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# Targeting Metabolic Vulnerabilities of Drug-Tolerant Persister Cells in Cancer

**Mohit Kumar**

mohitratti2@gmail.com

Mata Gujri College (Autonomous)

**mini-review**

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# Abstract

Drug-tolerant persister (DTP) cells survive anticancer treatment through reversible adaptive states and can contribute to tumour relapse and acquired resistance. This narrative review examines the metabolic dependencies that support persistence, focusing on mitochondrial oxidative phosphorylation, fatty acid oxidation, redox homeostasis and ferroptosis defence. It compares evidence across tumour types and treatment models to distinguish metabolic changes associated with persistence from dependencies demonstrated through genetic or pharmacological intervention. Selected persister populations rely on mitochondrial respiration and quality control, fatty acid oxidation or glutathione peroxidase 4 (GPX4)-mediated protection against lipid peroxidation. Preclinical studies suggest that targeting these processes can reduce persister survival in specific settings. Residual ferroptosis suppressor protein 1 activity can help some persister cells survive GPX4 inhibition. The effects vary with tumour type, prior treatment and cell state, and the clinical benefit of targeting these dependencies remains to be established. Functional profiling of residual disease, studies in patient-derived models and evaluation of treatment timing and toxicity could help identify which dependencies warrant clinical testing.


# 1. Introduction

Cancer therapy has improved substantially, yet drug resistance remains a major barrier to durable treatment responses. Following anticancer treatment, most sensitive tumour cells may die while a small fraction survives in a reversible drug-tolerant state. These drug-tolerant persister (DTP) cells can initially survive without stable resistance-conferring mutations and may regain drug sensitivity after treatment withdrawal. However, their persistence creates a reservoir from which tumour regrowth and more stable resistance can emerge, making DTP cells an important target for strategies aimed at preventing relapse [1, 2].

The modern concept of cancer DTP cells was strongly influenced by the study of Sharma and colleagues, who identified a small population of cancer cells capable of surviving otherwise lethal drug exposure through a reversible chromatin-mediated state involving lysine demethylase 5A (KDM5A) and insulin-like growth factor 1 receptor (IGF-1R) signalling [1]. Subsequent studies have shown that DTP survival can involve diverse epigenetic, phenotypic and metabolic adaptations. A particularly important example is the dependence of several persister populations on glutathione peroxidase 4 (GPX4), which protects membrane lipids from peroxidation. Hangauer and colleagues demonstrated that GPX4 inhibition selectively induced ferroptotic death in DTP cells and reduced tumour relapse in experimental models, providing proof that persister-specific metabolic dependencies can be therapeutically exploited [3].

DTP metabolism is highly heterogeneous and varies according to tumour type, treatment and cellular state. Reported adaptations include changes in mitochondrial oxidative phosphorylation, fatty-acid metabolism, antioxidant defence, glutathione metabolism and lipid-redox homeostasis. A key challenge is therefore to distinguish metabolic alterations that merely accompany persistence from pathways that are functionally required for DTP survival. This narrative review compares mitochondrial, lipid and redox dependencies across tumour types and treatments. It distinguishes evidence from direct genetic or pharmacological perturbation in DTP models from observations in related therapy-surviving states. It also examines which findings have been reproduced in animal models and what remains to be established before a persister-directed treatment can be tested clinically [2, 4].

## 2. Drug-Tolerant Persister Cells as a Reversible State of Therapy Resistance

# 2.1 Definition and Characteristics of DTP Cells

Drug-tolerant persister (DTP) cells are a small, phenotypically plastic population of cancer cells that survive otherwise effective anticancer treatment without initially requiring stable resistance-conferring mutations. The DTP state is generally transient and reversible. After removal of therapeutic pressure, surviving cells can resume proliferation and may again become sensitive to the original treatment. Many DTP populations are slow-cycling, quiescent or dormant-like during drug exposure, although this is not universal. Their defining features therefore include survival under otherwise lethal treatment, reversibility, marked heterogeneity and the ability to act as a reservoir from which more stable resistance can emerge [2, 4, 5].

DTP cells overlap with, but are not identical to, dormant tumour cells, cancer stem cells (CSCs) or permanently resistant clones. Dormant cells are mainly defined by low proliferative activity, whereas CSCs are defined by self-renewal and tumour-initiating capacity. In contrast, DTP cells are identified by their reversible survival under therapeutic pressure. Some persister populations can acquire stem-like, epithelial–mesenchymal transition (EMT)-associated or quiescent features, but these are context-dependent rather than universal characteristics. Stable resistant populations can maintain resistance through persistent genetic or epigenetic alterations, whereas early DTP survival can depend on reversible non-genetic adaptations [2, 5].

# 2.2 Emergence of DTP Cells During Therapy

Drug-tolerant persister cells generally become evident after a tumour is exposed to anticancer therapy. Treatment creates a strong selective pressure in which the majority of drug-sensitive cancer cells are eliminated, while a small fraction survives by entering adaptive cellular states. Current evidence suggests that DTP formation cannot always be explained by the simple selection of a permanently resistant clone that was already present before treatment. Instead, cancer cells can dynamically respond to therapeutic stress through changes in transcription, chromatin organisation, signalling, metabolism and cell-cycle activity. These adaptive responses can push individual cells into a temporary drug-tolerant state. The probability of entering this state may differ among cells because tumours are highly heterogeneous even before treatment. Thus, therapy can both select cells with favourable pre-existing characteristics and induce new adaptive programmes in surviving cells. This dynamic process helps explain why phenotypically different DTP populations can arise within the same tumour following exposure to anticancer drugs [2, 5].

The emergence of drug tolerance can involve both pre-existing cellular heterogeneity and treatment-induced adaptation. Even before therapy, genetically similar cancer cells can differ in gene expression, signalling activity and cellular state, making some cells more capable of surviving therapeutic stress than others. An important single-cell study in melanoma demonstrated that rare cells can transiently express resistance-associated genes before drug exposure. Following treatment, these favourable pre-existing states can be reinforced through drug-induced transcriptional and epigenetic reprogramming, allowing surviving cells to progress towards more persistent resistant phenotypes. Pre-existing variation and treatment-induced adaptation can cooperate. A transient cellular state present before treatment may increase the probability of survival, while continued drug exposure can stabilise or reshape that state. This combination of cellular heterogeneity and adaptive plasticity

provides an important framework for understanding how small persister populations emerge during cancer therapy [2, 6].

Epigenetic reprogramming is one of the best-characterised mechanisms supporting the emergence of the DTP state. Unlike a permanent DNA mutation, epigenetic regulation changes how genes are switched on or off without altering the underlying DNA sequence. Sharma and colleagues showed that drug-tolerant cancer cells acquire a distinct chromatin state requiring the histone demethylase KDM5A and involving IGF-1R signalling. These changes allowed a small population of cells to survive drug exposure while remaining capable of returning to a drug-sensitive state after treatment withdrawal. Subsequent studies have expanded this concept by showing that alterations in histone methylation, histone acetylation, chromatin accessibility and transcriptional programmes can help cancer cells rapidly adapt to therapeutic stress. Importantly, because many of these changes are reversible, epigenetic plasticity provides cancer cells with a flexible survival strategy before stable genetic resistance becomes established. Thus, chromatin remodelling can act as an early bridge between acute drug exposure and the development of a persistent drug-tolerant phenotype [1, 2, 4].

The DTP state is not controlled by a single universal pathway. Instead, cancer cells can combine several adaptive programmes to survive therapeutic stress, including changes in stress-response signalling, cell-cycle activity, cellular identity, stem-like features, epithelial–mesenchymal transition-associated programmes and metabolism. Metabolic adaptation is particularly important because persister cells must maintain energy production while limiting oxidative damage during prolonged drug exposure. Depending on the tumour and treatment context, DTP populations may rely on mitochondrial oxidative phosphorylation, lipid metabolism or antioxidant defence pathways. These metabolic changes can interact with epigenetic and transcriptional reprogramming, producing highly plastic cellular states that can shift during therapy. DTP emergence should therefore be viewed as a context-dependent process involving coordinated phenotypic, epigenetic, signalling and metabolic adaptations rather than one fixed molecular mechanism [2, 4, 5].

## 2.3 DTP Cells versus Genetically Resistant Cells

Drug-tolerant persister cells and genetically resistant cancer cells represent distinct but interconnected mechanisms of treatment failure. Genetically resistant cells typically survive because of stable, heritable alterations such as mutations affecting the drug target or activation of alternative survival pathways. In contrast, early DTP survival usually depends on reversible changes in transcription, chromatin regulation, signalling and metabolism. After drug withdrawal, at least some DTP-derived cells can regain sensitivity to the original treatment. Thus, the key distinction is whether survival is maintained by a stable heritable resistance mechanism or by a transient, plastic adaptive state [2, 5].

The temporary nature of the DTP state does not make it clinically unimportant. By surviving prolonged treatment, persister cells create a window during which further non-genetic and genetic changes can accumulate. In lung-cancer models, therapy-induced APOBEC3A activity in persistent cells increased mutagenesis and promoted progression towards acquired resistance, providing direct evidence that persister states can influence subsequent genetic evolution. This makes the DTP phase an important therapeutic window in which residual cells might be eliminated before stable resistance expands. Metabolic and redox dependencies, including GPX4 dependence in selected DTP populations, are therefore being investigated as vulnerabilities that could intercept this transition [2, 3, 5, 7].

## 2.4 Phenotypic Plasticity and Transition to Stable Resistance

Phenotypic plasticity allows cancer cells to change their behaviour without necessarily acquiring new DNA mutations. Under therapeutic pressure, this flexibility can enable transition from a drug-sensitive state to a temporary drug-tolerant state and, in some cases, towards stable resistance. Single-cell studies have shown that rare pre-resistant states can exist before treatment and may be reinforced by drug-induced transcriptional and epigenetic reprogramming. In melanoma, Shaffer and colleagues demonstrated that rare cells expressing resistance-associated genes could undergo stepwise reprogramming after treatment, illustrating how pre-existing variability and therapy-induced adaptation can cooperate during the evolution of resistance [2, 6].

DTP plasticity can also involve changes in cellular identity, including epithelial–mesenchymal transition-associated programmes, stem-like features and lineage switching. These states are not universal or necessarily permanent, but they can increase survival under therapeutic stress and provide multiple routes for persistence. During continued treatment, some persister cells may die or regain drug sensitivity, whereas others may survive long enough to acquire additional non-genetic or genetic alterations that support stable resistance. Thus, the transition from drug tolerance to resistance is a dynamic and non-linear process, making residual persister populations potential targets for interventions aimed at reducing the risk of tumour relapse [2, 4, 5]. The transition from drug sensitivity to reversible persistence and, in some cases, stable resistance is illustrated in Fig. 1.

## 3. Metabolic Reprogramming in DTP Cells

Metabolic reprogramming is an important component of DTP survival during therapeutic stress. Persister cells can alter energy production, nutrient utilisation and antioxidant defence to maintain cellular homeostasis despite prolonged drug exposure. Depending on tumour type and treatment context, reported adaptations include changes in mitochondrial oxidative phosphorylation, fatty-acid metabolism, glucose and glutamine utilisation, and lipid-redox homeostasis. However, these metabolic states are highly heterogeneous. A therapeutic objective is to identify which metabolic pathways become essential dependencies in specific persister populations [2, 3, 5].

The major metabolic adaptations and vulnerabilities associated with DTP-cell survival are summarised in Fig. 2.

# 3.1 Mitochondrial Metabolism and Oxidative Phosphorylation

Mitochondrial metabolism can become an important survival dependency in selected DTP populations. In lung adenocarcinoma models treated with inhibitors of the mitogen-activated protein kinase (MAPK) pathway, persister cells shifted from glycolysis towards oxidative phosphorylation (OXPHOS) and increased mitophagy mediated by PTEN-induced kinase 1 (PINK1), which helped maintain mitochondrial quality and redox homeostasis. Genetic depletion of PINK1 or pharmacological disruption of mitophagy reduced drug tolerance, indicating that mitochondrial maintenance can be therapeutically important in OXPHOS-dependent persister states. Similar findings have been reported in estrogen receptor-positive breast cancer, where cells tolerant to inhibition of cyclin-dependent kinases 4 and 6 (CDK4/6) showed increased mitochondrial content, respiration and reactive oxygen species (ROS), and became more sensitive to mitochondrial complex I inhibition [8, 9].

However, increased OXPHOS is not a universal feature of therapy-surviving cancer-cell states. In cisplatin-surviving polyploid cancer cells, metabolic-flux analysis showed reduced oxidative phosphorylation together with increased glycolysis and pentose phosphate pathway activity, redirecting glucose- and glutamine-derived carbon towards production of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and antioxidant defence. Together with the contrasting DTP studies above, these findings highlight substantial metabolic diversity among

therapy-surviving states. Mitochondrial targeting should therefore be restricted to DTP populations in which mitochondrial dependence has been functionally demonstrated, ideally using metabolic profiling or biomarkers capable of identifying OXPHOS-dependent residual disease [2, 10].

## 3.2 Lipid Metabolism and Fatty-Acid Dependence

Lipid metabolism can support DTP survival by providing energy, membrane components and metabolic intermediates. In BRAF-mutant melanoma, Shen and colleagues showed that persister cells surviving combined BRAF and MEK inhibition increased peroxisomal fatty acid oxidation (FAO) regulated by peroxisome proliferator-activated receptor alpha (PPARα) and acyl-CoA oxidase 1 (ACOX1). Genetic suppression of ACOX1 or pharmacological inhibition of this pathway reduced oxidative respiration and persister emergence, while combined metabolic and targeted therapy produced more durable tumour control in mice. These findings identify peroxisomal fatty-acid oxidation as a functional dependency in selected melanoma persisters [11].

Fatty-acid oxidation may also support the rare subset of persister cells that continues to proliferate during treatment. Oren and colleagues identified cycling persisters across multiple cancer models and found that they displayed stronger fatty-acid oxidation and antioxidant-response programmes than non-cycling persisters. Experimental manipulation of fatty-acid oxidation altered the cycling-persister fraction, supporting a functional role for FAO in maintaining proliferative capacity under drug pressure. Thus, lipid metabolism can contribute not only to survival but also to persistence-associated proliferation [12].

Altered lipid storage and mobilisation can simultaneously create ferroptosis vulnerability. In paclitaxel-tolerant head and neck cancer cells, increased lipid droplets, fatty-acid oxidation and lipid regulation mediated by progesterone receptor membrane component 1 (PGRMC1) were associated with enhanced susceptibility to ferroptosis induced by system $x_c^-$ inhibition. Inhibition of system $x_c^-$ promoted lipophagy and release of stored fatty acids, increasing lipid oxidative stress. These findings illustrate the dual role of lipid metabolism in DTP cells: fatty-acid utilisation can support persistence, whereas accumulation or mobilisation of oxidisable lipids can increase sensitivity to lipid-peroxidation-driven cell death. The therapeutic value of targeting lipid metabolism is therefore likely to depend on the balance between lipid utilisation, storage and antioxidant protection in each persister state [2, 3, 13].

## 3.3 Redox Homeostasis and Antioxidant Defence

Redox homeostasis is an important survival requirement for many DTP populations because anticancer therapy can increase ROS and oxidative damage. Persister cells can adapt by strengthening antioxidant pathways involving reduced glutathione (GSH), GPX4 and other redox-protective mechanisms [4]. Related evidence from cisplatin-surviving polyploid cancer cells shows that glucose can be redirected through the pentose phosphate pathway, while glutamine-derived carbon supports NADPH production and antioxidant metabolism. Although this model is not a classical DTP system, it illustrates how therapy-surviving cancer-cell states can reorganize central carbon metabolism to maintain redox balance [10].

This enhanced antioxidant capacity can itself become a therapeutic vulnerability. Hangauer and colleagues showed that several DTP models acquired a strong dependence on GPX4, whereas a recent pancreatic cancer study identified increased antioxidant responses involving GPX4, SLC7A11 and pathways associated with nuclear factor erythroid 2-related factor 2 (NRF2) during treatment and relapse. These findings suggest that some DTP populations may survive close to a redox threshold and become vulnerable when the antioxidant system on

which they depend is disrupted. However, the dominant redox dependency is likely to vary between tumour types and treatment contexts [2–4, 14].

## 3.4 Glutathione and GPX4 Dependence

GPX4 is one of the best-established metabolic vulnerabilities in DTP cells. This selenium-containing enzyme uses GSH to detoxify phospholipid hydroperoxides and prevent ferroptotic cell death. Hangauer and colleagues showed that persister cells generated from multiple cancer types and treatments acquired a selective dependence on GPX4. Genetic or pharmacological GPX4 inhibition increased lipid reactive oxygen species and preferentially killed persister cells, while GPX4 loss also reduced tumour relapse in experimental models [3].

The cystine/glutamate antiporter system $x_c^-$ imports cystine through its SLC7A11 and SLC3A2 subunits, supporting cysteine and GSH synthesis. NADPH-dependent systems regenerate GSH. Blocking cystine uptake or GSH synthesis can therefore weaken GPX4-mediated defence [15]. Cancer cells can also limit lipid peroxidation through ferroptosis suppressor protein 1 (FSP1), which maintains reduced coenzyme Q, or through a mitochondrial pathway involving dihydroorotate dehydrogenase (DHODH) [16, 17]. In experimental DTP models, cells that survived GPX4 inhibition depended on residual FSP1 activity, identifying an escape mechanism in a subset of persisters [18]. The evidence for DHODH comes from broader ferroptosis models [17].

## 3.5 Ferroptosis Vulnerability

Ferroptosis is an iron-dependent form of regulated cell death driven by accumulation of membrane lipid peroxides. DTP cells can become particularly vulnerable to ferroptosis when they operate under high oxidative and lipid-peroxidation stress and therefore depend strongly on GPX4-mediated protection. Hangauer and colleagues showed that GPX4 inhibition selectively increased lipid reactive oxygen species and induced ferroptotic death across several persister-cell models, establishing ferroptosis as a major metabolic vulnerability in therapy-persistent cancer cells [3, 15].

These findings extend the ferroptosis-vulnerability concept beyond classical DTP models and suggest that therapy-resistant mesenchymal states can also acquire dependence on lipid-peroxide defence because their lipid composition can favour accumulation of oxidisable polyunsaturated phospholipids. Viswanathan and colleagues demonstrated that high-mesenchymal therapy-resistant cancer cells across several models were selectively dependent on GPX4, linking phenotypic plasticity with ferroptosis sensitivity. These findings suggest that resistance-associated cellular states can simultaneously promote survival under conventional therapy and create susceptibility to lipid-peroxidation-driven death [19].

These findings support testing ferroptosis-directed treatments against residual persister cells after initial therapy has reduced the drug-sensitive tumour population. Susceptibility is likely to vary across models and treatments. Most DTP-directed ferroptosis evidence remains preclinical, and clinical translation will require biomarkers, tumour-selective delivery and strategies to overcome heterogeneity and alternative ferroptosis-defence mechanisms [2, 3, 20–22].

## 3.6 Other Emerging Metabolic Dependencies

Beyond OXPHOS, lipid metabolism and GPX4-dependent redox defence, therapy-surviving cancer-cell states can acquire additional context-specific metabolic dependencies. Cisplatin-surviving polyploid cancer cells have been shown to increase glycolysis and pentose phosphate pathway activity while redirecting glucose- and glutamine-

derived carbon towards NADPH-supported antioxidant metabolism [10]. In a longitudinal model of adaptation along a resistance continuum, functional screening identified emerging dependencies involving the pentose phosphate pathway, nucleotide metabolism, cholesterol biosynthesis, oxidative phosphorylation, iron homeostasis and NRF2-associated antioxidant programmes. Highly adapted cells also became increasingly vulnerable to combined glutaminase inhibition with CB-839 and olaparib [23]. Although these models do not represent a single universal DTP phenotype, they illustrate the broader metabolic plasticity that can accompany therapy persistence and adaptation [10, 23].

Aldehyde dehydrogenase (ALDH) activity represents another vulnerability in selected persister populations. Raha and colleagues found that drug-tolerant cancer cells with elevated ALDH activity were preferentially affected by ALDH inhibition, which increased oxidative stress and DNA damage and delayed tumour relapse when combined with targeted therapy. In gastric cancer, ALDH1A3 also supported gastric cancer persister survival and was associated with regulation of the mTOR pathway. However, ALDH dependence varies between tumour types and should therefore be considered a context-specific rather than universal DTP marker [24, 25].

The major metabolic vulnerabilities identified across representative DTP models are summarised in Table 1.

Table 1
Metabolic vulnerabilities identified in representative persister models

| Study | Tumour and model | Treatment context | DTP metabolic feature | Target or strategy | Major finding |
|---|---|---|---|---|---|
| Hangauer et al. [3] | Multiple cancer cell models and mouse xenografts | Different anticancer therapies | Increased dependence on lipid-peroxide defence | GPX4 inhibition | GPX4 loss induced ferroptosis in persisters and prevented relapse in a mouse model. |
| Shen et al. [11] | BRAF-mutant melanoma cells and mouse tumour models | BRAF + MEK inhibition | Increased peroxisomal fatty-acid oxidation | PPARα–ACOX1 / FAO | ACOX1 suppression or FAO inhibition reduced persister emergence |
| Oren et al. [12] | Multiple cancer models | Targeted therapies | FAO and antioxidant programmes in cycling persisters | Fatty-acid oxidation | Manipulating FAO altered the fraction of cycling persisters. |
| You et al. [13] | Head and neck cancer cells | Paclitaxel | Lipid droplets, FAO and PGRMC1-dependent lipid metabolism | System $x_c^-$ inhibition/ ferroptosis | Persisters showed increased ferroptosis sensitivity associated with lipophagy and PGRMC1 |
| Li et al. [8] | Lung adenocarcinoma cells and organoids | MAPK-pathway inhibition | OXPHOS and mitochondrial quality control | PINK1-mediated mitophagy | Disrupting PINK1-mediated mitophagy reduced drug tolerance. |
| Abdel Hadi et al. [14] | Pancreatic cancer models and mouse xenografts | Chemotherapy / residual disease | Increased oxidative stress and antioxidant defence | ROS induction + GSH depletion | Arsenic trioxide plus BSO prevented relapse during the observation period in two xenograft models. |
| Higuchi et al. [18] | Multiple cancer cell persister models | Oncogene-targeted therapy | Residual FSP1 activity after GPX4 inhibition | GPX4 and FSP1 perturbation | Persisters surviving GPX4 inhibition depended on residual FSP1. |

Abbreviations used in the table are defined in the manuscript.

# 4. Therapeutically Targeting Metabolic Vulnerabilities of DTP Cells

Metabolic adaptations that support DTP survival can also create therapeutic vulnerabilities. This has led to a two-stage treatment concept in which conventional therapy first eliminates the drug-sensitive tumour population,

followed by a second intervention directed against dependencies acquired by surviving persister cells. Candidate targets include GPX4-dependent ferroptosis defence, fatty-acid oxidation, mitochondrial metabolism, mitophagy and antioxidant pathways. Because these dependencies vary across tumour types and treatments, effective metabolic targeting will require identification of the dominant vulnerability within each residual persister population rather than a single universal strategy [2, 3, 8, 11].

A conceptual overview of therapeutic strategies designed to exploit metabolic vulnerabilities in DTP cells is shown in Fig. 3.

Representative therapeutic strategies targeting these metabolic vulnerabilities, together with their supporting evidence and major limitations, are summarised in Table 2.

Table 2
Experimental strategies targeting metabolic vulnerabilities in persister cells

| Target | Example intervention | Mechanism | Evidence in persisters | Main advantage | Main limitation |
|---|---|---|---|---|---|
| GPX4-dependent lipid-peroxide defence | RSL3, ML210 (experimental) | GPX4 inhibition impairs phospholipid hydroperoxide detoxification and promotes ferroptosis. | GPX4 dependence in several DTP models [3] | Preferential killing in selected persister models | Preclinical compounds. Residual FSP1 can support escape in selected persisters [18]. |
| System $x_c^-$ and GSH | Erastin-like inhibition, GSH depletion | Reduces cystine/GSH supply → weakens GPX4 defence | Direct evidence in paclitaxel-tolerant persisters [13]. Broader mechanistic evidence [15, 26]. | Targets upstream ferroptosis defence | Alternative antioxidant pathways may compensate |
| Peroxisomal FAO (PPARα–ACOX1 axis) | ACOX1/PPARα pathway inhibition | Reduces fatty-acid-derived metabolic support | Direct evidence for the axis in melanoma [11]. FAO also supported cycling persisters [12]. | Targets a defined metabolic dependency | Not all DTPs are FAO-dependent |
| Mitophagy/OXPHOS | PINK1 disruption or chloroquine in experimental models | Impairs mitochondrial quality control | Strong in selected lung adenocarcinoma DTP models [8] | Exploits mitochondrial dependence | Chloroquine also affects broader lysosomal and autophagy pathways. |
| Redox defence | Arsenic trioxide + BSO | Raises oxidative stress while lowering glutathione defence | In vivo PDAC evidence [14] | Prevented relapse during follow-up in two PDAC xenograft models. | Potential normal-tissue toxicity |
| Lysosomal iron / ferroptosis | Fentomycin-1 | Activates redox-active lysosomal iron → lipid oxidation | Direct recent DTP evidence [27] | Targets iron-rich persistent states | Preclinical evidence. Tolerance can emerge. |

# 4.1 Targeting GPX4

Direct disruption of GPX4-dependent lipid-peroxide defence is one of the most extensively studied approaches for targeting DTP metabolism. Experimental compounds including RSL3 and the selective cellular GPX4 inhibitor ML210 can promote lipid-peroxide accumulation and ferroptotic death. Hangauer and colleagues showed that loss of GPX4 function selectively eliminated persister cells and prevented tumour relapse in experimental

models, providing strong proof of principle for GPX4 dependence in therapy-persistent cancer cells. However, currently available GPX4-targeting compounds remain preclinical research tools, and clinical translation will require agents with suitable pharmacological properties, tumour selectivity and an acceptable therapeutic window [3, 21].

GPX4 defence can also be weakened indirectly by reducing cystine uptake or glutathione availability. Inhibition of system $x_c^-$ lowers cysteine supply for GSH synthesis and can thereby reduce GPX4-mediated protection from lipid peroxidation. Erastin and related experimental ferroptosis inducers exploit this upstream mechanism by limiting cystine-dependent glutathione synthesis. However, DTP sensitivity to this approach is context dependent, and alternative antioxidant pathways may compensate for disruption of the cystine–GSH–GPX4 axis [15, 26].

## 4.2 Inducing Ferroptosis

Ferroptosis can also be induced by increasing iron-dependent lipid peroxidation rather than directly inhibiting GPX4. Cañeque and colleagues developed fentomycin-1, which activates redox-active lysosomal iron and promotes oxidative membrane damage. The compound preferentially targeted iron-rich CD44-high cancer-cell states, eradicated DTP cells *in vitro* and reduced tumour growth in an experimental metastasis model. CD44-high cancer-cell populations may be particularly relevant because CD44-mediated iron uptake can increase lysosomal iron availability and create susceptibility to ferroptotic damage [27, 28].

However, cancer cells can adapt to ferroptotic stress. Exposure to sublethal fentomycin-1 generated a ferroptosis-tolerant state associated with altered iron homeostasis, changes in mesenchymal markers and reinforced antioxidant responses, including increased GPX4 in lysosome-enriched fractions. These findings indicate that ferroptosis-directed therapy may itself induce adaptive ferroptosis-tolerant states. Combination strategies and optimised treatment schedules may therefore be required to prevent survival of ferroptosis-tolerant residual populations [27, 29].

## 4.3 Targeting Lipid Metabolism

Targeting fatty-acid metabolism represents a potential strategy for eliminating persister populations that become dependent on lipid oxidation. In BRAF-mutant melanoma, Shen and colleagues showed that persister cells surviving combined BRAF and MEK inhibition relied on PPARα–ACOX1-regulated peroxisomal fatty-acid oxidation. Genetic or pharmacological disruption of this pathway reduced persister emergence, and combining FAO inhibition with targeted therapy produced more durable tumour control in mice [11].

FAO may also support the rare persister cells that continue to proliferate during therapy. Oren and colleagues found that cycling persisters displayed stronger fatty-acid oxidation and antioxidant programmes than non-cycling populations, and FAO inhibition reduced the fraction capable of continued proliferation under drug pressure. These findings suggest that lipid metabolism can provide both energetic and redox support to selected proliferative persister states [12].

However, FAO dependence is not universal, and normal tissues also rely on fatty-acid metabolism [30]. In addition, DTP cells may compensate for FAO inhibition by increasing their use of alternative fuels such as glucose or glutamine. Therefore, clinical targeting of lipid metabolism will require selective inhibitors, functional evidence of FAO dependence and biomarkers capable of identifying the appropriate residual-disease population. Interpretation of experimental studies is further complicated by off-target effects of commonly used inhibitors such as etomoxir at higher concentrations, including inhibition of mitochondrial complex I [31].

## 4.4 Targeting Mitochondrial Metabolism

Mitochondrial metabolism can be targeted in DTP populations that acquire dependence on oxidative phosphorylation and mitochondrial quality control. In MAPK-inhibitor-treated lung adenocarcinoma models, PINK1-mediated mitophagy supported mitochondrial homeostasis and persister survival. Genetic depletion of PINK1 or pharmacological disruption of lysosomal function with chloroquine reduced drug tolerance and enhanced the response to MAPK-pathway inhibition, providing proof of principle that mitochondrial quality control can represent a therapeutic vulnerability in selected DTP states [8].

However, mitochondrial targeting is unlikely to be broadly applicable. Oxidative phosphorylation is required by many normal tissues, which can restrict the therapeutic window of respiratory-chain inhibitors. Clinical experience with the complex I inhibitor IACS-010759 illustrates this problem: phase I trials revealed a narrow therapeutic index and dose-limiting toxicities including elevated blood lactate and neurotoxicity, preventing establishment of a recommended phase II dose [32]. In addition, therapy-surviving cancer-cell states are metabolically heterogeneous and may rely on glycolysis, glutamine metabolism or other pathways rather than increased OXPHOS. Therefore, mitochondrial-directed strategies should be restricted to residual tumours with experimentally demonstrated mitochondrial dependence and ideally guided by biomarkers or functional metabolic profiling [2, 10].

## 4.5 Targeting Redox Balance

Redox balance may represent a therapeutic vulnerability in DTP populations that adapt to therapy-induced oxidative stress by strengthening antioxidant defence. In pancreatic ductal adenocarcinoma (PDAC) models, Abdel Hadi and colleagues found that therapy-persistent and relapsing tumours showed increased ROS together with stronger antioxidant defences. Arsenic trioxide increased oxidative stress, while combined treatment with buthionine sulfoximine (BSO) reduced glutathione synthesis. In combination, these agents prevented relapse during the reported observation period in two xenograft models. These findings support the concept that simultaneously increasing oxidative stress and weakening antioxidant capacity can expose a redox vulnerability in selected DTP states [14].

However, redox-targeted therapy requires careful control because ROS and antioxidant systems are also essential in normal tissues. DTP populations can rely on different protective pathways, including GPX4, glutathione and NRF2-associated programmes, so indiscriminate ROS induction is unlikely to be effective or safe. Clinical translation will therefore require identification of the dominant antioxidant dependency in residual disease and strategies that create a therapeutic window between persister cells and normal tissues [14, 33].

# 5. Combination Strategies to Prevent Tumour Relapse

Drug-tolerant persister cells provide a rationale for combination or sequential treatment because therapies that efficiently kill the bulk tumour may leave behind a metabolically distinct residual population. A second intervention can therefore be directed against vulnerabilities that emerge during persistence, including GPX4-dependent ferroptosis defence, fatty-acid oxidation, mitochondrial quality control or antioxidant metabolism. Preclinical studies support this principle, showing that targeting these dependencies can reduce persister survival and delay tumour regrowth in selected models [2, 3, 14].

The timing of persister-directed therapy is likely to be an important determinant of treatment efficacy. A simultaneous strategy may be useful when the relevant vulnerability is already present or appears early during therapy, whereas a sequential strategy may be more appropriate when metabolic dependencies emerge only after the residual persister state has formed. Sequential treatment also creates an opportunity to profile residual disease before selecting the second therapy. However, delaying intervention for too long could allow persister cells to progress towards stable resistance [2, 20].

Because DTP populations are heterogeneous, combination therapy will likely require biomarker-guided selection rather than a uniform approach. Molecular profiling, single-cell analysis and functional metabolic measurements could help identify whether residual cells depend predominantly on GPX4, OXPHOS, FAO or another pathway. Clinical implementation must also balance efficacy with toxicity because many metabolic targets are important in normal tissues, and additional therapeutic pressure may also favour the emergence of further adaptive states. The objective should therefore be to match the appropriate persister-directed treatment to the correct residual-cell state and therapeutic window [2, 4, 5, 20, 27].

## 6. Challenges in Translating DTP Metabolism into Cancer Therapy

Translating metabolic vulnerabilities of drug-tolerant persister cells into clinical therapy remains difficult because DTP populations are rare, heterogeneous and highly plastic. Their phenotype can differ between tumour types, treatments and even within the same tumour, and no universally accepted biomarker currently identifies DTP cells in patients. Single-cell and spatial approaches can detect rare residual states, whereas liquid-biopsy methods such as circulating tumour DNA may help monitor residual disease, but these approaches do not yet directly reveal which metabolic dependency a persister population relies upon. Reliable biomarkers and longitudinal monitoring will therefore be essential for identifying when DTP cells emerge and which vulnerabilities are therapeutically relevant [2, 5, 20].

Experimental models also incompletely reproduce the complexity of human residual disease. Two-dimensional cultures lack stromal, immune and extracellular-matrix interactions, while organoids, spheroids and xenografts each capture only part of the tumour microenvironment. These interactions can directly alter DTP metabolism. For example, in lung cancer with mutations in the epidermal growth factor receptor (EGFR), an $RGS5^{+}MYL9^{+}$ cancer-associated fibroblast population was reported to accept damaged mitochondria from persister cells through tunnelling nanotubes, thereby supporting DTP survival during EGFR-targeted therapy. Such findings indicate that metabolic dependencies identified in isolated cancer cells should be validated in physiologically relevant and patient-derived systems [2, 4, 34].

Metabolic plasticity and therapeutic selectivity create additional challenges. Metabolic programmes can vary substantially across therapy-persistent and therapy-surviving cancer-cell states. Selected DTP populations show increased dependence on oxidative phosphorylation, whereas related therapy-surviving states can display increased glycolysis and pentose phosphate pathway activity together with enhanced NADPH production and reduced oxidative phosphorylation [8, 10]. These contrasting findings emphasise that no single metabolic programme should be assumed to characterize all DTP populations. Inhibition of one metabolic pathway may also permit compensatory use of alternative metabolic routes. At the same time, many proposed targets, including mitochondrial respiration, glutathione metabolism, fatty-acid oxidation and ferroptosis-defence pathways, are also important in normal tissues. Successful therapy will therefore require functional evidence of

DTP-specific dependence, biomarker-guided patient selection and strategies that create an acceptable therapeutic window [2, 10, 22, 35].

Finally, most metabolic DTP-targeting strategies remain preclinical. Demonstrating that a pathway can eliminate persister cells in culture or animal models is not sufficient for clinical translation; candidate therapies must also show appropriate pharmacological properties, acceptable toxicity and selective activity against residual tumour cells. Prospective studies using samples collected before treatment, during residual disease and at relapse will be important for determining whether experimentally identified dependencies are reproduced in patients. Ultimately, trials specifically designed around the residual-disease window will be required to test whether targeting metabolically vulnerable persister cells can delay acquired resistance and improve durable treatment responses [2, 20].

# 7. Future Directions

Future progress in DTP metabolism research will require longitudinal analysis of residual disease rather than reliance on single time-point measurements. Samples collected before therapy, during residual disease and at relapse should be integrated with single-cell transcriptomics, spatial profiling and metabolic measurements to identify how persister states evolve over time. Patient-derived organoids, co-culture systems and relevant *in vivo* models can then help determine which metabolic vulnerabilities are reproducible across experimental and clinical settings. A major goal should be to develop biomarkers that distinguish true persister populations from dormant cells, cancer stem cells and genetically resistant clones and that identify the dominant metabolic dependency within each residual tumour [2, 5, 36].

Combining lineage tracing with single-cell and multi-omic profiling will be particularly valuable for linking the evolutionary origin of persister cells with their metabolic state. Recent studies have revealed multiple trajectories towards persistence and resistance, including treatment-associated programmes involving stress responses, lipid metabolism and signalling changes. Integrating these approaches with functional metabolic measurements could determine whether a vulnerability is present before treatment, induced by therapy or acquired during prolonged persistence [36–38].

Future studies should also distinguish metabolic alterations from true survival dependencies. Transcriptomic or metabolomic changes alone do not prove that a pathway is essential. CRISPR-based screens, pharmacological perturbation and metabolic-flux analysis can directly test which pathways are required during different stages of adaptation. Functional screening has already identified dependencies involving oxidative phosphorylation, glutaminolysis, cholesterol biosynthesis, iron homeostasis and NRF2-associated antioxidant programmes across drug-adaptation states. Similar approaches applied specifically to DTP populations, ideally in models containing stromal and immune components, should improve identification of actionable vulnerabilities [2, 4, 23, 36].

Computational and machine-learning approaches may help integrate increasingly complex single-cell, spatial and functional datasets and prioritise candidate therapies. For example, single-cell RNA sequencing combined with machine learning has been used to identify candidate drugs against machine-learning-classified DTP cells in patient-derived colorectal tumour organoids, although computational predictions still require experimental validation. Ultimately, the clinical value of DTP-directed metabolic therapy must be tested in biomarker-guided trials focused on the residual-disease window. Such trials should determine whether eliminating metabolically

vulnerable persister cells can delay acquired resistance and improve the durability of treatment responses [2, 20, 36, 39].

The major challenges limiting translation of DTP-directed metabolic therapies and key priorities for future research are summarised in Fig. 4.

## 8. Conclusion

Drug-tolerant persister cells represent an important barrier to durable cancer therapy because they allow a small fraction of tumour cells to survive treatment through reversible adaptive mechanisms. Increasing evidence shows that this survival can depend on metabolic reprogramming involving mitochondrial function, fatty-acid metabolism, antioxidant defence, glutathione metabolism and GPX4-mediated protection from ferroptosis. These adaptations can therefore act as both survival mechanisms and therapeutic vulnerabilities.

Among currently identified targets, GPX4 dependence and ferroptosis sensitivity provide some of the strongest preclinical evidence for selective elimination of persister cells. However, DTP metabolism is highly heterogeneous, and no single pathway is likely to represent a universal target. Successful translation will require functional identification of the dominant dependency within each residual tumour, together with reliable biomarkers, physiologically relevant models and carefully timed combination strategies.

Future studies should focus on the residual-disease window between initial treatment response and stable resistance. Targeting metabolically vulnerable DTP cells during this period may provide an opportunity to reduce the residual-cell reservoir from which acquired resistance and tumour relapse can emerge.

## Abbreviations

ACOX1 (acyl-CoA oxidase 1) • ALDH (aldehyde dehydrogenase) • BSO (buthionine sulfoximine) • CDK4/6 (cyclin-dependent kinases 4 and 6) • CSC (cancer stem cell)

DHODH (dihydroorotate dehydrogenase) • DTP (drug-tolerant persister) • EGFR (epidermal growth factor receptor) • EMT (epithelial–mesenchymal transition) • FAO (fatty acid oxidation)

FSP1 (ferroptosis suppressor protein 1) • GPX4 (glutathione peroxidase 4) • GSH (reduced glutathione) • IGF-1R (insulin-like growth factor 1 receptor) • KDM5A (lysine demethylase 5A)

MAPK (mitogen-activated protein kinase) • NADPH (reduced nicotinamide adenine dinucleotide phosphate) • NRF2 (nuclear factor erythroid 2-related factor 2) • OXPHOS (oxidative phosphorylation) • PDAC (pancreatic ductal adenocarcinoma)

PGRMC1 (progesterone receptor membrane component 1) • PINK1 (PTEN-induced kinase 1) • PPARα (peroxisome proliferator-activated receptor alpha) • ROS (reactive oxygen species)

## Declarations

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# Author Contributions

Mohit Kumar conceived the review, carried out the literature search and synthesis, and prepared the manuscript and original schematics.

# Competing Interests

The author declares no competing interests.

# Ethics Approval

Not applicable. This review synthesises previously published literature.

# Consent to Participate

Not applicable.

# Consent to Publish

Not applicable.

# Data Availability

No new datasets were generated or analysed for this review.

# AI Use Declaration

ChatGPT (OpenAI) assisted with manuscript structure, drafting, language refinement, reference organisation and figure planning. The original schematics were assembled in Microsoft PowerPoint and redrawn for this version from the supplied figures using ChatGPT-assisted vector drawing code. The author is responsible for the accuracy of the text, citations and illustrations.

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# Figures

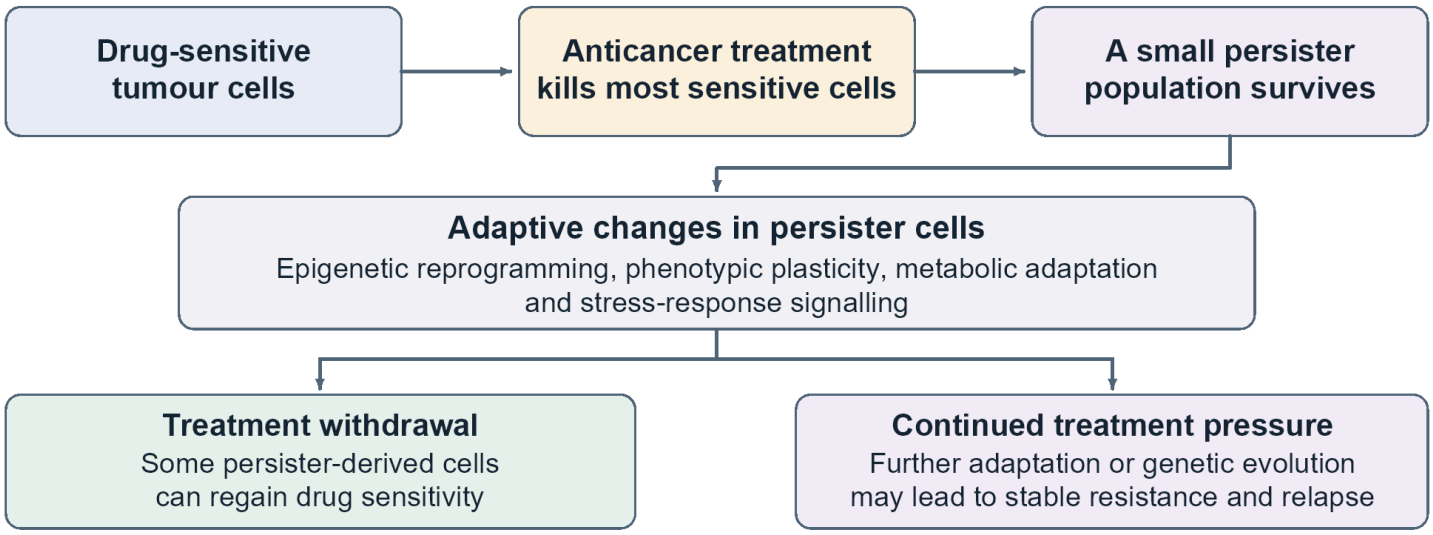


**Figure 1**

Evolution of drug-tolerant persister cells during cancer therapy. Treatment eliminates most sensitive cells. A small persister population survives and may undergo epigenetic, phenotypic and metabolic adaptation. After treatment withdrawal, some persister-derived cells regain drug sensitivity. Continued treatment can favour further adaptation, stable resistance and tumour relapse

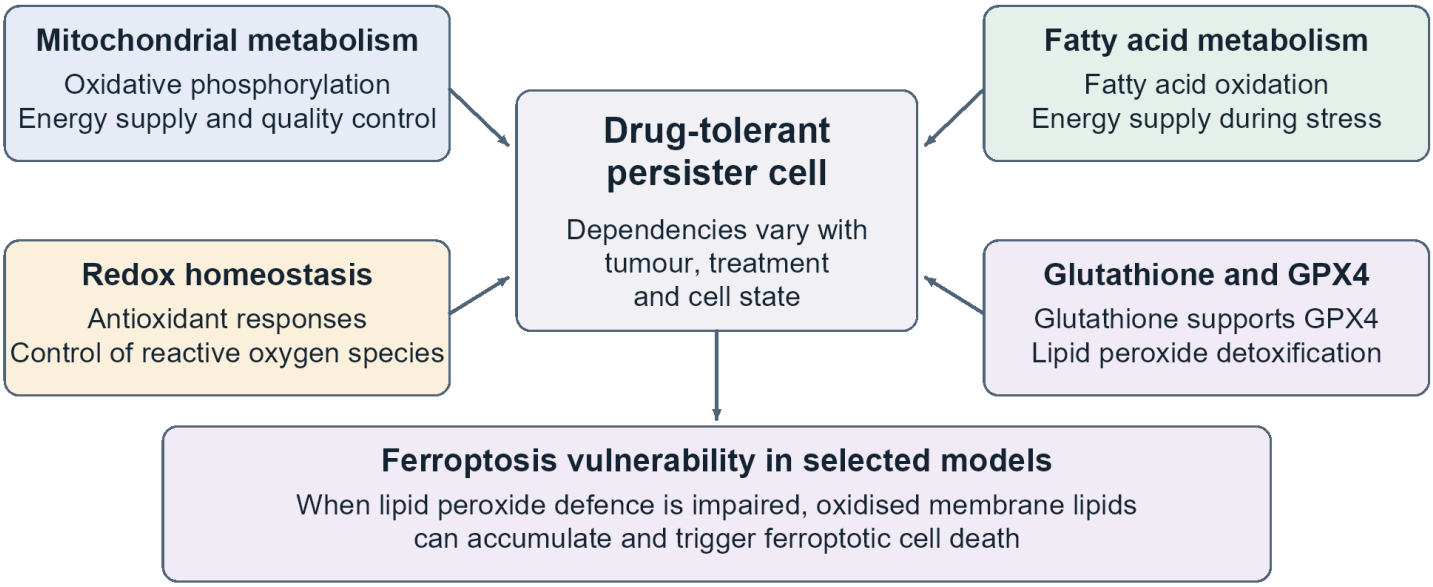


**Figure 2**

Context-dependent metabolic adaptations in drug-tolerant persister cells. Mitochondrial respiration, fatty acid oxidation, antioxidant responses and glutathione peroxidase 4 (GPX4) can support survival. In susceptible states, loss of lipid peroxide defence can trigger ferroptosis

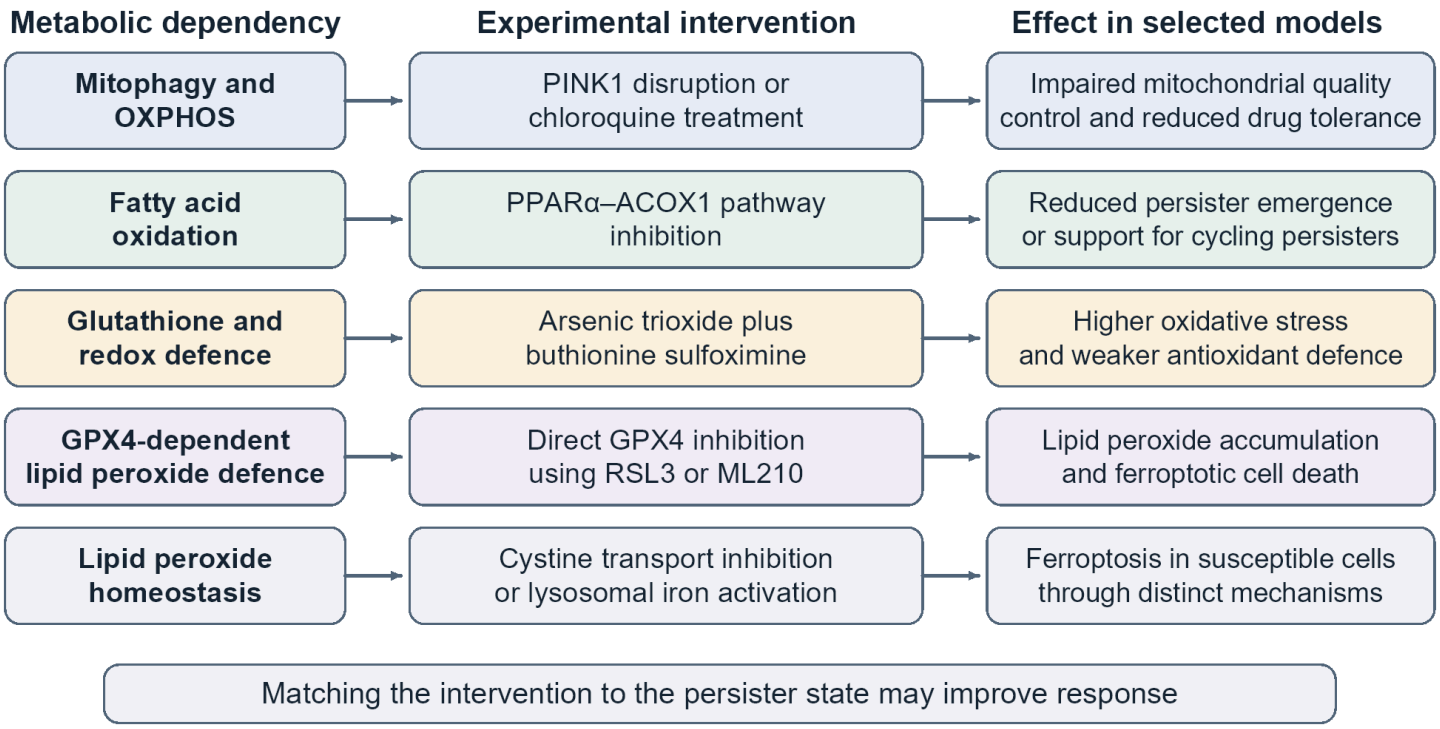


**Figure 3**

Experimental strategies targeting metabolic dependencies in drug-tolerant persister cells. PINK1 disruption, chloroquine, PPARα–ACOX1 inhibition, arsenic trioxide plus buthionine sulfoximine, GPX4 inhibitors, and interventions that alter cystine transport or lysosomal iron are shown with their effects in selected models. Abbreviations are defined in the text

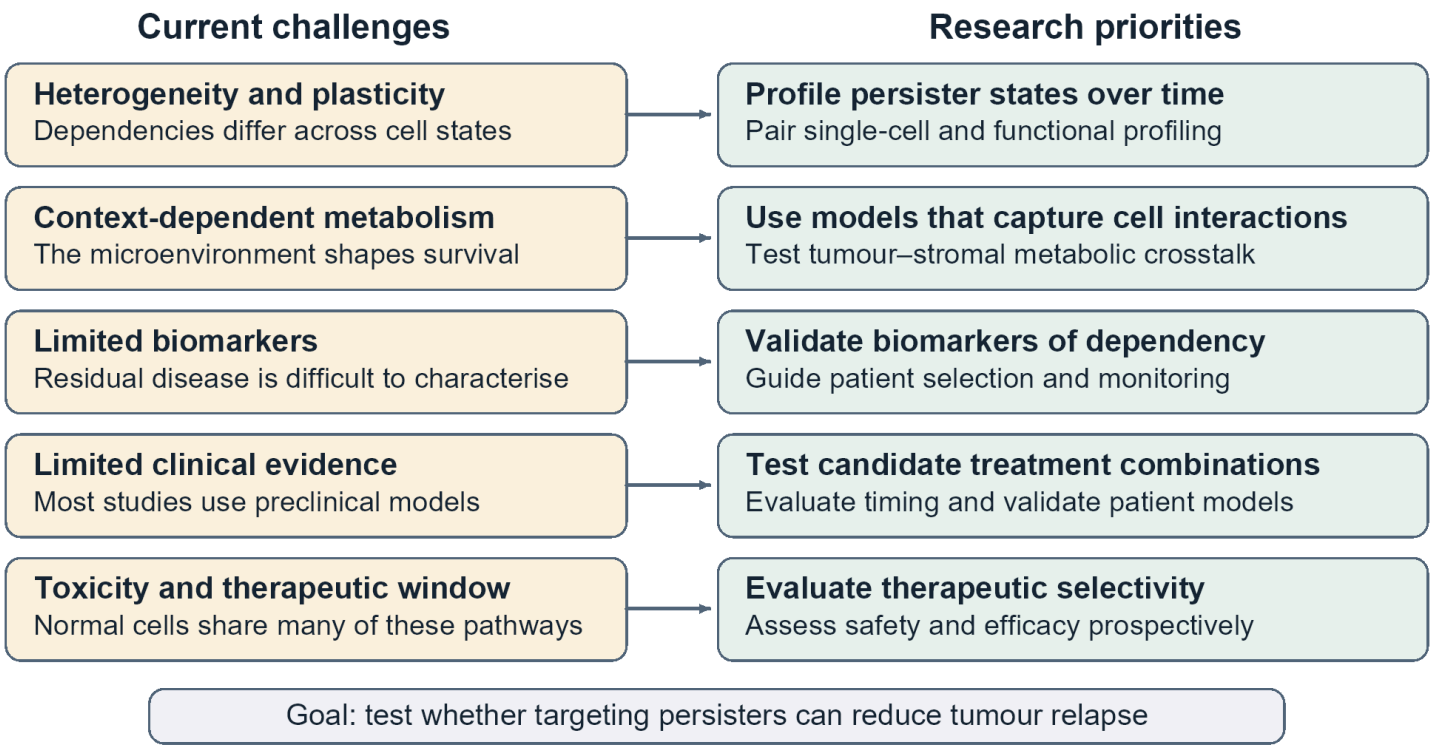


**Figure 4**

Barriers and research priorities for targeting drug-tolerant persister cells. The schematic links heterogeneity, microenvironment-dependent metabolism, limited biomarkers, a predominantly preclinical evidence base and toxicity concerns with approaches for testing candidate vulnerabilities