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Review

Targeting Drug-Tolerant Persister Cells in Cancer: Progress From Modeling to Targeting Fragility Studies

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Abstract

Drug-tolerant persister (DTP) cells are a major cause of cancer treatment failure and tumor relapse. This review provides a systematic summary of recent advances in research on DTP cells in cancer, including their biological features, model systems, and targeting strategies. Current studies show that formation of DTP cells is a complex and dynamic process, involving cell cycle arrest, epigenetic remodeling, and extensive transcriptional and metabolic reprogramming. The drug tolerance of DTP cells arises from a cooperative molecular network that integrates metabolic adaptation, reactivation of key signaling pathways, and resistance to cell death programs. To investigate the DTP state, researchers have developed a variety of *in vitro* and *in vivo* models, including patient-derived xenograft (PDX) models, drug-treated cancer cell lines, and organoid systems. These models have become essential tools for identifying vulnerabilities in DTP cells, such as sensitivity to ferroptosis induction, dependence on specific metabolic pathways, and expression of distinct surface antigens. However, the field still faces important challenges. Existing models have limitations in mimicking the complex tumor microenvironment and in capturing the transient nature of DTP cells. In addition, the clinical value of targeting strategies identified with experimental models remains to be validated in large-scale trials. The high level of heterogeneity of DTP cells and the lack of standardized research protocols also hinder the comparison and integration of findings across studies. The future development of more physiologically relevant models, the use of advanced technologies to better characterize DTP cells, and the design of clinical trials aimed at eliminating DTP cells are critical steps in the transition from tumor control to durable cure.

Keywords: DTP cells; models; vulnerabilities; targeting

1. Introduction

A durable response refers to the sustained therapeutic effect of tumor regression or stable disease after antitumor treatment. It serves as a core metric for assessing the clinical value of treatment. Durable responses to cancer therapy are often disrupted by the emergence of drug-tolerant persister (DTP) cells. These cells can survive the pressure of targeted therapy or chemotherapy by entering a reversible slow-cycling or quiescent state. They later serve as a source of acquired resistance and tumor relapse [1]. The generation of DTP cells is not driven by a single mechanism. Two main hypotheses have been proposed: clonal selection and drug-induced adaptation [2]. Maintenance of the drug-tolerant phenotype depends on a complex and cooperative network involving epigenetic remodeling, metabolic reprogramming, reactivation of key signaling pathways, and resistance to programmed cell death. Additionally, resistance is driven by permanent genetic and epigenetic alterations in tumor cells, with a slow onset mediated by the accumulation of mutations and clonal selection. This results in a complete and irreversible loss of cellular drug sensitivity, ultimately leading to treatment failure and the need to switch the therapeutic regimen. In contrast, tolerance is a reversible phenotypic adaptation of tumor cells that is independent of genetic mutations and characterized by rapid emergence within days. It induces transient cellular insensitivity to drugs and is closely associated with the development of residual post-treatment disease, thereby predisposing tumors to subsequent recurrence.

The drug-tolerant phenotype is a plastic and reversible adaptive state. Under long-term drug selection pressure, tolerant cells may acquire genomic mutations and further convert to a state of permanent drug resistance, which is a key step in the development of clinically acquired drug resistance [3]. The drug-tolerant epigenetic phenotype (DTEP) is a non-mutational and reversible drug-tolerant state implicated in cancer chemoresistance. It is fundamentally distinct from classical mutation-driven drug resistance. DTP cells are the cellular basis of DTEP, and are predominantly dormant or slow-cycling subpopulations

that survive initial drug treatment. Notably, a subset of dormant DTPs can be activated to develop into drug-tolerant expanded persisters (DTEPs). These cells retain the capacity to proliferate even under sustained drug pressure. Due to their preserved phenotypic plasticity, they are able to revert to a drug-sensitive state following drug withdrawal [1].

To better understand this dynamic and heterogeneous cell state, researchers have developed diverse model systems, including patient-derived xenograft (PDX) models, drug-treated cancer cell lines, and organoid models. These systems are not only essential for defining the biological features of DTP cells, but also serve as key platforms for identifying their vulnerabilities. Based on findings from these models, new targeting strategies are being actively explored, such as interfering with metabolic dependencies (e.g., sensitivity to ferroptosis) [4], targeting epigenetic regulatory nodes, and cell death pathways. The goal of these approaches is to eliminate residual disease at its source and prevent tumor recurrence.

Despite these advances, the field still faces major challenges. Current models have inherent limitations in reproducing the tumor microenvironment (TME), especially the immune components, and in capturing the transient dynamics of the DTP state. There is also a large gap between results from preclinical models and therapeutic benefit in patients. In addition, the high heterogeneity of DTP cells and the lack of standardized research protocols hinder the development and evaluation of targeting strategies. This review systematically summarizes the biological features of DTP cells, the methods used to model them, recent progress in vulnerability-based targeting strategies, and an analysis of current limitations. The aim is to provide a theoretical framework to inform future research aimed at developing more effective and durable cancer treatments.

2. Chapter 1: Biological Characteristics and Functional Analysis of DTP Cells

2.1 Formation of DTP Cells

The origin of DTP cells is still debated, with two hypotheses proposed (see Fig. 1). The clonal selection hypothesis posits that a subpopulation of cancer cells with intrinsic DTP traits is already present in untreated tumors and is selectively preserved and clonally expanded upon drug exposure. This phenotypic state of DTPs arises from a stochastic gene expression model, whereby a subset of cells transiently activates specific transcriptional programs to establish a pre-existing drug-tolerant state. This mechanism also accounts for the phenotypic and functional heterogeneity observed in these cells, even when they share an identical genetic background. Such drug-tolerant cells are ultimately selected for survival during drug treatment [5,6]. However, a critical question arises: how is the pre-existence of subpopulations, as posited by the clonal selection hypothesis, compatible with the well-established reversibility of the DTP state? Recent single-cell lineage-tracing studies have provided key mechanistic insights. For example, Sharma et al. (2010) [1] first identified a chromatin-mediated, reversible drug-tolerant state in cancer cell subpopulations. This tolerant state is not dependent on stable genetic alterations, but is achieved through reversible epigenetic remodeling, such as changes in histone methylation. Importantly, subsequent studies employing DNA barcode-based lineage tracing have confirmed that under drug pressure, not all pre-existing clones with tolerant potential survive. Moreover, the progeny of those that do survive can regain drug sensitivity and revert to a non-tolerant phenotype upon drug withdrawal [7]. Thus, clonal selection does not favor permanently resistant clones, but rather selects for cellular subpopulations capable of rapidly activating plastic transcriptional programs to achieve transient tolerance under specific microenvironmental or pharmacological pressures. This reversible adaptive capacity may itself be an intrinsic, heterogeneous trait of tumor cells that can be enriched through clonal selection.

The second hypothesis, known as the drug-induced hypothesis, proposes that DTP cells arise from drug-sensitive cells after short-term drug exposure, ranging from a few days to weeks [8]. Induced transformation refers to the process in which the drug resistance of cancer cells is enhanced through a cellular stress-induced mutagenesis response (SOS)

and stress-induced mutagenesis mechanism under external pressures and drug action, with DTP cells considered a significant source of drug resistance [2,9].

The mechanisms by which cancer cells develop into DTP cells are complex and multifaceted, involving molecular modification, regulation of signaling and transcriptional pathways, remodeling of the TME, metabolic reprogramming, and redox regulation. These mechanisms do not operate in isolation, nor do they function under exclusive or singular conditions. In most cases, once cancer cells enter the DTP state, multiple mechanisms work together. One mechanism can strengthen another, leading to sustained drug resistance of DTP cells and making them difficult to treat and eliminate [3]. DTP cells are derived from drug-sensitive cell populations and develop after short-term drug exposure. This process is accompanied by complex cell cycle dynamics, with most cells showing arrest in the G1 phase. A small fraction remains in the S phase, displaying cell cycle diversity [10]. It typically involves efficient cell cycle exit to form a reversible drug-tolerant state dependent on CDKN1B (p27/Kip1), the stabilization of which is driven by AKT-mediated phosphorylation [11]. This molecular axis raises a critical question: is cell cycle arrest the initiator or a consequence of DTP induction? Recent evidence indicates that cell cycle arrest may be an initiating trigger for DTP formation. AKT-stabilized CDKN1B forces G1 arrest and restricts endoreduplication in patient-derived circulating tumor cells, which is required to initiate the reversible drug-tolerant

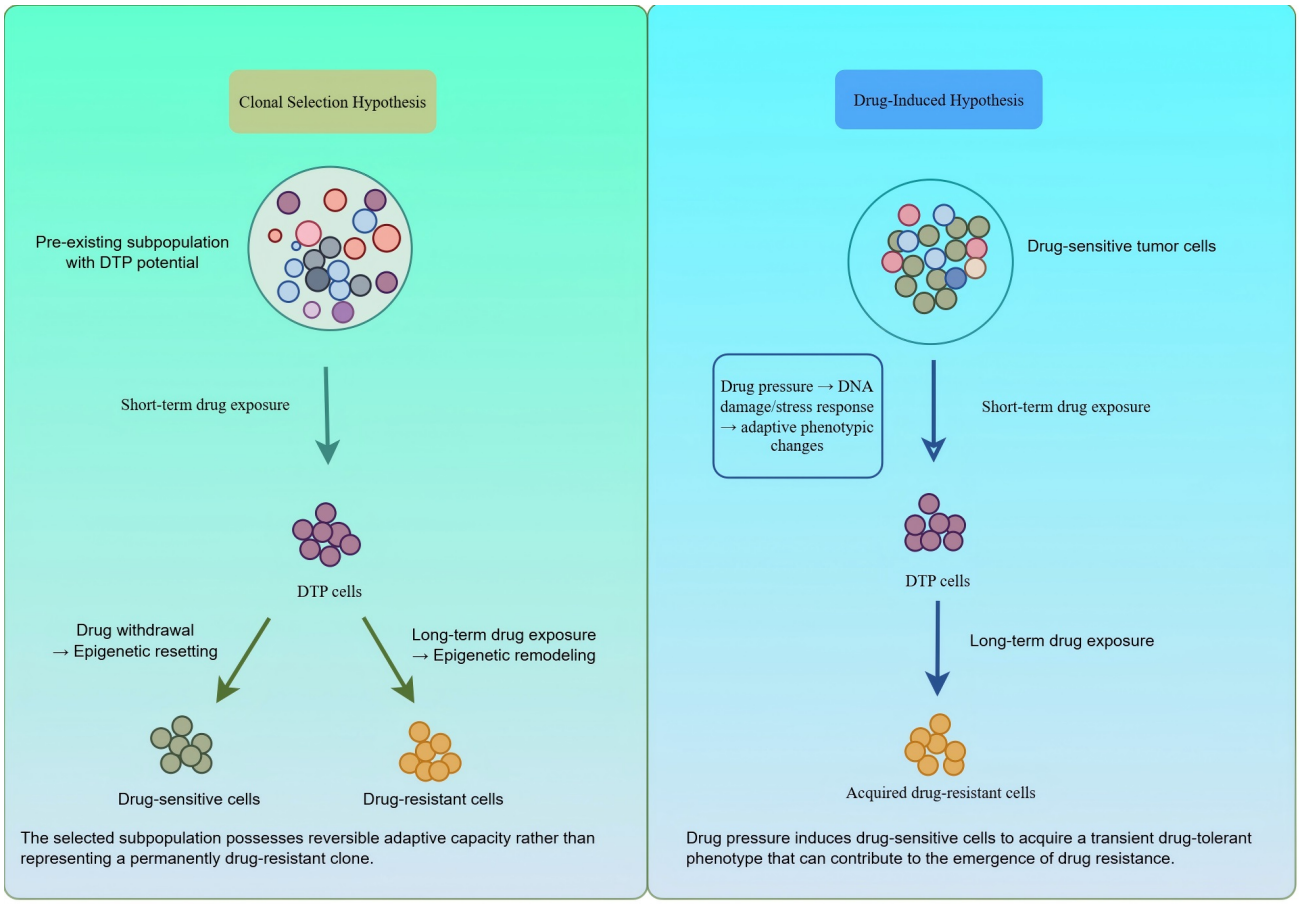

Fig. 1. Schematic diagram illustrating two postulated origins of drug-tolerant persister (DTP) cells. The Clonal Selection Hypothesis proposes selection of a pre-existing, epigenetically plastic subpopulation. The Drug-Induced Hypothesis posits adaptive transformation of sensitive cells under stress. Both paths lead to a reversible, drug-tolerant state. Note: this figure was created with FigDraw (www.figdraw.com).

state upon exposure to mitotic inhibitor [11]. This arrest creates a quiescent state that enables activation of adaptive tolerance programs, with cell cycle diversity (G1 arrest vs. S phase retention) foreshadowing formation of the DTP subpopulation under drug pressure [12]. Conversely, the phenotypic plasticity of DTPs also implies cell cycle arrest as a downstream consequence: drug-induced stochastic activation of tolerance-related transcriptional programs may secondarily drive AKT/CDKN1B-mediated cell cycle exit to reinforce drug insensitivity [12], locking in the tolerant state by reducing proliferative vulnerability. Collectively, these findings point to bidirectional crosstalk, where CDKN1B-dependent cell cycle arrest acts as both an early priming event for DTP induction, as well as a late adaptive mechanism to sustain the drug-tolerant phenotype [11,12].

At the molecular level, epigenetic adaptation is widely recognized as a core survival mechanism underlying emergence of the DTP state [12]. Early chromatin remodeling events are characterized by coordinated changes in histone modifications, including increased repressive marks such as histone H3 lysine 9 trimethylation (H3K9me3) and histone H3 lysine 27 trimethylation (H3K27me3), as well as dynamic regulation of histone acetylation states [12]. These changes are mediated by multiple epigenetic enzymes, including histone methyltransferases (e.g., G9a/EHMT2, enhancer of zeste homolog 2 [EZH2]), histone deacetylases (HDACs), and chromatin-remodeling complexes such as switch/sucrose non-fermentable (SWI/SNF), which collectively reshape chromatin accessibility and transcriptional potential [13]. As a consequence, DTP cells undergo extensive transcriptional reprogramming, marked by the suppression of cell-cycle progression and DNA replication programs, alongside activation of stress response, cell communication, and cytokine signaling pathways [12]. Importantly, these chromatin state transitions ultimately converge on the activation of specific transcription factor networks that function as downstream effectors of epigenetic remodeling. Transcription factors such as activator protein 1 (AP-1), interferon regulatory factor/signal transducer and activator of transcription (IRF/STAT), and nuclear factor kappa-B (NF-κB) are preferentially recruited to newly accessible or reprogrammed enhancers, thereby reinforcing the DTP

transcriptional profile and stabilizing the tolerant phenotype [14]. FOSL1 promotes the transition of cancer cells to the persister state by binding to genomic enhancers and orchestrating the transcriptional reprogramming of cancer cells [15]. Moreover, ATF4 plays a critical role in maintaining DTP survival.

Additionally, stress response mechanisms (e.g., upregulation of ATF3 expression) and partial epithelial-mesenchymal transition (EMT) are associated with the formation of more invasive DTP cell subpopulations [16]. Notably, ATF3 was recently implicated in the transition from dormant DTPs to proliferating DTEPs [14]. Moreover, ATF4 plays a critical role in maintaining DTP survival [17]. In terms of cell phenotype, DTP cells often exhibit morphological changes such as cytoplasmic expansion and enhanced mitochondrial biosynthesis [18]. Specific protective mechanisms are also activated to support establishment of the DTP state, such as PINK1-mediated mitophagy, which is crucial for maintaining mitochondrial homeostasis in DTP cells [19]. In EGFR-mutant lung cancer, EGFR inhibition rapidly induces APOBEC3 expression and activity, promoting the survival of DTP cells and driving their subsequent evolution through genetic variation [20]. Additionally, certain signaling pathways, such as Notch activation, have been associated with osimertinib-induced DTP states [21]. The evolutionarily conserved mechanism of diapause was found to regulate the survival of DTP cells. Tumor cells can exploit this mechanism to enter a reversible DTP state, thereby evading therapeutic elimination and driving cancer relapse. Studies using colorectal cancer models have confirmed that all tumor cells possess an equal potential to adopt the DTP state, and that DTPs exhibit high transcriptional and functional similarities to diapause. The discovery of a conserved developmental mechanism provides a key rationale for developing novel therapeutic strategies that target DTPs [22].

2.2 Molecular Network of Drug Resistance Mechanisms

Studies have shown that persistent tumor cells are characterized by slow or arrested growth, highly flexible cellular metabolism, highly plastic cellular phenotypes, adaptability to the tumor immune microenvironment, resistance to apoptosis, and sensitivity to ferroptosis. These features make them important drivers of tumor relapse and treatment resistance [23]. At the same time, research into the mechanisms of drug resistance in these cells has identified several contributing factors. During the formation of DTP cells, most cells exhibit cell cycle arrest, while others are in the S phase. This diversity of the cell cycle continues during subsequent stages of formation [10]. Additionally, DTP cells use various molecular mechanisms to achieve drug resistance, laying the foundation for residual disease after targeted therapy and chemotherapy. The key feature is entry into a reversible dormant or slow-cycling state, characterized by metabolic reprogramming, enhanced DNA repair capabilities, and altered immune evasion mechanisms, thereby avoiding clearance by different therapies [24]. At the metabolic level, DTP cells exhibit significant adaptability. For example, in lung cancer, DTP cells switch to oxidative phosphorylation and upregulate fatty acid metabolism, with DPP4 promoting fatty acid uptake and oxidation via CPT1A, and maintaining mitochondrial function through the MEK-Nrf2 pathway [25]. This also explains how rewired transcriptional networks and altered metabolism can promote the survival of DTPs. For instance, in gastroesophageal cancer, NRF2 activation is essential for the formation of DTPs induced by anti-HER2 agents, and NRF2-mediated glutathione metabolism serves as a core mechanism for DTP generation [26]. Similarly, inhibition of NPC1L1, a direct downstream target of NRF2, disrupts the adaptive response of DTPs to chemotherapy [27]. In colorectal cancer, overexpression of LPIN1 promotes lipid droplet formation to sequester free fatty acids. Inhibition of this process can induce ferroptosis [28]. Similarly, reprogramming of lipid metabolism mediated by the FABP4/UCP2 axis helps cancer cells survive in a dormant state, contributing to resistance to cetuximab [29]. In glioblastoma, the super-enhancer-driven PPP1R15B/EIF2A axis leads to temozolomide resistance by maintaining oxidative phosphorylation [30].

Reconnection and abnormal activation of signaling pathways are another key mechanism. Although EGFR signaling in osimertinib-treated lung cancer DTP cells is initially blocked, ribosomal synthesis and protein translation-related pathways are upregulated. Moreover, EGFR downstream pathways, along with anti-apoptotic mechanisms such as YAP1 and mTOR-BAD, are reactivated. Changes in kinase networks are also crucial. For example, increased phosphorylation of CDK1 substrates and their mediation of SAMHD1 activation are essential for the growth and survival of DTP cells [31]. In breast cancer, proliferative DTP cells undergo a shift in the EGFR-Src family kinase axis, where EGFR expression is downregulated while Src kinases like Lyn and Fyn are upregulated. This creates a hyperphosphorylation state that over-activates downstream pathways such as STAT3, AKT, and MAPK [32]. Key survival regulators such as YAP are activated in DTP cells from various cancer types (e.g., lung cancer, gastrointestinal stromal tumor [GIST]), with increased nuclear localization promoting survival under drug pressure [33]. Proliferative DTPs upregulate antioxidant pathways, such as NRF2-mediated glutathione metabolism, and undergo metabolic reprogramming, including enhanced fatty acid oxidation (FAO). Collectively, these mechanisms enhance the resistance of DTPs to ferroptosis. As a key ferroptosis suppressor, FSP1 further strengthens this protective effect by maintaining lipid redox homeostasis in proliferative DTPs [34]. Sensitivity to ferroptosis is a hallmark of quiescent DTPs, stemming from their glutathione depletion and dependence on GPX4. Due to the lack of survival programs involving

FSP1 upregulation, non-proliferative DTPs remain vulnerable to ferroptosis inducers [8]. Certain secreted factors, such as GDF15, are crucial for DTP cell survival, and targeting them can enhance therapeutic responses [35]. From an evolutionary perspective, the DTP state, as a precursor to acquired resistance, has inherent genetic heterogeneity and plasticity that contribute significantly to the emergence of drug-resistant clones [20].

2.3 Knowledge Gaps and Unresolved Issues

Many unanswered questions remain regarding DTP cells, and the mechanisms supporting the existence of these cells are still not fully understood. Moreover, the specific molecular processes that drive DTP cell formation, metabolic reprogramming (such as lipid metabolism), evolution (such as APOBEC activity), and transition to acquired drug resistance in different cancer types have yet to be clearly defined [19,20,21,28]. In addition, there are major limitations related to research models and sample accessibility. The difficulty in obtaining DTP cells directly from patients restricts study of their *in vivo* behavior and driving mechanisms [15]. In addition, the lack of cell models that truly reflect clinically relevant residual disease and capture molecular heterogeneity hinders the understanding of DTP cell proliferation mechanisms in specific cancer subtypes, such as triple-negative breast cancer (TNBC) [32]. Furthermore, DTP cells represent a highly heterogeneous population, and their precise characteristics and functional diversity among such subpopulations remain unclear. This complexity creates significant challenges for the accurate identification and effective targeting of DTP cells [10].

DTP cells do not survive by having a single gene mutation or an isolated signaling pathway. Instead, they adapt to drug pressure through coordinated changes at multiple levels, including metabolic reprogramming, dynamic remodeling of signaling pathways, and fine control of cell death programs. Together, these mechanisms drive DTP cells into a highly plastic, reversible, and survival-advantaged state, enabling them to persist for long periods and ultimately fuel tumor relapse. At the same time, molecular descriptions alone are insufficient to fully capture the biological nature of DTP cells. Consequently, in-depth investigation of DTP cells requires reliable and clinically relevant model systems that can reproducibly recapitulate their formation, maintenance, and progression toward acquired resistance under controlled conditions. Additionally, multi-level validation strategies are needed to reveal their key vulnerabilities. Consequently, Chapter 2 focuses on the construction and validation of DTP cell models. This chapter systematically introduces *in vitro* and *in vivo* models, patient-derived systems, and their associated functional, mechanistic, and technical validation approaches, thereby providing an experimental and methodological foundation for subsequent therapeutic development.

3. Chapter 2: Model Construction and Validation Systems for DTP Cells

Several complementary *in vitro* and *in vivo* models have been constructed to investigate DTP cells in cancer, and a variety of validation approaches have been used.

3.1 Construction of In Vitro and In Vivo Models for DTP Cells

Researchers have developed diverse model systems to simulate the formation, maintenance, and targeted intervention of DTP cells in different cancer types.

Drug treatment-induced models are a common strategy for constructing DTP models in various solid tumor types. For instance, in TNBC, taxane drugs can induce drug-tolerant cells *in vivo*, which are then used to evaluate the efficacy of nanomedicines [36]. DTP cells in GIST can be generated *in vitro* by treating imatinib-sensitive cell lines [33]. In non-small cell lung cancer, osimertinib-treated cell lines and PDX models are widely used to assess targeted strategies [37]. Resistance and relapse models have also been constructed for specific treatment scenarios. In glioblastoma, multiple rounds of irradiation are used to engineer radioresistant cells from stem cells, thereby simulating recurrence after radiotherapy [38]. In high-grade serous ovarian cancer (HGSOC), isogenic clones of treatment-naive and cisplatin-resistant persister cells are generated to simulate the persistent cell state [16]. Repeated carboplatin treatment of ovarian cancer models, both *in vivo* and *in vitro*, can successfully induce and enrich cell populations with platinum-tolerant features [39]. It is important to note that *in vitro* DTP cell models induced by short-term, high-concentration drug exposure primarily mimic the reversible drug-tolerant state that emerges during the early stages of treatment. These models are characterized by the ability of cells to resume proliferation and regain drug sensitivity following drug withdrawal [1]. However, when continuous therapeutic pressure is applied to *in vivo* models, these initial DTP cell populations may serve as evolutionary precursors for the acquisition of stable drug-resistant mutations, thereby driving the evolution of tumors into irreversible drug-resistant clones [40].

To reliably generate and study DTP cells, Szebényi et al. [41] developed a standardized *in vitro* model using short-term, high-dose (IC_{30}) exposure of breast cancer cell lines (MCF7, T47D, MDAMB468) to chemotherapeutics such as doxorubicin or olaparib for five days. This treatment recapitulates the acute therapy response, with most cells dying. However, surviving cells enter a DTP state marked by G1 arrest, DNA damage, EMT-like morphology, and transient P-gp upregulation in a subpopulation. Following drug withdrawal, DTPs remain quiescent for weeks before reversibly resuming proliferation and fully recovering their drug sensitivity. This reproducible pulse-withdrawal-recovery system, with well-defined phenotypic transitions, enables detailed analysis of DTP dynamics. Szebényi et al.

[41] further validated the role of DTPs in residual disease and acquired resistance *in vivo* using a Brca1$^{-/-}$p53$^{-/-}$ PDX mouse model that underwent longitudinal treatment. Collectively, this integrated platform supports the systematic investigation of DTP biology and testing of targeted strategies, such as extended P-gp inhibition during drug holidays [41].

PDXs are crucial for enhancing the clinical relevance of experimental studies. Temozolomide-resistant persister cells in glioblastoma can be obtained directly from patient-derived organoid cultures. In addition, PDX models from high-grade ovarian cancer have been used to generate tumors that are either carboplatin-sensitive or intrinsically resistant. Through longitudinal treatment, sampling, and relapse tracking, these models faithfully recapitulate the full disease course from initial response, through tumor residual disease, to the development of acquired resistance [42]. In pancreatic cancer, organoid models established from patient samples before and after chemotherapy were used to identify specific drug-tolerant phenotypes [43]. PDX models of melanoma were used to simulate the minimal residual disease (MRD) stage after targeted therapy [44]. Review articles have also highlighted that PDX models of EGFR-mutant lung cancer are key *in vivo* platforms for studying persistence mechanisms after osimertinib treatment [45]. Furthermore, a detailed protocol was recently reported for generating DTP cells *in vivo* through long-term erlotinib treatment using EGFR-mutant lung adenocarcinoma PDX models [46].

The value of PDX and organoid models lies in their ability to recapitulate the continuous progression of clinical drug resistance. Through parallel modeling and longitudinal tracking of samples collected at the pre-treatment stage, and from residual and recurrent lesions post-treatment, two scenarios are clearly distinguished. The first is MRD driven primarily by the reversible DTP state and characterized by the potential for tumor resensitization following drug withdrawal. The second is acquired drug resistance driven by de novo genetic mutations and where the tumor no longer responds to the original regimen, even after drug withdrawal [47]. For example, in PDX models of EGFR-mutant lung cancer, initial residual tumors following long-term erlotinib treatment typically represent the DTP state, whereas subsequent recurrent tumors often harbor drug-resistant mutations such as T790M [34].

Intervention models aimed at eradicating or exploiting the DTP state are also being developed. In mouse models of pancreatic ductal adenocarcinoma (PDAC), a combination of RAS inhibitors and CDK4/6 inhibitors can drive drug-tolerant cells into a senescence-like state [48]. In a study of GIST, an *in vitro* model was constructed to test ferroptosis inducers by analyzing the metabolic changes in imatinib-derived persister cells [49]. Interestingly, these methodologies have been extended to the field of bacterial infections, where a general approach has been developed to analyze the dynamics of persistent bacterial cell populations in mouse models [50]. In summary, current research models for DTP cells have developed into a diversified system, ranging from *in vitro* induction of drug sensitivity and engineered construction, to patient-derived organoid and PDX models, and assessment of *in vivo* intervention. These models provide key tools for uncovering the biological characteristics of DTP cells and for testing novel targeted strategies.

3.2 Validation Systems for DTP Cells

To clarify the biological characteristics of DTP cells and validate the reliability of related models, researchers have established a multi-layered and integrated validation system. This can be summarized into two core aspects: phenotypic validation and molecular marker profiling.

3.2.1 Phenotypic Validation of the DTP State

Functional validation serves as the cornerstone for assessing the DTP state. Key phenotypic hallmarks include proliferation arrest, apoptosis resistance, and cross-tolerance, which are assessed by testing sensitivity to specific inhibitors (e.g., YAP inhibitors) [33]. To evaluate the sensitivity of DTP cells to inhibitors that target key nodes like YAP, studies in this field rely primarily on standardized, *in vitro* viability-based assays. High-content microscopy screening serves as a core methodology. It utilizes automated imaging to quantitatively analyze post-treatment cell confluence (representing proliferation/survival) and apoptosis rates, thereby allowing direct assessment of the impact of inhibitors on DTP cell viability (e.g., testing of the TEAD inhibitor VT-104). The CellTiter-Glo 3D Cell Viability Assay is commonly employed to quantify drug responses in patient-derived organoids (PDOs), as three-dimensional models can better recapitulate clinical samples. A critical experimental strategy is to include a comparative design that tests the same inhibitor in parallel against parental cells, DTPs, and acquired resistance cells. This enables assessment of selective or enhanced efficacy specifically against the DTP state, providing an essential *in vitro* pharmacodynamic rationale for subsequent *in vivo* combination therapy studies [33].

In drug-induced models, tolerant cells exhibit stem-like functional traits, such as enrichment of ALDH-positive populations and enhanced sphere-forming capacity [39]. Advanced assessments of dormancy or slow-cycling characteristics are conducted through monitoring of tumor volume, cell-cycle analysis, and fluorescent ubiquitination-based cell cycle indicator (FUCCI) systems. Furthermore, specific vulnerabilities can be exploited for validation. For example, sensitivity to ferroptosis has been identified as a hallmark of certain quiescent DTP populations. Gene expression profiles of residual tumor cells consistently show cell-cycle suppression alongside the upregulation of specific survival programs, providing a molecular basis for their quiescent and tolerant behavior [42]. Importantly, re-

versibility of the DTP state upon drug withdrawal is a critical criterion, distinguishing it from permanent genetic resistance.

3.2.2 Profiling of Molecular Markers in DTPs

Molecular profiling across cancer models reveals diverse, context-dependent markers of DTPs that can be functionally categorized. However, no universal DTP marker has yet been reported, and phenotypic validation therefore remains essential. Key examples include the upregulation of cell-surface receptors such as FZD7, which activates survival pathways like Tp63 and glutathione metabolism (e.g., GPX4) [39]. Other examples include regulatory proteins like cIAP2 [44], and super-enhancer-associated signaling axes (e.g., PPP1R15B/EIF2A) [30]. DTPs also exhibit metabolic reprogramming, such as suppressed glucose metabolism [49], transcriptional/epigenetic features including enhanced interferon responses, EMT [16,42,46], and elevated expression of proteins like CEACAM6, CRYAB, and SOX2 [42], alongside broader chromatin state alterations.

In summary, this integrated validation system combines functional characterization, mechanistic analysis, technical tracking, *in vivo* efficacy, and clinical correlation. It forms a complete loop from model discovery to clinical relevance, thereby advancing the biological understanding and targeted development of DTP cells.

3.3 Limitations and Future Directions

Current research on DTP cells in cancer faces fundamental constraints in model construction and validation systems, thereby limiting our understanding of the basic mechanisms and potential for clinical translation.

First, existing model systems struggle to replicate the complex physiological environment of the human body. Most *in vitro* models and some *in vivo* models fail to fully recapitulate the complex extracellular matrix, heterogeneous cell composition, and dynamic biochemical signaling networks present in human tumors. Many models, especially xenograft models using immunodeficient mice, lack a complete immune system. This prevents study of the roles of adaptive and innate immunity in the recognition and shaping of DTP cells, thus missing critical tumor-immune interactions. Several strategies have been explored to address the current limitations. One approach involves the generation of human cytokine–expressing mouse models, whereby human-specific cytokine genes are introduced into immunodeficient mice (e.g., NSG mice) to support the survival, differentiation, and function of human immune cells [51]. Another strategy is the combined transplantation of multiple immune components, in which hematopoietic stem cells are co-transplanted with selected immune cell populations (e.g., tumor-associated macrophage precursors, specific T-cell subsets) or cytokines, enabling rapid reconstruction of defined immune microenvironments [52].

Second, these model limitations lead to technical bottlenecks in the study of dynamic mechanisms. The DTP state is inherently dynamic and reversible, but current methods are unable to continuously and in real-time track the fate of individual DTP cells from their formation and maintenance through to their exit. Although single-cell sequencing technology has begun to reveal the heterogeneity of DTP subtypes, it remains challenging to accurately link these transcriptional features to functional outcomes such as the development of relapse or resistance.

Furthermore, the predictive value of various models still needs to be confirmed in the translational validation phase. Combination therapies that are effective in preclinical models, such as by targeting specific pathways or cells, require large-scale clinical trials to validate their efficacy. While patient-derived models (e.g., PDX or organoids) can be used to improve clinical relevance, they rely on fresh tissue and are unable to represent all patient subtypes. Hence, the conclusions drawn from a small number of samples may not be universally applicable.

Finally, the lack of standardization in this field has added considerable confusion. Different studies vary significantly in the conditions used to generate DTP cells (e.g., drug concentrations, treatment duration). This means that DTP cells defined in different laboratories may have quite different biological states, severely hindering the comparison, integration, and eventual consensus of research findings. The current shortcomings create a closed-loop challenge, from “insufficient physiological relevance in basic models” to “limited dynamic capture technologies”, to “uncertainty about clinical predictive value”, and ultimately trapped by “a lack of standardization”. Future breakthroughs will require the development of more physiologically relevant models and advances in live cell tracking technologies. Together with the establishment of widely recognized construction and evaluation standards, these advances should eventually drive the field toward more reliable clinical translation.

In summary, research on DTP cells has established a multi-layered modeling framework that integrates *in vitro* induction models, *in vivo* relapse and intervention models, and patient-derived systems. When combined with complementary functional, mechanistic, technical, and efficacy-based validation strategies, this integrated platform enables systematic characterization of the formation, biological state, and clinical relevance of DTP cells. In addition to serving as reliable tools to define this distinct drug-tolerant state, such models and validation systems have laid a solid foundation for identifying critical dependency pathways and potential therapeutic weaknesses. Building on this framework, the next chapter will focus on the biological vulnerabilities of DTP cells and the corresponding targeting strategies. We further explore how discoveries from experimental models and mechanisms can be translated into interventions with therapeutic potential.

4. Chapter 3: Vulnerabilities of DTP Cells and Targeting Strategies

4.1 Biological Vulnerabilities and Therapeutic Strategies for DTP Cells

DTP cells exhibit a complex array of survival mechanisms that also represent key vulnerabilities for therapeutic targeting. These mechanisms are largely driven by specific molecular programs that can be actively modulated. At the metabolic level, DTP cells often have reprogrammed metabolic pathways. For instance, in ovarian cancer, DTP cells increase FAO activity through CEBPB, providing a survival advantage [53]. Mantle cell lymphoma DTPs depend on the tricarboxylic acid (TCA) cycle for biosynthesis, while GIST DTPs downregulate glucose metabolism and exhibit reduced glutathione levels, making them susceptible to ferroptosis [49]. Similarly, acute myeloid leukemia DTPs uniquely utilize glutamine for pyrimidine synthesis, bypassing the TCA cycle [49], while drug-resistant melanoma clones depend on oxidative phosphorylation [54]. DTP cells also display specific regulatory vulnerabilities at the epigenetic level. In head and neck squamous cell carcinoma, DTP formation depends on the histone demethylase KDM5D, which regulates AURKB [55]. In the context of PARP inhibitor resistance, DTPs rely on the histone chaperone NASP to maintain histone homeostasis [56]. Inhibition of peroxiredoxin 5 (PRDX5) and its interaction with NRF2 has been shown to induce apoptosis in prostate cancer-derived DTPs [57]. Stress responses are another hallmark of DTP cells, and their vulnerability in this aspect has therapeutic implications. In HGSOC, DTPs express high levels of the stress marker ATF3 and undergo EMT [16]. Sublethal mitochondrial damage triggers an integrated stress response, inducing an ATF4-dependent DTP phenotype [17]. In lung adenocarcinoma, integrin-linked kinase (ILK) mediates resistance and maintenance of the DTP phenotype by impairing YAP activation [58]. DTP cells also present vulnerabilities at the level of cell cycle regulation and programmed cell death. In circulating breast tumor cells, stabilization of CDKN1B inhibits mitosis, promoting the formation of a reversible DTP state [5]. Cells resistant to pro-apoptotic drugs show impaired caspase-8 activation and elevated RIPK3 levels, making them more sensitive to necroptotic apoptosis [12]. Several therapeutic strategies have been developed to exploit these biological vulnerabilities. The targeting of key molecules that regulate DTP states, such as inhibiting CDKN1B in breast cancer, can reduce DTP numbers and prevent tumor regrowth [11]. In head and neck cancer, AURKB inhibitors like Barasertib selectively kill DTP cells [55], while targeting ILK in lung adenocarcinoma restores sensitivity to osimertinib [58]. Targeting metabolic pathways such as FAO in ovarian cancer, oxidative phosphorylation in melanoma, and glutamine metabolism in acute myeloid leukemia have also shown promise in eliminating residual DTP cells [53,54,59]. Furthermore, inhibiting de novo pyrimidine synthesis with DHODH inhibitors, such as brequinar, suppresses resistant tumor cell growth and enhances chemotherapy efficacy [60]. DTP cells are also susceptible to ferroptosis, a form of programmed cell death. Inducing ferroptosis in combination with inhibitors targeting the FSP1-mediated resistance pathway can comprehensively eliminate DTPs. This strategy is particularly effective in GIST and lung cancer [17,49]. Exploiting the differential sensitivity of DTP cells to necroptosis by alternating between apoptosis and necroptosis inducers can further enhance cytotoxic effects [12]. In prostate cancer, pharmacological inhibition of PRDX5 with C6-STA triggers apoptotic programs in DTP cells [57]. Combination therapies are crucial for overcoming DTP cell plasticity. For example, pairing AURKB inhibitors with cisplatin in HGSOC can enhance treatment efficacy [55]. Targeting DTPs before giant cell differentiation in mantle cell lymphoma is also a promising therapeutic approach [61]. Studies suggest that timing optimization, such as knocking down stress markers like ATF3 to enhance cisplatin sensitivity, is vital for maximizing treatment effects [16]. Furthermore, sequential treatments with PARP inhibitors and WEE1 inhibitors have been shown to selectively eliminate DTP cells with high replication stress, while sparing normal cells [62].

In conclusion, therapeutic strategies for DTP cells have been developed to address their dynamic and plastic nature, combining interventions that target metabolic, epigenetic, and death signaling pathways. Recent research has uncovered key biological vulnerabilities of DTP cells, with their survival shown to be highly dependent on the BRD2 protein and the antioxidant stress response pathway, particularly the expression of genes such as *GPX2*, *ALDH3A1*, and *MGST1*. Although DTP cells undergo metabolic reprogramming and upregulate their defense mechanisms against oxidative stress, they nevertheless exhibit high sensitivity to BET protein inhibition. BET inhibitors suppress expression of the aforementioned antioxidant genes, leading to the lethal accumulation of reactive oxygen species (ROS) within DTP cells and subsequent induction of apoptosis. *In vivo*, BET inhibitors effectively eliminate residual DTP cells and significantly delay tumor relapse in melanoma and lung cancer models. This strategy provides a promising new therapeutic direction for targeting DTP cells and overcoming clinical drug resistance [63].

4.2 Remaining Challenges

Current research on the vulnerabilities of tumor DTP cells and the development of targeted strategies faces several interconnected challenges.

First, the inherent limitations of current research systems constitute an initial barrier to the understanding of DTP cells. Most key mechanistic discoveries rely on patient-derived cells, cell lines, or mouse xenograft models. These systems cannot fully recapitulate the complex TME and drug pharmacokinetics in humans, raising fundamental

questions about the clinical efficacy and safety of identified vulnerability targets, even before translation.

Second, beyond model limitations, the highly dynamic and heterogeneous nature of DTP cells presents a core scientific challenge. DTP is recognized as a reversible cellular state with heterogeneous subpopulations. Resistant clones may therefore evolve from different precursors. The core regulatory networks that drive the formation, maintenance, and interconversion of DTP cells have not yet been systematically elucidated, and tools capable of tracking their dynamic evolution *in vivo* at the single-cell level are still lacking. Consequently, our mechanistic understanding of the DTP state remains fragmented.

Third, incomplete mechanistic knowledge leads to bottlenecks in clinical application. Although several potential molecular markers associated with the DTP state have been identified, they are still far from being reliable predictive or classification tools in the clinic, and their utility requires validation in prospective studies. Importantly, techniques for non-invasive, real-time monitoring of DTP cell dynamics in patients are also lacking, leaving treatment without a critical navigator.

Together, these challenges make it difficult to develop practical, targeted strategies. Three main problems remain. First, the potential therapeutic window is narrow, as drugs targeting specific metabolic or epigenetic vulnerabilities may cause off-target toxicity in normal tissues. Second, the optimal timing and combination strategies are difficult to determine, since alternating therapy or interventions within specific time windows may be theoretically advantageous, but extremely challenging to implement in clinical practice. Third, targeting a single vulnerability may drive the emergence of new resistance. Such interventions could apply selective pressure similar to traditional therapies, promoting further tumor evolution and more complex resistance mechanisms.

In summary, the imperfect model systems, dynamic and complex core mechanisms, lack of clinical monitoring tools, and practical limitations in targeted therapy remain as major shortcomings in DTP research. There is clearly an urgent need to develop more physiologically relevant models and dynamic analytical tools, as well as a systematic paradigm for clinical translation.

5. Chapter 4: Conclusion and Perspectives

Research on DTP cells has evolved from initial phenomenological descriptions into a frontier field that integrates fundamental biology, disease modeling, and therapeutic translation. This review systematically summarizes the core advances from model construction to the targeting of DTP vulnerabilities.

It is now clear that DTP cells are not a homogeneous entity, but instead represent a highly heterogeneous and dynamic plastic state shaped by both clonal selection and drug-induced mechanisms. Their formation and maintenance involve a coordinated molecular network, including key epigenetic remodeling (e.g., *H3K27me3* modification), profound metabolic reprogramming (e.g., shift toward oxidative phosphorylation), and reactivation of pro-survival signaling pathways such as YAP/TAZ. These biological features enable DTP cells to evade conventional therapy by entering a reversible slow-cycling or quiescent state, placing them at the core of MRD and acquired resistance.

To dissect the complex DTP state, researchers have developed diverse model systems, ranging from relatively simple drug-treated cell lines to PDX and organoids that retain tumor architecture. These models have become indispensable tools, not only for validating the biological properties of DTP cells, but also as platforms for vulnerability screening. They have allowed the discovery of a promising set of targets for eradicating DTP cells, including specific metabolic dependencies (e.g., ferroptosis sensitivity, reliance on FAO), epigenetic regulatory nodes (e.g., *KDM5D/AURKB* axis, histone chaperone NASP), and cell surface antigens (e.g., TROP2, CD70). Based on these findings, novel strategies such as combination targeting (e.g., TKI plus ferroptosis inducers) or temporally optimized therapies have been developed for clearing residual disease and delaying relapse.

However, critical bottlenecks remain in the process from discovery to cure. First, the existing models and known mechanisms cannot fully recapitulate the complexity of the human TME, particularly tumor-immune interactions, thereby limiting our understanding of DTP immune editing and escape mechanisms. Moreover, current technologies are unable to track single-cell fate transitions of DTP cells in real time and over long periods, making it difficult to fully understand the highly plastic nature of regulatory networks. Second, major gaps remain between experimental models and patient reality at the translational level. Targeted combinations that show efficacy in PDX models require large-scale prospective trials to validate clinical efficacy and safety. The inherent heterogeneity of DTP cells and the lack of non-invasive, dynamic monitoring biomarkers make it extremely challenging to achieve accurate patient stratification and optimal treatment timing. Moreover, the targeting of specific vulnerabilities may risk off-target toxicity or induce bypass resistance. Finally, the field lacks consensus standards for DTP cell generation, identification, and efficacy assessment, severely affecting the ability to compare and integrate research findings.

The core challenges in current DTP research are being addressed by emerging technologies such as spatial transcriptomics, single-cell multi-omics (e.g., scRNA-seq, scATAC-seq), and high-parameter proteomics [64]. These technologies can reveal the complex spatial niches inhabited by DTP cells and their interactions with immune and stromal cells, which is key to understanding immune evasion and drug resistance [65]. Furthermore, the integration of transcriptomic and chromatin accessibility results at

single-cell resolution may enable dissection of the dynamic regulatory networks driving DTP state transitions [66]. Additionally, high-dimensional analysis of immune cells and proteomes from peripheral blood, and direct discovery of cell-surface protein markers from tissues, offer the potential to develop non-invasive, dynamic monitoring tools [67]. Recent studies have demonstrated that spatial multi-omics can reveal the spatial distribution of specific cell subpopulations associated with therapy resistance, while machine learning models based on peripheral blood multi-omics can infer the intra-tumoral immune state [68]. Collectively, these advances provide a technical foundation for bridging the gap between models and clinical reality, enabling precise DTP profiling and targeted intervention.

Looking ahead, the elimination of DTP cells is a major therapeutic challenge that will require a multidisciplinary and multi-level shift in research paradigms. From a technical perspective, future efforts should focus on developing more physiologically relevant, humanized animal models and complex organoid systems that integrate immune and stromal components. The use of dynamic single-cell multi-omics and spatial transcriptomics in DTP research should also be expanded. In terms of clinical translation, priority should be given to dynamic monitoring of DTP cells using liquid biopsy approaches, and to the design of clinical trials that intervene at the stage of MRD. With respect to standardization, there is a clear need for preliminary consensus guidelines to define DTP cells, as well as for model construction and evaluation.

In summary, the conquering of DTP cells is unlikely to be achieved by a single approach, but will require the integration of innovative model systems, advanced analytical technologies, and more rational clinical development strategies.

Author Contributions

YPZ: original draft preparation, creation of figures and tables, literature retrieval and synthesis. XQD: design and investigation; YFS: literature search and data analysis; JC: supervision, substantial involvement in study design and methodology refinement. MRL: Conception. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflicts of Interest

The authors declare no conflicts of interest.

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