy can induce minimal residual disease negative remission in 67% of patients (32/53 patients) [102]. Overall, these studies show that despite MHC class I downregulation, T-cell-mediated cytotoxicity remains effective in dormant tumor cells. T-cell therapy could therefore be a promising approach to target these cells. However, we lack knowledge of the DTC antigen repertoire and, specifically, a common putative target antigen. The role of the innate immune system and notably NK cells has been explored in the preclinical setting.

Another explanation provided by a preclinical trial for the persistence of DTCs, despite their sensitivity to T-cell cytotoxicity, is a distance problem. Indeed, this study showed that DTCs may persist simply because CD8+ T cells do not encounter DTCs due to their scarcity and therefore cannot exert their cytotoxic effect [103]. Increasing the T cell:DTC ratio and particularly improving T-cell penetration in the different niches appear as promising paths to leverage the role of the immune system in clearing minimal residual disease.

The role of immune checkpoint inhibitors on dormant tumor cells has not been particularly explored. For example in melanoma, in both the early and advanced setting, checkpoint inhibitors have enabled more than 50% of patients to remain cancer-free several years after the end of systemic therapies [104,105]. It should therefore be evident that immune checkpoint inhibitors have a role in controlling minimal residual disease. It is not clear whether this effect is achieved through maintaining tumor mass dormancy, or by exerting a direct effect on the dormant tumor cell level (either by maintaining a non-proliferative state, or by cytotoxic effect).

6. Targeting the Niche

6.1. The Bone Marrow Niche

The most studied DTC niche is the bone. Bone-modifying agents such as bisphosphonates and RANKL antibodies have been used in several clinical trials, with variable impacts on cancer-related survival and bone metastasis prevention. Preclinical data have shown that bisphosphonates also exert antitumoral effects by limiting tumor cell adhesion and invasion, angiogenesis, and by increasing immunosurveillance by γδ T cells [106].

In the adjuvant setting, bone-modifying agents are currently only recommended for breast cancer. Bisphosphonates in the adjuvant setting in early breast cancer are currently recommended by the European guidelines in women without ovarian function (postmenopausal or receiving ovarian function). The guidelines are however contradictory on the aim of this treatment (prevention of bone loss or prevention of tumor recurrence) [107]. In prostate cancer, another tumor type with a high propensity for bone metastasis, bisphosphonates in the early setting are not recommended for the prevention of tumor recurrence. The STAMPEDE trial in particular did not report improved long-term outcomes when zoledronic acid was added to radiotherapy and endocrine therapy [108].

In breast cancer, the effect of bisphosphonates has been tested specifically in bone marrow DTC-positive patients after primary breast cancer treatment. A trial by Banys et al. compared standard adjuvant treatment versus adjuvant treatment combined with zoledronic acid. The results showed that all patients receiving bisphosphonates became DTC-negative at 24 months (40/40 patients) compared to 84% in the standard adjuvant therapy group (39/46 patients). The relapse rate was higher in the control group versus the zoledronic acid group (15% versus 8%). Persistent DTCs after treatment were significantly associated with worse survival [59]. A prospective pilot trial by Vidula et al., testing the effect of zoledronic acid in breast cancer patients with DTC-positive bone marrow, also showed a significant reduction in DTC levels with zoledronic acid (32% complete DTC response at 12 months, 26% at 24 months) [70]. Another trial identified bisphosphonates as an independent prognostic factor for disease-free survival in patients with DTC-positive luminal breast cancer [74]. Long-term outcomes with bisphosphonates are heterogeneous. The AZURE trial randomized patients with early breast cancer regardless of endocrine receptor positivity to zoledronate versus placebo. In the postmenopausal group only, an improvement in disease-free survival was seen (HR 0.77; (95%CI 0.63–0.96) [109]. On the contrary, the ABCSG-12 trial, which tested bisphosphonates in hormone receptor-positive breast cancer in premenopausal patients receiving ovarian function suppression, did not show a statistically significant DFS benefit in this younger population [110]. The Royal Marsden trial was the only trial with bone metastasis as a primary endpoint; this trial was positive with a 31% decrease in bone metastasis incidence [111]. Overall pooled data in the EBCTCG meta-analysis showed that bisphosphonates significantly reduce bone metastasis, distant disease recurrence, and cancer-related mortality in the postmenopausal population [112]. To put into perspective the benefit of bisphosphonates, the 3% breast cancer mortality risk reduction is similar to the effect seen of anthracycline addition to chemotherapy regimens.

RANK-L inhibitors such as denosumab are another class of bone-modifying agents. The data on the benefit of adjuvant denosumab in breast cancer are not consistent and denosumab is therefore not recommended in the current guidelines. Indeed, the ABCSG18 trial reported an improvement in DFS, whereas the D-CARE trial was negative for DFS [113,114]. The influence of RANK-L inhibitors on DTCs is not as clear as for bisphosphonates [73].

Preclinical data have shown that the bone marrow microenvironment in the perivascular niche protects DTC from chemotherapy, through integrin-mediated interactions. The inhibition of these interactions interestingly did not trigger proliferation in dormant

cells [115]. Targeting these integrin interactions therefore in order to chemosensitize DTCs is an appealing approach to eradicate the DTC reservoir. Subsequently, a study in breast-cancer murine models tested the effect of gedatolisib, a Pi3K inhibitor, downstream of integrin-ß-1, with or without genotoxic therapy, on DTC burden and prevention of metastasis. This study was negative [116].

Targeting the homing strategy of circulating tumor cells to the bone marrow niche could also be a strategy to limit seeding in distant sites. Mouse models have shown that prostate cancer cells compete with hematopoietic stem cells (HSC) in the bone marrow niche. Interestingly, it has been shown that HSC mobilization protocols with G-CSF also mobilized prostate cancer cells in the peripheral blood, suggesting that HSCs and disseminated tumor cells share similar homing and mobilization strategies [117]. To our knowledge, there are no reported clinical data on the effect of G-CSF on the bone marrow DTC pool.

We also searched the literature for the potential effects on dormant tumor cells of Radium-223. Radium-223 is a calcium mimetic isotope that can home into bone tissue and exert an antitumoral effect by inducing double-strand breaks in the bone tumor microenvironment. The ALSYMPCA trial tested radium-223 versus standard of care in men with metastatic castration-resistant prostate cancer, and showed a decrease in bone turnover with a 30% decrease in serum alkaline phosphatase levels, with a positive effect on overall survival (median OS 14.9 months versus 11.3 months; hazard ratio, 0.70; 95% CI, 0.58 to 0.83; $p < 0.001$) [118]. Observational data from the retrospective EPIX study, which included data from patients with metastatic castration-resistant prostate cancer receiving Ra-223, identified long-term responders with this treatment, with 15% of the included patients surviving longer than 2 years [119]. Characteristics of the patients with longer survival included less use of prior chemotherapy. Trials on treatment sequencing have not been performed; therefore, it is difficult to extrapolate whether earlier use of Ra-223 could have a longer effect, potentially by impacting tumor dormancy in the bone niche. Exosomal transcriptome analyses from patients treated with Ra-223 showed a decrease in WNT/β, BMP, and RANK signaling in patients with favorable outcomes (defined as survival longer than 24.3 months) [120]. These pathways have been associated with tumor dormancy in bone [36]. [177Lu]Lu-PSMA-617 is another radionuclide that has been approved for the treatment of metastatic castration-resistant prostate cancer [121]. Preclinical data on the effect of this treatment on the bone niche are lacking. It is also unclear whether PSMA is expressed on dormant prostate cancer cells.

6.2. The Liver Niche

The liver has also been described as a potential niche for dormant tumor cells, particularly in colorectal cancer [122]. Data on this specific site relating to tumor dormancy in the clinical setting are lacking, in part due to the technical difficulty in accessing the liver.

The role of surgery in triggering cancer proliferation has been controversial. In colorectal cancer, resection of liver metastasis can prolong overall survival. Nevertheless, despite appropriate patient selection, more than 50% of patients will experience relapse after surgery in the liver or other distant sites. It has been suggested that patients undergoing hepatectomy can experience tumor growth through a transient increase in cytokines and growth factors, subsequently triggering a transition in remnant cells from a dormant to a proliferative state [123,124]. This has not been described in other tumor resections and can be explained by the liver's intrinsic ability to regenerate [125]. Chemotherapy-induced toxicity in the liver (steatosis, steatohepatitis, sinusoidal obstruction) impairs regeneration, and could potentially limit the proliferative effect of liver regeneration in the perioperative setting [126]. The level of evidence for the risk of progression after surgery is however debatable. Clinical trials have yet to demonstrate whether further perioperative

therapies (chemotherapy, targeted therapies, radioembolization, etc.) can limit the risk of postoperative tumor relapse.

In the liver, natural killer (NK) cells have been shown to maintain tumor cells in a dormant state in preclinical models. The pool of NK cells is controlled by the liver microenvironment, namely, hepatic stellate cells, through the effect of CXCL12. High CXCL12 leads to a decrease in NK-cell-mediated immunity and an emergence of DTCs [89]. The CXCL12/CXCR4 pathway is also affected in the pathogenesis of liver fibrosis. This study therefore mostly highlights the direct role of the liver microenvironment in maintaining dormancy, and provides a rationale for the association between lifestyle-related injuries on the liver (alcohol, obesity, and smoking) and tumor relapse.

7. Conclusions

In conclusion, the clinical implications of tumor dormancy still remain an area of significant uncertainty and complexity. Despite advancements, we have significant technical limitations for the detection of minimal residual disease, and in particular, dormant tumor cells. As long as detection techniques do not capture the full population of dormant cells, the challenges will persist in integrating tumor dormancy in clinical trials. So far, disseminated tumor cells (DTCs) are the most robust marker, but they require invasive procedures, limiting their practical application. Furthermore, ethical considerations have limited trials looking into residual tumor cells. It is difficult, ethically, to randomize patients with residual disease, to receive a treatment or to receive no treatment. Preclinical models have identified cell- and microenvironment-related factors associated with tumor dormancy but fail to accurately depict host-related factors (such as aging, inflammation, stress, alcohol, smoking, and hormonal variations). Considering the multitude of variables that influence tumor dormancy, it seems unlikely that identifying a single target will solve the minimal residual disease problem. Targeting tumor dormancy and residual disease seems to be an important component of an effective strategy against cancer. Clinical trials testing drugs in the early setting with an aim to leverage dormant tumor cells run the risk of exposing patients to toxicity, which can be drug-related or procedure-related, but also to the psychological burden of knowing of the presence of minimal residual disease. If we implicate tumor dormancy in our clinical practice, clinical trials must therefore imperatively be designed with clinically meaningful endpoints such as quality of life and overall survival.

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