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# Targeting Cancer Drug-Tolerant Persister Cells in Minimal Residual Disease

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**Abstract:** Cancer cells that survive therapeutic drug pressure are a significant cause of disease relapse and progression, impeding curative cancer treatment. Drug-triggered Darwinian selection and the emergence of subclones harbouring specific mutations that confer resistance have been well documented and extensively studied. However, these genetic alterations, while important, do not fully explain clinical observations where some patients, after a drug holiday, regain sensitivity to the same treatment despite previous disease progression. This phenomenon highlights the possibility that drug resistance may not solely rely on genetic mutations but could also involve reversible, non-genetic mechanisms. Recent studies have highlighted the existence of drug-tolerant persister cells (DTPs), a subpopulation of cancer cells that can survive short-term therapeutic pressure without acquiring resistance-associated genetic alterations. These cells exhibit a temporary yet reversible tolerance to the initial treatment while also acquiring cross-tolerance to other anti-cancer therapies. The presence of DTPs underscores a dynamic and complex plasticity in tumours, wherein cancer cells can utilise epigenetic rewiring, metabolic reprogramming, and specific signalling pathways to transit between drug-tolerant and drug-sensitive states to adapt to environmental pressures. Furthermore, this adaptive resilience enables DTPs to act as a reservoir for the development of genetically stable resistance, resulting in cancer therapy failure and eventual relapse. In this mini-review, we examine recent evidence on DTPs to provide an overview of their characteristics, development, and survival mechanisms.

**Keywords:** minimal residual disease; drug-tolerant persister cells; drug resistance; drug tolerance; ferroptosis

## 1. Introduction

Minimal residual disease (MRD) refers to the small population of cancer cells that persist in a patient after treatment. These residual cells can evade therapy, posing a significant risk of tumour recurrence and treatment failure. Therefore, eradicating MRD is considered essential for curing cancer. MRD primarily arises from cancer cells that withstand therapeutic drugs. These cells were previously believed to be drug-resistant due to certain genetic mutations, mainly through two mechanisms: first, drug-driven Darwinian selection of intrinsically resistant subgroups (primary resistance); and second, the development of acquired

resistant mutations along the therapeutic continuum (secondary resistance).

As resistant mutations develop, discontinuing the initial therapy and switching to alternative treatments often become necessary. This approach is exemplified in the development of epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC). Initially, EGFR mutations such as the exon 19 deletions and L858R sensitise tumours to EGFR-targeted therapies, such as erlotinib. However, under therapeutic pressure, cancer cells evolve by developing secondary mutations like T790M, which conferred resistance to the initial treatment. In response, third-generation EGFR inhibitors, such as osimertinib, were developed to effectively target these mutations. Despite their efficacy, resistance arises again due to further EGFR mutations (e.g., C797S), and there is currently no clinically approved inhibitor used to overcome such resistance [1]. These observations highlight the challenge of developing drug that keep pace with the unpredictable evolution of mutations. Therefore, targeting the mechanisms that allow cancer cells to survive under drug pressure prior to generation of new genetic mutations may be feasible to overcome this uncertainty.

While genetic alterations account for drug response in some patients, clinical observations show that some patients regain responsiveness to the same therapy after a drug holiday, a period of deliberate pause in medication, even following relapse or progression during the initial treatment [2]. This phenomenon suggests that not all residual cancer cells result from genetic mutations; instead, they may arise from reversible, non-genetic mechanisms within tumours. Supporting this notion, Sharma and colleagues provided early preclinical evidence identifying a small population of lung cancer cells capable of surviving a lethal dose of anti-EGFR drugs [3]. These cells, termed drug-tolerant persister cells (DTPs), entered a dormant state, exhibiting temporary tolerance to the drug but regained drug sensitivity once the treatment was withdrawn. Following this pioneering study, the DTP phenotype has been observed across multiple cancer types in response to chemotherapy and targeted therapy [4]. Moreover, DTPs serve as a reservoir for the eventual development of stable genetic mutation if drug exposure persists (Figure 1) [5]. In this review, we will discuss the key characteristics, origins, and survival mechanism of DTPs, aiming to deepen our understanding of these cells and highlight the challenges they pose in cancer therapy.

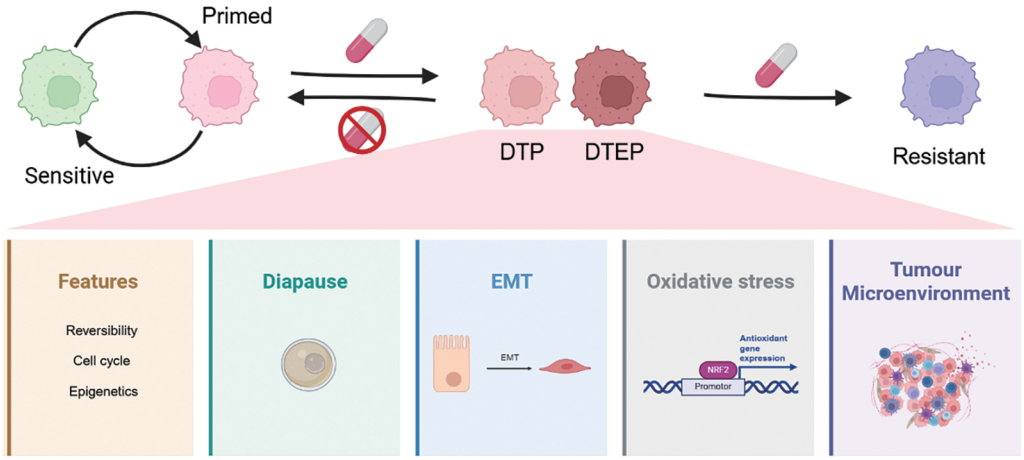


**Figure 1.** Drug-sensitive cancer cells transition through distinct stages, including primed cells, DTPs, cycling DTPs (also referred to as drug-tolerant expanded persister cells, DTEPs), and ultimate resistant cells. Key features of DTPs include their ability to revert to a sensitive state, undergo reversible cell cycle stagnation, and adapt epigenetically. The survival strategies of DTPs encompass embryonic diapause, epithelial-mesenchymal transition (EMT), oxidative stress regulation, and interactions with the tumour microenvironment. These adaptations enable DTPs to withstand therapeutic stress and drive cancer persistence and relapse. Created with BioRender.com.

## 2. Characteristics of DTPs

### *2.1. Reversibility and Non-Clonal Dependency*

Initially, it was hypothesised that the surviving cell population consisted of small drug-resistant clones

capable of withstanding drug selection, implying primary resistance with certain genetic mutations. However, subsequent evidence from three aspects, namely drug-response reversibility, nonclonal dependency and genetic consistency, supports that these cell population do not arise from the clones with genetic mutations.

Firstly, after drug withdrawal, DTPs can proliferate with a comparable doubling time and exhibit drug sensitivity akin to their untreated parental cells, as well as equivalent epigenetic, transcriptional, and translational profiles [3,6–9]. Secondly, the induction of the DTP state is not confined to specific clones but is reproducible from randomly established single clones across various cancer types [3,10]. Supporting this, single-cell barcode tracking has shown no enrichment of specific cell clones across repeated experiments [11]. Thirdly, whole-exome sequencing (WES) reveals no significant patterns of single nucleotide variations (SNVs) or known resistance-associated mutations occurring in DTPs [10,11]. A recent study further confirmed this finding using high-depth whole-genome sequencing (WGS) on breast cancer samples from the same patient and anatomic sites before and after treatment progression [12]. Collectively, these findings indicate that DTPs are not derived from pre-existing drug-resistant subpopulations with certain genetic mutations.

### *2.2. Cell Cycle Suspension and Progression*

DTPs exhibit reduced proliferation and frequently display G1/S phase arrest, described as "dormancy" or "quiescence" in certain contexts [12–15]. However, treatment with cell cycle inhibitors, such as CDK4/6 inhibitors and Aurora kinase inhibitors, fails to completely resemble the transcriptional profile of DTPs [10]. Specifically, while these inhibitors halt cell proliferation, they do not induce the broader features observed in DTPs, such as biosynthetic quiescence, suppression of redox stress, and upregulation of cell-ECM interaction pathways [10]. This highlights that proliferative quiescence alone is insufficient to account for the complex molecular adaptations associated with DTPs, which can emerge under drug stress regardless of prior cell cycle arrest. This suggests that DTPs harbour broader features beyond G1/S arrest. In line with this, DTPs show broad suppression of transcriptional and translational programmes [5,16]. Given the close link between cell cycle arrest and senescence, it is unsurprising that DTPs share some senescence-associated characteristics [9,17,18]. However, unlike senescent cells, DTPs have distinct drug vulnerabilities, underscoring that they are not merely senescent-like cells resulting from cell cycle arrest [19].

Under prolonged drug exposure, DTPs can even re-enter the cell cycle and resume proliferation without acquiring resistance-associated mutations, forming the proliferative drug-tolerant population termed drug-tolerant expanded persister cells (DTEPs) [3,15,20]. Although a small subset is within the dormant DTP population, DTEPs have been directly confirmed through single-cell division tracking [15]. Evidence suggests that DTEPs are awakened from dormant DTPs rather than arising from pre-existing proliferative heterogeneity. Barcoding tracking experiments have shown that the labels enriched in emerging DTEPs are not reproducible across repeated experiments [12], indicating that DTEPs do not originate from pre-existing clones with a proliferative advantage. Furthermore, upon drug removal, both cycling DTEPs and dormant DTPs regain parental drug sensitivity and exhibit similar cell doubling times [15], supporting the improbability of inherent proliferative heterogeneity. The emergence of cycling DTEPs within isogenic DTP populations [3] further supports this conclusion. Importantly, DTEPs can give rise to clinically relevant drug-resistant populations over long-term treatment [21].

Under drug pressure, DTPs sporadically attempt to re-enter the cell cycle; if unsuccessful, they may die or return to the persistent state [12]. These attempts might reflect non-genetic adaptive evolution during treatment. Consistent with this, the concept of a "resistance continuum" has been recently proposed, illustrating the gradual cellular adaptations in which prior drug exposure at lower doses enables the emergence of proliferative populations that increasingly tolerate higher doses [22]. Collectively, these findings highlight the dynamic and adaptive nature of DTPs in response to therapeutic stress.

### *2.3. Epigenetic Alteration*

The plasticity of DTPs, characterised by their ability to switch between drug-sensitive and drug-tolerant states, implicates the involvement of epigenetic mechanisms that reprogramme gene expression to enable cells to adapt to therapeutic stress. Indeed, DTPs exhibit a global decrease in acetylation of histone H3 lysine residues (H3KAc) and histone H3 lysine 4 trimethylation (H3K4me3), along with increased methylation of

histone H3 lysine 9 (H3K9) and histone H3 lysine 27 (H3K27) [3,23–25]. These modifications create a repressive chromatin environment, aligning with the broad transcriptional suppression seen in DTPs. Notably, targeting the enzymes responsible for these histone modifications significantly reduces DTP formation [3,23,25,26], suggesting that these chromatin changes causally drive the DTP phenotype rather than being passive consequences. These findings underscore the critical role of epigenetic reprogramming in the formation and maintenance of DTPs, highlighting that targeting epigenetic regulators could be a promising strategy to eliminate DTPs.

## 3. Ontogeny of DTPs and DTEPs

As previously discussed, if DTPs do not arise from specific resistant subclones**,** they may instead arise stochastically. Barcoded tracking approaches support this notion. DNA-barcode clonal tracking in triple-negative breast cancer (TNBC) cell lines transplanted into mice showed that chemotherapy-induced tumour persistence was not driven by any pre-existing clones, as no particular barcodes were enriched in residual tumours across individual mice [10]. Similar results were observed in patient-derived TNBC [6] and colorectal cancer (CRC) xenografts [11]. In HER2-positive breast cancer cell lines, barcode diversity persisted after HER2-targeted treatment, far exceeding the observed survival rate of residual cells [18]. Consistently, DTPs induced by anti-EGFR therapy in lung cancer maintain barcode diversity comparable to untreated controls [15], as did endocrine therapy-induced DTPs in estrogen receptor (ER)+ breast cancer cells [12]. Since each barcode represents an individual cell or its progeny, the retention of diverse barcodes suggests that drug tolerance arises randomly across the population.

These findings imply that DTP emergence is stochastic, ruling out the existence of heritable resistant subclones prior to drug treatment. However, this also raises the question of why some cells persist while others perish even in a genetically identical background. It is possible that, due to cellular plasticity and stochastic variations in gene expression, genetically identical cells can randomly shift to different cell states. Differential gene expression results in phenotypic diversity, stimulating varied responses to environmental stress without genetic mutations. This is demonstrated by the observation that well-differentiated luminal or basal TNBC cells can spontaneously develop into stem-like cells [27]. Subsequent studies have shown that stochastic gene and protein expression fluctuations may determine cell fate under drug exposure [16,28–31].

Certain transient expressions of markers such as EGFR, nerve growth factor receptor (NGFR), and AXL receptor tyrosine kinase have been observed in rare subsets of single-clone derived melanoma cells, conferring a survival advantage under BRAF-targeted therapy [28,32]. Similarly, melanoma cells expressing the H3K4-demethylase KDM5B demonstrate multidrug tolerance but can fully restore drug sensitivity upon drug withdrawal, reverting to a state similar to their parental cells [33, 34]. These transient expressions are stochastic, lacking stability or heritability, and do not represent pre-existing resistant subpopulations. Collectively, these observations suggest that stochastic gene expression fluctuations might temporarily provide cells with a survival advantage under therapeutic stress.

## 4. Surviving Mechanism and Vulnerabilities of DTPs

### *4.1. Evolutionarily Conserved Mechanisms in DTPs*

DTPs not only develop tolerance to the initial drug treatment but also exhibit cross-resistance to other drugs with different mechanisms of action, suggesting that the DTP state confers a broad resilience against various therapies [3,35]. Remarkably, non-drug stressors, such as hypoxia or nutrient starvation, can similarly induce multidrug resistance in melanoma cells [24], suggesting that cancer cells may employ evolutionarily conserved strategies to withstand diverse stresses.

One of the compelling parallels to the DTP state is embryonic diapause, a reversible and dormant state that embryonic cells adopt in response to environmental stress [10,11,36]. In DTPs, pathways associated with embryonic diapause, such as MYC and mTOR signalling, are downregulated. Notably, MYC suppression is functionally important for provoking the DTP state, as MYC inhibition alone can induce a diapause-like, drug-tolerant phenotype. Supporting this, inhibition of bromodomain containing 4 (BRD4), an upstream regulator of MYC, has been shown to enhance cancer cell persistence during drug treatment [10,19]. Interestingly, in oesophageal adenocarcinoma DTPs induced by anti-HER2 agents, transcription factors associated with early intestinal development, hepatocyte nuclear factor 4 alpha (HNF4A) and KLF

transcription factor 5 (KLF5), are enriched in the DTP state [8]. These findings indicate that DTPs may utilise conserved developmental programmes to withstand stress conditions.

Another critical survival mechanism contributing to the DTP phenotype is epithelial-mesenchymal transition (EMT), a process through which cells transit from an epithelial state to acquire mesenchymal characteristics [37]. EMT is frequently activated in DTPs across a variety of cancer types [9,15,38,39]. While the precise role of EMT in the DTP state is not fully understood, it likely contributes to cellular plasticity and resistance to apoptosis. For instance, in lung cancer DTPs treated with anti-EGFR inhibitors, EMT activation is evident, and exposure of EMT-inducing factor TGF-β confers temporary tolerance to EGFR inhibitors, mirroring the behaviour of DTPs [40]. Key regulators of the Hippo signalling pathway, Yes1 associated transcriptional regulator (YAP1) and TEA domain transcription factor (TEAD), drive the expression of the EMT-associated transcription factor SLUG, preventing drug-induced apoptosis in DTPs by repression of pro-apoptotic BMF [9]. Similarly, EMT signatures are highly activated in DTPs survived from pro-apoptotic BH3 mimetics, supporting the role of EMT in anti-apoptotic mechanisms [35]. Consistently, a study revealed that EMT mediates resistance to EGFR inhibitors in EGFR-mutant NSCLC by suppressing apoptosis through downregulation of BIM. Mechanistically, the EMT transcription factor ZEB1 directly represses BIM transcription by binding to its promoter, reducing BIM's pro-apoptotic activity and enabling survival despite EGFR inhibition. Notably, this EMT-BIM axis was also observed in KRAS-mutant NSCLC, indicating its broader significance in therapy resistance [41].

### *4.2. Anti-Oxidant Defence in DTPs*

Chemotherapy and targeted therapies can significantly increase oxidative stress in cancer cells by elevating reactive oxygen species (ROS), leading to widespread damage and cell death [42]. DTPs appear to rely heavily on antioxidant defences to survive oxidative stress. In prostate cancer DTPs induced by androgen receptor (AR) inhibitors, the antioxidant thioredoxin/peroxiredoxin pathway is upregulated, and inhibiting the antioxidant enzyme peroxiredoxin 5 (PRDX5) markedly reduces the number of DTPs [43]. Similarly, DTPs induced by EGFR-targeted and BRAF-targeted therapies show increased glutathione S-transferase (GST) activity, indicating activation of glutathione (GSH) pathways that mitigate ROS [44]. Supporting this, the addition of antioxidants like N-acetylcysteine (NAC) has been shown to promote cell survival during certain ROS-generating drug treatments [14,45]. These findings underscore the essential role of antioxidant mechanisms in DTP survival.

A key player in antioxidant defence is nuclear factor erythroid 2-related factor 2 (NFE2L2), also known as NRF2 [46]. NRF2 activation promotes the expression of genes involved in metabolic pathways, including GSH metabolism, pentose phosphate pathway (PPP) and glutamine metabolism [47]. GSH, a major antioxidant that counteracts cellular ROS, is regulated by NRF2, which transactivates key enzymes involved in GSH synthesis, including glutamate-cysteine ligase catalytic subunit (GCLC), glutamate-cysteine ligase modifier subunit (GCLM), and glutathione reductase (GSR). Although NRF2 has been extensively studied in the context of chemoresistance, its role in the DTP state is less well reported. Recent evidence identified NRF2 activation in a subpopulation of ovarian cancer cells capable of tolerating high-dose PARP inhibitors [22], suggesting a role in promoting cell survival. In line with this, our recent work found that NRF2 mediates the persistent adaptation of oesophageal adenocarcinoma cells to HER2 inhibition by increasing glutathione (GSH) levels [48]. Similarly, in multidrug-resistant (MDR) DTPs, NRF2 activation led to the upregulation of NPC1-like intracellular cholesterol transporter 1 (NPC1L1), which protects against chemotherapy by reducing oxidative stress [45]. Furthermore, NRF2 has been linked to the awakening of DTEPs during anti-EGFR treatment in lung cancer [15]. DTEP cells display heightened NRF2 activity, which upregulates antioxidant genes, enhancing their capacity to neutralise ROS. Consistently, increasing antioxidant capability, either by knocking out KEAP1 (a negative regulator of NRF2) or by adding the reducing agent NAC, increases the fraction of DTEPs. These findings suggest that NRF2 plays a crucial role in enabling DTEP cells to withstand therapeutic pressures by enhancing antioxidant defences. Targeting the NRF2 pathway could be a potential strategy to eliminate these resilient cancer cell populations and improve treatment outcomes. Collectively, these findings suggest that antioxidant mechanisms contribute to the survival of DTPs during drug treatment.

### *4.3. Vulnerabilities of DTPs: Ferroptosis and Beyond*

Ferroptosis is an iron-dependent form of cell death driven by lipid peroxidation, which leads to extensive membrane damage. Glutathione peroxidase 4 (GPX4) utilises GSH to reduce lipid peroxides to non-toxic substance, and thus inhibiting GPX4 with compounds like RSL3 and ML210 induces ferroptosis [49]. Hangauer et al. discovered that HER2+ breast cancer DTPs, induced by anti-HER2 agents, are highly susceptible to the ferroptosis inducer RSL3 due to reduced antioxidant gene expression and decreased GSH levels. This ferroptotic sensitivity has been extended to various cancer types, indicating a broad vulnerability of DTPs to ferroptosis [35,48,50].

The increased sensitivity of DTPs to ferroptosis might also be related to their EMT-associated properties. As discussed earlier, EMT activation is frequently observed in DTPs. The EMT-related transcription factor zinc finger E-box binding homeobox 1 (ZEB1) can shift cellular metabolism towards lipid dependency, increasing polyunsaturated fatty acids (PUFAs) in cell membranes and enhancing lipid peroxidation [51,52]. Elevated levels of ferrous iron in DTPs [13] further promote lipid peroxidation via the Fenton reaction, amplifying susceptibility to ferroptotic damage. Additionally, metabolic alterations in DTPs might further contribute to this vulnerability. A recent study demonstrated that shifting cells towards oxidative phosphorylation (OXPHOS) by inhibiting glycolysis sensitises CRCs to ferroptosis [53]. Since DTPs often rely on OXPHOS or a hybrid of OXPHOS and glycolysis [54], this metabolic shift might underlie their increased sensitivity to ferroptosis.

Beyond ferroptotic sensitivity, few recent studies have systematically identified other vulnerabilities in DTPs. Chen et al. found that inhibition of bromodomain-containing protein 2 (BRD2) disrupts antioxidant defences, effectively eliminating DTPs induced by anti-EGFR therapies [19]. Similarly, Criscione et al. reported that DTPs induced by anti-EGFR inhibitors are vulnerable to inhibition of Aurora Kinase B (AURKB) and TEAD [7]. The involvement of AURKB in dormant DTPs is intriguing, as AURKB is primarily known for its role in mitosis regulation, suggesting pleiotropic functions of AURKB in regulating DTP survival beyond cell division [55]. TEAD, a critical component of the Hippo pathway, aligns with previous findings supporting its crucial role in DTP survival under drug pressure by preventing apoptosis [9]. Additionally, Dhimolea et al. found that cyclin-dependent kinase 9 (CDK9) inhibition sensitises DTPs to chemotherapy [10]. Although the precise mechanism is not fully understood, CDK9 is thought to promote transcriptional elongation, potentially contributing to DTP resilience through gene regulation [56].

### *4.4. DTPs and Tumour Microenvironment*

The tumour microenvironment (TME) plays a pivotal role in shaping cancer adaptation and fostering drug resistance. As a dynamic network of stromal cells, immune infiltrates, extracellular matrix (ECM), and soluble factors, the TME could promote intratumoural heterogeneity, metabolic reprogramming, and phenotypic plasticity, all of which contribute to the emergence and persistence of DTPs [57,58]. These selective pressures shape the adaptive behaviour of cancer cells, enabling flexibility critical for DTP survival under therapeutic stress.

Within the TME, hypoxia, nutrient deprivation, and acidosis drive metabolic reprogramming in cancer cells, allowing DTPs to rely on OXPHOS or glycolytic pathways for survival. This metabolic adaptability allows DTPs to conserve energy and mitigate oxidative damage in response to stress, enhancing their resilience and ability to endure prolonged drug exposure. Cancer-associated fibroblasts (CAFs), one of the primary stromal components, further enhance this adaptability by secreting metabolites like lactate and pyruvate, which serve as alternative energy sources and sustain cancer cell metabolism even in nutrient-deprived environments [59,60].

Inflammation within TME also plays a critical role in regulating DTPs. CAF subpopulations undergo phenotypic switches from myCAFs to inflammatory CAFs that activate signaling pathways such as NF-κB and IL-6/JAK/STAT3 signaling, promoting DTP formation [61]. Additionally, the immunosuppressive nature of the TME provides a sanctuary for DTPs. Regulatory T cells, myeloid-derived suppressor cells (MDSCs), and tumour-associated macrophages (TAMs) infiltrate the tumour site and secrete anti-inflammatory cytokines, such as IL-10 and TGF-β, which suppress anti-tumour immunity and create an environment conducive to DTP survival. These factors not only reduce immune surveillance but also interfere with T-cell

activation, further insulating DTPs from immune-mediated destruction [62,63]. Furthermore, DTPs can actively modulate immune responses by upregulating immune checkpoint ligands like PD-L1, effectively silencing cytotoxic T-cell activity and promoting immune evasion. This immunomodulatory feedback loop strengthens the immune-privileged niche in the TME, facilitating DTP persistence.

In addition, the ECM undergoes significant remodelling within the TME, driven primarily by CAFs and other stromal elements. Increased stiffness resulting from excessive deposition of collagen and fibronectin activates integrin-mediated survival pathways, including the FAK-Src and ERK/MAPK cascades, which promote drug resistance and DTP persistence [34,64]. These ECM changes create not only a physical barrier to drug penetration but also biochemical signalling hubs that reinforce cell survival by activating pro-survival pathways. The ECM also serves as a reservoir for growth factors, such as VEGF and TGF-β, that further enhance tumour cell resilience. By creating localized protective niches, the ECM limits therapeutic efficacy and supports DTP survival. Additionally, drug stress amplifies these effects by inducing a pro-survival secretome within the TME, comprising cytokines, growth factors, and extracellular vesicles. These secreted factors reinforce survival pathways, enhance the proliferation of resistant clones, and sustain the activation of critical signalling axes such as PI3K/AKT/mTOR [63, 65]. This dynamic crosstalk between the TME and cancer cells not only protects existing DTPs but also promotes the emergence of resistant clones, driving relapse and long-term therapeutic failure. Together, these interactions underscore the central role of the TME in supporting the persistence of DTPs and fostering the emergence of drug-resistant populations.

### *4.5. Challenges in Treating DTPs*

Despite significant progress in understanding DTP biology, several challenges impede the development of targeted therapies. The heterogeneity and dynamic behaviour of DTP populations under varying conditions complicate the identification of specific vulnerabilities. Additionally, many DTP mechanisms, such as biosynthetic quiescence and epigenetic plasticity, overlap with essential processes in normal cells, raising concerns about safety and side effects. The majority of current studies focus solely on the intrinsic mechanisms of DTPs, making the translation of preclinical insights into clinical applications more challenging due to the context-dependent behaviours of DTPs within the tumour microenvironment. Accordingly, advancing experimental models and integrative approaches holds promise for bridging this gap and enhancing therapeutic strategies.

## 5. Concluding Remarks

As research into drug-tolerant persister cells (DTPs) advances, several fundamental questions remain unanswered. For instance, what triggers the transition from dormant DTPs to cycling DTEPs, and are these transitions synchronized across cells or entirely random? Do disseminated dormant cancer cells in distant organs and DTPs share parallel awakening mechanisms? When a dormant cell re-enters a cycling state, does it promote awakenings in neighbouring cells, or does it simply outcompete them? Additionally, post-transcriptional and post-translational modifications warrant further investigation, as mechanisms involving RNA processing and protein modifications are less understood than DNA modifications in DTPs. Mathematical modelling has emerged as a powerful tool to address some of these complexities [66]. By simulating the stochastic and dynamic behaviour of DTPs, mathematical models offer a quantitative framework to study their population dynamics, transitions between states, and responses to therapeutic interventions. For instance, mathematical frameworks have helped clarify the balance between dormancy, cycling, and death under drug pressure, offering predictive insights that are difficult to achieve through experiments alone [67,68]. These models complement experimental data, providing insights into the mechanisms driving DTP survival and guiding the optimisation of treatment strategies to prevent relapse and resistance.

In summary, this review underscores the complexities of DTP biology, including their characteristics, origins, and vulnerabilities. Looking forward, integrating approaches that target metabolism, screen for vulnerabilities, modulate epigenetic states and leverage quantitative modelling holds promise for overcoming therapeutic resistance and improving clinical outcomes.

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original draft preparation; W. Z.: visualization, investigation; J. Z.: supervision; H. C.; M. M.: writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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