Trends in Cancer

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Opinion

Persisting cancer cells are different from bacterial persisters

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The persistence of drug-sensitive tumors poses a significant challenge in cancer treatment. The concept of bacterial persisters, which are a subpopulation of bacteria that survive lethal antibiotic doses, is frequently used to compare to residual disease in cancer. Here, we explore drug tolerance of cancer cells and bacteria. We highlight the fact that bacteria, in contrast to cancer cells, have been selected for survival at the population level and may therefore possess contingency mechanisms that cancer cells lack. The precise mechanisms of drug-tolerant cancer cells and bacterial persisters are still being investigated. Undoubtedly, by understanding common features as well as differences, we, in the cancer field, can learn from microbiology to find strategies to eradicate persisting cancer cells.

Highlights

Residual disease comprises a small subset of cancer cells that temporally tolerate various drugs, resulting in therapy failure and eventually recurrence.

Residual tumor cells have been thought to resemble bacterial persisters, a subpopulation of bacteria that survive lethal doses of antibiotics.

The exact mechanisms of residual disease and bacterial persistence remain elusive and appear to involve diverse mechanisms ranging from quiescence, epigenetic modifications, transcriptional adaptations, to an altered metabolism.

Although residual cancer cells and bacterial persisters share similarities, their underlying evolution is different: cancer cells persist for self-preservation, whereas bacterial persistence is a survival strategy at the population level.

We suggest using the term drug-tolerant cells (DTCs) instead of drug-tolerant persisters (DTPs) in oncology.

Residual cancer cells are a major challenge in oncology

The concept of minimal residual disease (MRD) has garnered significant interest in the field of oncology for many years. MRD refers to the subset of cancer cells that remain after various types of treatment, whether at the primary site, in the bloodstream as circulating tumor cells, or in distant organs as disseminated tumor cells [1–3]. Although a tumor may be initially sensitive to the treatment, not all tumor cells are eradicated, leading to recurrence at the primary site and/or metastatic sites. Understanding the mechanisms underlying MRD is crucial, given its association with high mortality rate and poor prognosis. Moreover, since these residual cells are continuously exposed to therapeutic interventions, they eventually acquire secondary resistance mechanisms resulting in refractory disease and pan-resistance [4–6]. Hence, successful mitigation of transient drug tolerance could potentially herald cures for patients with drug-sensitive malignancies.

In the pursuit of unraveling the mechanisms of MRD, scientists have often compared residual cancer cells to bacterial persisters. Bacterial persistence, a well-established phenomenon in microbiology, refers to the ability of a subpopulation (known as persisters) within a bacterial population to survive lethal doses of antibiotics [7]. In recent years, the term 'persister' cell is also commonly used in oncology and it is often employed as a synonym for 'dormant', 'tolerant', or 'quiescent' cells [3]. Although residual cancer cells are a subpopulation of tumor cells that survive the therapy onslaught, the question arises as to whether they use similar mechanisms as bacterial persisters. We aim to explore the comparability between residual disease in cancer and bacterial persistence.

How do residual cells persist?

MRD is a recurring challenge across diverse cancer types following various therapeutic regimens. Cancer cells will obviously survive if drug exposure is eluded, exemplified by brain metastases sheltered by the blood–brain barrier or pancreatic cancer islets shielded by a robust stromal component

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[8]. Here, we focus on tumors subjected to equitable drug exposure, where a significant fraction of tumor cells succumbs to treatment. Another obvious mechanism contributing to residual survival is secondary drug resistance. This encompasses refractory cells that persist in the presence of drugs due to (epi-)genetic alterations. Educative examples comprise alterations in drug targets, as observed in BRAF inhibitor resistance within melanoma [9], or the restoration of homology-directed DNA repair culminating in PARP inhibitor (PARPi) resistance in *BRCA*-mutated cancers [10,11]. A crucial question pertains to the temporal origin of such mutant cells: whether they pre-exist prior to treatment or emerge during therapy. Genetically defined model systems, such as the elegant CRISPRa-based CATCH tool employed by Umkehrer *et al*. [12], illuminate these dynamics by capturing and tracking rare tumor cell clones. Their findings support that kinase inhibitor resistance likely arises *de novo* under drug pressure, rather than being selected from pre-existing resistant clones. Akin observations manifest in our genetically engineered mouse models for *BRCA1*-mutated breast cancer, where deep sequencing or immunohistochemical tissue analysis does not detect inactivating mutations in *53bp1* or *Rev7*, resulting in partial restoration of homologous recombination and PARPi resistance, to be present in primary tumors [13,14]. This concurs with data obtained from therapy-sensitive patient-derived xenografts of BRCA1-deficient breast cancer [15]. Mutations resulting in BRCA1 restoration differ among individual mice, which argues against a shared pre-existing mutation that would otherwise be seen in several animals [15]. Together, this underscores the presence of underlying non-mutational biological mechanisms that mediate drug tolerance, acting as a transitory phase preceding fully fledged drug refractoriness caused by mutations. It also explains the circumstantial clinical observation that relapsing tumors may respond again to the same therapy and refractory disease emerges only after repeated dosing.

Also in microbiology, there is a clear distinction between drug-resistant bacteria that grow in the presence of antibiotics and the presence of a small subpopulation of cells that are drug-tolerant within an otherwise antibiotic-susceptible clonal population [16]. The latter are called bacterial persisters and they are characterized by multidrug tolerance that contributes to the antibiotic recalcitrance of biofilm infections. In general, persistence employed by various microbiological organisms is described as a bet-hedging strategy [17]. In evolutionary biology, this strategy relies on phenotypic heterogeneity to ensure survival in diverse environments. The nongenetic stochastic differentiation leads to different phenotypes, each with unique fitness-related traits in an isogenic population. Although these traits come at a cost that does not benefit most individual clones, they offer an advantage when environmental conditions abruptly change. This allows the surviving persisters to propagate the shared genome and secure the long-term survival of the colony [18–20]. Among microbiological organisms, bacteria have been extensively studied for employing persistence as a bet-hedging strategy. In dense populations with fluctuating nutrients and environmental conditions, bacteria need mechanisms to endure. During evolution, bacteria became versed to make use of contingency loci to adapt to changing habitat by increasing their fitness. In a similar manner, antibiotic treatments drastically alter surrounding conditions, leading to the emergence of nongrowing dormant cells with a survival advantage. While one might expect all bacteria to adopt this phenotype for enhanced fitness, it comes at the cost of reduced proliferation, which is unfavorable under normal conditions. Therefore, bacterial persistence is an altruistic behavior of a small subset of bacteria to ensure the long-term survival of the colony [19]. This persistence arises either spontaneously or is triggered by stress signals from various sources; the latter being the more frequent form of persistence [21]. Interestingly, unlike resistance mechanisms, it implies that the potential to become a persister exists in every bacterium. The persisters can repopulate the environment after antibiotic clearance, thereby sustaining infection.

In the literature, residual cancer cells are often likened to bacterial persisters. Undoubtedly, there are several parallels (Figure 1) and in the cancer field we can learn from drug-tolerant bacteria. The

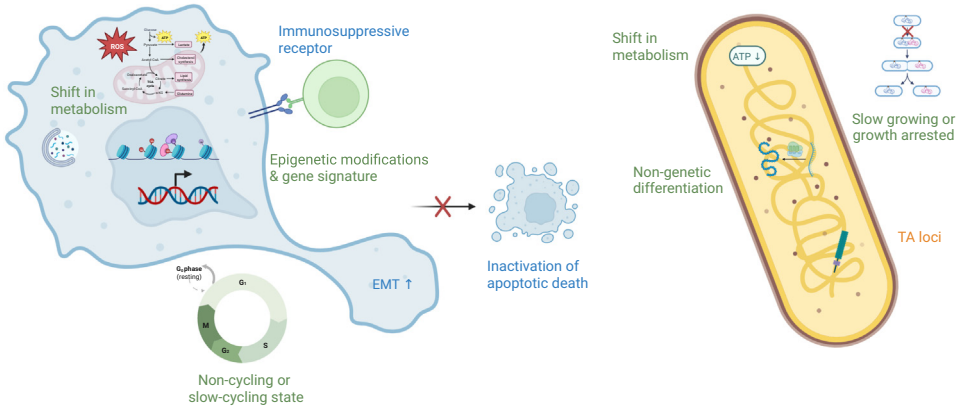

Figure 1. Comparison of the intrinsic mechanisms of drug tolerance in residual cancer cells and in bacterial persisters. Residual cancer cells can rely on different intrinsic mechanisms of drug tolerance. Many of them (in green) are shared with bacterial persisters. However, some of them are unique to each cell type (in blue for cancer cells, in orange for bacteria). Abbreviations: EMT, epithelial-to-mesenchymal transition; TA, toxin–antitoxin.

underlying mechanism of drug tolerance in persisting cells may be intrinsic to the cancer cells or bacterial persisters themselves or facilitated by their specific microenvironment. This section provides a brief overview of the intrinsic and extrinsic mechanisms that have been put forward to explain such drug tolerance. We only highlight some key concepts, acknowledging that these mechanisms are often context-dependent, tissue- or strain-specific, and can be influenced by the therapy employed.

Cell cycle effects and dormancy

Bacterial persisters constitute only a tiny frequency in a bacterial population (between 10^{-6} and 10^{-2}), which makes their study challenging. Nevertheless, it is widely accepted that bacterial persisters are slow-growing or growth-arrested [22] and dormancy is believed to be one of the key mechanisms of persister formation [23]. This trait makes them particularly resistant to antibiotics, which are primarily designed to target pathways active in growing bacteria. By entering a dormant state, persisters inactivate these targeted pathways, effectively neutralizing the impact of antibiotic treatments.

The emergence of dormancy in bacteria can occur through several pathways, especially under hostile conditions, such as antibiotic exposure. One such pathway involves the SOS response, a well-conserved DNA repair mechanism. This response serves a dual role: on the one hand, it can help bacteria recover from the antibiotic onset by efficient repair of the damaged cell, preventing cell death [24]. DNA damage can therefore induce persister formation [25]. On the other hand, the SOS response can induce genome plasticity and/or activate genes of a toxin–antitoxin (TA) module [23]. TA loci are ubiquitously present in the bacterial genome and typically consist of a toxin inhibiting essential cellular processes, including cell wall synthesis, ATP production, transcriptional and translational processes, and an antitoxin countering its effects. Historically, much of our understanding of TA systems was derived from studies in *Escherichia coli* K-12 laboratory strains, with a particular focus on type II RNase toxins as key regulators [26–28]. Regarding the TA regulation, further studies suggested a stochastic accumulation of the 'stress alarmone' (p)ppGpp in persister cells, activating the Lon protease through polyphosphate synthesis. This would trigger the proteolysis of antitoxins, releasing toxins and halting global translation. Such (p)ppGpp-dependent TA-induced 'dormancy' would allow a small subset of cells to transiently withstand antibiotic

treatments and resume growth once the antibiotics are removed [29]. Moreover, this (p)ppGpp alarmone can be induced by nutrient starvation and by antibiotics themselves and can alter various metabolic pathways, promoting persister formation [23,24,30].

The TA model has recently been challenged as a contingency mechanism [31]. Further studies found that, in the case of the ten RNase toxins in *E. coli*, various abiotic stresses, such as antibiotics or heat shock, induce the transcription of TA operons without leading to toxin-specific RNA cleavage. This suggests that toxins may not be liberated from TA complexes under these stress conditions. Transcriptional upregulation may instead result from the relief of autoregulation due to antitoxin degradation. Antitoxin mutants lacking DNA-binding activity and Lon protease mutants no longer display transcriptional activation under stress conditions. In essence, the role of chromosomally encoded TA systems in stress responses and antibiotic persistence appears to be less significant than previously thought [31]. A recent study, utilizing microfluidics time-lapse microscopy and fluorescent reporters, identified a subpopulation of bacteria with low ATP levels that survived antibiotic treatment [32]. Lowering the energy level of the cell inhibits translational processes independently of TA or (p)ppGpp expression, suggesting that 'these low ATP cells are formed stochastically as a result of fluctuations in the abundance of energy-generating components' [32]. The question remains whether there is a genetic program selected during evolution that regulates such stochastic fluctuations.

Not surprisingly, growth arrest or dormancy is linked to drug tolerance in oncology as well. A cornerstone of anticancer strategies centers on targeting the cell cycle by impeding DNA metabolism or mitotic spindle formation. Such therapeutic approaches include alkylating agents, platinum drugs, topoisomerase inhibitors, inhibitors of DNA metabolism, and taxanes. The consequence of these interventions is the stalling of pivotal enzymes implicated in replication, transcription, or mitosis, eventually triggering cellular demise. Therefore, it has long been known that chemotherapy mainly targets cells in S, G2, and M phase, whereas G1 or noncycling (G0) cells manifest heightened resilience [33]. G0 or G1 cells can rectify the inflicted damage prior to S phase entry, thereby evading therapy damage. The noncycling status (or quiescence or dormancy) may be transient and, when the drug is gone, tumor cells re-enter the cell cycle. Even senescent tumor cells may partake in cell cycle resurgence [34–37]. A crucial question that emerges is whether the quiescence is a therapy-induced response or a pre-existing trait? Here, the responses to the maximum tolerated dose (MTD) of cisplatin in a mouse model for *BRCA1*-mutated breast cancer provide useful insights. In this model, restoration of BRCA1 function is impossible due to a large deletion of the *Brca1* gene. In contrast to PARP inhibition, the MTD of cisplatin inflicts higher levels of DNA damage that the BRCA1-deficient cells cannot compensate for without restoring BRCA1 function, eliminating the emergence of secondary resistance. Yet, these tumors evade eradication, they eventually regrow, and they show repeated susceptibility to cisplatin exposure [38]. Hence, under these conditions we can clearly exclude the presence of cells with acquired mechanisms causing secondary drug resistance among the residual cells. Scrutinizing these residual tumors revealed a population akin to G0 cells, which are enriched post-MTD cisplatin exposure, despite its near-total tumor cell eradication [38]. This presence of quiescent cells in the primary tumor contrasts with the aforementioned emergence of secondary resistance-causing mutations observed in PARPi resistance, as exemplified by *53bp1* or *Rev7* loss.

Importantly, genetically engineered mouse models of *BRCA1*-mutated breast cancer show that drug tolerance fostered by cell cycle effects is surmountable. If sufficient DNA damage is inflicted, drug-tolerant residual cells give in. Intensified cisplatin treatment, achieved by curbing its *Lrrc8d*-mediated uptake in nontumoral cells, doubled the MTD and effectively cured the mice, a feat replicated with the MTD of nimustine, a DNA crosslinker inducing greater interstrand crosslinks than

cisplatin [38,39]. Although these observations may be specific for BRCA-deficient tumors that lack homology-directed DNA repair, we think the basic concept is important: if a more intensified chemotherapy can be achieved in a safe manner in patients, patients with drug-sensitive cancers may be cured and drug tolerance can be overcome. Indeed, studies of patients with stage III *BRCA1/2*-associated breast cancer support this concept [40,41]. In parallel, this concept raises the question whether the increased dose is just enough to kill dormant cells or whether there is more to residual disease than just cell cycle arrest.

Phenotypic plasticity and metabolic shift

Our understanding of bacterial persister formation has undergone significant revision in recent years as the role of TA systems in stress responses and antibiotic persistence is now questioned [31,42]. Current research suggests that bacterial persistence encompasses more than just a state of dormancy [43]. Persisting cells, while often dormant, are not merely passive; they exhibit active characteristics even under antibiotic treatment [23]. Consequently, it is crucial to consider both genetic and epigenetic factors in understanding persister formation. Genetic changes, while influential in creating a high persister state, do not solely account for persistence. Phenotypic plasticity is also pivotal in conferring drug tolerance [44]. This idea aligns with the bet-hedging strategy's transient and reversible nature.

DNA methylation, a well-known epigenetic regulatory mechanism in bacteria, influences the expression of various genes, including those related to persister formation. Studies have established a direct association between DNA methylation patterns and the potential for persister formation [45]. Beyond this epigenetic modulation of gene expression, bacterial persisters also undergo significant metabolic shifts [23,30,46,47]. They transition into a repressed yet still active metabolic state, characterized by reduced ATP and energy levels [48,49], reduction of oxidative phosphorylation [30], alterations in amino acid and lipid metabolism [30,48], use of different carbon sources, and accumulation of insoluble proteins [49]. Such variations could be a response to nutrient deprivation or, in the case of intracellular persisters, the exploitation of metabolites from the host cell [30]. This metabolic profile distinguishes persister from fully dormant cells, which do not regrow once the antibiotic is gone. Further emphasizing the complexity of persister cells, various mechanisms, such as upregulation of membrane proteins (including drug efflux pumps) and the formation of nonwalled cells [23], have been observed. These findings underscore that dormancy in bacterial persisters can manifest at different levels.

Despite these insights, the precise mechanisms governing bacterial persistence remain somewhat elusive. Studying the mechanisms of stochastic cellular fluctuations of metabolites may be a useful avenue in deciphering the precise mechanisms behind bacterial persistence. Moreover, the metabolic state of bacteria significantly impacts the efficacy of antibiotics. Bacteria in their exponential growing phase have high metabolic needs, accompanied by metabolic stress, and those stresses can affect the pathways targeted by the used antibiotics [46]. As Lopatkin *et al*. [50] noted, ‘ … antibiotic lethality uniformly depends on the bacterial metabolic state at the time of treatment, rather than growth rate.’ Hence, targeting metabolic pathways, or reprogramming the metabolic shift (e.g., by supplementing the missing nutrients [30]), present promising therapeutic strategies to enhance treatment outcomes.

In the field of oncology, the relevance of epigenetic changes in drug tolerance emerged from the work of Sharma *et al*. [51], who explored the response of an *EGFR*-mutant non-small cell lung cancer cell line to tyrosine kinase inhibition. Despite the effective elimination of most cells, a small subpopulation displaying remarkable resilience emerged under drug concentrations exceeding the IC_{50} by 100-fold. These survivors, termed ‘drug-tolerant persisters’ (DTPs), account

for 0.3–5% of the cell population and, notably, lack stable resistance. Withdrawal of the drug restores their susceptibility and, the authors emphasized, the resemblance to bacterial persisters [51]. Delving into the intricacies of DTPs revealed widespread shifts in chromatin modifier enzymes' activity, such as elevated KDM5A/Jarid1A (a histone H3K demethylase) and histone deacetylases (HDACs) in lung cancer [51] or elevated BRD4 in breast cancer [52]. More recently, residual cells in the same PC9 lung cancer cell line used by Sharma *et al*. [51] were further studied using lineage tracing with a barcode library with fluorescent reporters and subsequent single-cell RNA sequencing analysis [53]. This study showed that, similar to bacterial persisters, there are cycling and noncycling persisting cells that arise from different cell lineages with distinct transcriptional and metabolic programs. The cycling persisting cells are characterized by an upregulation of antioxidant gene programs and a metabolic shift to fatty acid oxidation. Alleviating reactive oxygen species (ROS) levels further increased the number of cycling persisting cells [53]. This correlated with an increased abundance of carnitine-linked fatty acids in the cycling residual cells, which are substrates of mitochondrial β-oxidation. Intriguingly, this protective program of the cycling persisting state is transient and the progeny of these cells, as well as the progeny derived from the noncycling residual cells, regain drug sensitivity [53]. This suggests the presence of two independent, reversible, transcriptional programs among residual cells, that can both enable cell survival and foster regrowth of the tumor. While noncycling residual cells avoid damage induction by lying low, there are also cycling cells that manage to decrease therapy-induced damage. A mechanism regulating these lineage directions has not been discovered and it is likely that these phenotypes emerge from the inherent heterogeneity within the cancer cell population [54]. This finding highlights the importance of looking beyond dormancy in understanding residual disease, also in oncology.

That only a small fraction of cancer cells survive therapy spurred the cancer stem cell (CSC) hypothesis [55,56]. This hypothesis delineates a hierarchical structure within tumors, highlighting a subset of cells with self-renewal potential capable of repopulating heterogeneous tumors [57]. CSCs share traits akin to normal stem cells, such as self-renewal potential, temporal quiescence, epithelial-to-mesenchymal transition (EMT), and heightened resilience to external stress [58]. Consequently, targeting CSCs, as opposed to rapidly proliferating non-CSCs or postmitotic differentiated tumor cells, appears essential for effective tumor eradication. Despite two decades of intensive research about the CSC concept, the development of a convincing therapy targeting CSC in solid cancers remains elusive. Instead, more studies indicate that cancer stemness is a dynamic and plastic phenomenon [59–62], implying that there is a reciprocal phenotypic shift between CSC and non-CSC to which transient mechanisms such as quiescence, EMT, or epigenetic alterations of transcriptional or metabolic programs contribute. Moreover, proving the existence of CSCs in real tumors poses challenges. The gold-standard assay, involving transplantation of limiting dilutions of tumor cell populations into immunodeficient mice to assess tumor-initiating capacity, may primarily measure cell survival post-tumor dissociation and xenograft efficacy [56,62]. Useful markers to unambiguously identify CSCs are also limited, especially in solid tumors [56,60,62]. For some tumors, it is plausible that most tumor cells exhibit self-renewal plasticity, rendering the CSC concept less applicable. Consequently, recent research in the context of residual disease has shifted focus towards understanding how phenotype switching and plasticity provide survival advantages [3,63].

In the context of carcinomas, one prominent epigenetically regulated phenomenon is EMT [61]. While the relevance of a complete transition to a mesenchymal phenotype remains debated in human cancer, increasing evidence supports the presence of a partial EMT, especially in invasive and metastatic tumors [60,61,64]. In tumor tissues, distinguishing residual mesenchymal cancer cells from reactive stroma can be challenging. Nevertheless, the temporal and spatial regulation of

EMT aligns with the transient and plastic nature of residual cancer cells. Like bacterial persisters, residual cells are characterized by an altered metabolism that favors survival. Those metabolic shifts include evasion of apoptotic death [65–69], alterations in mitochondrial and energy metabolism, alterations in mitochondrial oxidative phosphorylation and ROS levels, lipid metabolism, and autophagy [69–75].

More recently, a novel phenotype of tumor cell plasticity has emerged in colon, breast, and prostate cancers: the presence of a transient embryonic diapause-like state [66,76]. This state resembles the developmental delay observed under stress conditions and is characterized by a distinct transcriptional signature including downregulated MYC and mTOR signaling alongside upregulated autophagy in residual tumors.

Hence, like phenotypic alterations and metabolic shifts in bacterial persisters, these data suggest that understanding the dynamics of tumor cell plasticity is crucial in comprehending tumor resilience and devising innovative therapeutic strategies. It shows the complexity of residual disease and that there are various sources of cancer cell persistence, even in the absence of gene mutations that cause refractory disease. These sources include both quiescent cells as well as cycling cells with epigenetically driven variations resulting in protective metabolic programs, both of which are already present before treatment.

The role of immune system and microenvironment

Bacterial persisters are adept at not only withstanding antibiotics, but also evading host defense mechanisms [45]. A common example of this is found in intracellular persisters, which can inhibit phagolysosome maturation or acidification, allowing them to survive post phagocytosis. Immune cells of the host can also trigger persister formation by sequestering nutrients, known as nutritional immunity [77]. Furthermore, ROS, produced by the immune system during infection, are considered key stressors that can trigger bacterial persistence [78–80].

In many cases, bacterial colonies are enclosed in a biofilm: 3D structures composed of an extracellular polymeric matrix [81]. These biofilms, housing slow-cycling bacteria and persisters, act as formidable barriers against both immune cell infiltration and antibiotic penetration [19,82]. Moreover, they provide protection against oxidative stress, a critical mechanism employed by certain antibiotics to kill bacteria [83]. Within the biofilm, a new microenvironment with different nutrients and oxygen gradients is shaped, sustaining a niche favorable for persister formation [24]. The formation of the biofilm can be further assisted by different components such as fibronectin, whose expression is enhanced during the SOS response [24], or by the regulation of DNA methylation [84]. Other strains of bacteria form structures like granulomas or pseudocapsules, which similarly create a hypoxic, nutrient-deprived environment, shielded by fibrin to limit antibiotic diffusion and protect against phagocytosis [85].

Parallels can be drawn with residual tumor cells, which, akin to bacterial colonies, may reside within specialized niches that support their tolerant phenotype. The tumor microenvironment (TME) plays a pivotal role in this context. It significantly influences the delicate balance between a state of quiescence and the potential for proliferation and regrowth in residual disease [86–91].

While not fully understood, the mechanisms governing immune surveillance and immune escape are central to the therapy response of residual tumor cells [88,92]. Despite the emergence of new therapeutic approaches combining immunotherapy with conventional cancer therapy, complete eradication of residual tumors remains a challenge [93]. This problem may stem from redundant immunosuppressive factors expressed by both tumor cells and TME components. Residual

tumor cells can modulate various inflammatory and innate immune response pathways, including IL-6 and -10, type 1 interferon, JAK/STAT, TGF-β, and NF-kB [3,93–95], as well as the expression of PD-L1, CTLA-4, CD47, CD80, and CD86 [88,93,95–97]. While some of these factors exert antitumoral effects, others promote tumor cell survival. For example, PD-L1 or CD47 inhibit effector immune cells, thus shielding tumor cells. Type 1 interferon exhibits context-dependent dual roles, even inducing cancer cell reprogramming via histone-demethylase activity [98]. Moreover, immunosuppressive tumor cells not only create an immune-evading niche, but they may also get support from TME components [99]. Usually, T cells are recognized as key players in cancer immunoediting, yet recent studies have highlighted the roles of other immune cell types in the intricate tumor–TME crosstalk. Tumor-associated macrophages (TAMs), often associated with tumor promotion [88,99], display immunosuppressive effects on T cells, dendritic cells, and natural killer (NK) cells [96]. Notably, SPP1+ macrophages can hinder immune cell infiltration by fostering a desmoplastic environment together with FAP+ fibroblasts [100]. Targeting immunosuppressive macrophages has therefore shown promise as an alternative to immune checkpoint inhibitors [101]. TAMs have also been implicated in activating EMT and invasion processes [96,102], while NK cells appear to regulate dormancy and outgrowth of disseminated tumor cells in breast cancer [103].

Besides the immune system, various stromal cell types within the TME significantly contribute to mechanisms that likely foster drug tolerance in cancer cells. A prime example is cancer-associated fibroblasts (CAFs). These cells, in combination with cytokines produced by tumor-associated inflammatory cells such as TAMs, release signaling molecules that promote specific cancer cell phenotypes, including EMT [61,100]. They also exhibit a dual role in modulating immune responses, mediating both immune cell recruitment and immunosuppression [104]. Additionally, CAFs play a pivotal role in (re-)shaping the extracellular matrix, influencing cancer cell processes, chemoattractant gradients, tumor stiffness, and intratumoral pressure [3,60,99]. These factors collectively impact tumor vascularization, blood supply, and drug accessibility. Tumor vascularization is not only relevant for immune cell recruitment [99] but it also contributes to tumor cell dormancy, when insufficient [88]. The endothelium has also been shown to influence the entry into and exit of cellular dormancy [105]. Lastly, a feature of the TME that mirrors the conditions found in bacterial biofilms is hypoxia. Hypoxia in the TME aids in sustaining processes such as tumor vascularization, immune evasion, and the maintenance of cancer cell dormancy, among others [100,106,107].

To summarize, it is baffling how little we know and that mechanisms found in one cell system often do not generalize to other cell systems, even within bacteria or cancer cells. Hence, one cannot speak of the mechanism of drug tolerance, very much as the parable of the blind men describing an elephant. Regarding cancer, dynamic plasticity is induced by therapy and influenced by the TME. Notably, these events appear to be stochastic, driven by intratumoral heterogeneity and individual cell responses to therapy within the tissue [108]. Unlike a regulated genetic program, cancer cells seem to adapt opportunistically to external stress, promoting tumor regrowth.

Similar yet different

The term ‘persister cells’ has found its way into oncology due to the striking parallels between bacterial persister cells and residual cancer cells (Table 1, Key table). Both represent small subsets of the original population that persist after treatment, leading to recurrence. They share a nonproliferative or slow-growing phenotype that is refractory to various drugs and the mechanisms appear stochastic with distinct transcriptional changes and metabolic shifts. Moreover, the phenotypes of both bacterial persisters and residual tumor cells are reversible, suggesting

Key table

Table 1. Comparability between residual cancer cells and bacterial persisters

Drug-tolerant tumor cell	Bacterial persister
Represents a small subset of the original population	
Precise underlying mechanisms remain elusive	
Mainly nongenetic mechanisms	Persister genes have been suggested
Emerges from a heterogeneous pool of cancer cells	Altruistic program to enhance fitness of relatives within a colony
Nonproliferative or slow-growing state	
Phenotype is transient and reversible	
Phenotype switching driven by intratumoral heterogeneity	Phenotype switching as bet-hedging strategy
Repressed metabolic status arising, in part, from fluctuations in the nutrients and environmental conditions	
Immunosuppressive and hypoxic niche	
Immunoregulatory receptors or cytokines	Formation of biofilm, granuloma, or pseudocapsule
Recruitment of immunosuppressive cells from the tumor microenvironment	Intracellular persisters
Shielded by stroma	
Refractory to various drugs	
Fostering the emergence of secondary drug resistance	
Can persist for years without causing symptoms	

they are not genetically selected populations. Similar to residual disease, bacterial persisters can remain for years in the body without causing apparent disease and this persisting pool of bacteria can encourage the emergence or the acquisition of resistance via secondary mutations and/or via horizontal gene transfer [19,109,110]. An additional commonality is their dependence on the environment. They both create an immunosuppressive niche that is usually hypoxic, nutrient-deprived, and difficult for the drugs to reach. The formation of these persisting cells can be triggered by a range of stressors, both endogenous (such as those encountered during normal growth) and exogenous (including the therapeutic agents themselves). The complexity of these scenarios is further compounded by the fact that different stressors can activate similar persistence mechanisms and a single stressor can trigger various responses, resulting in a heterogeneous population [111]. This heterogeneity significantly complicates the development of effective therapeutic strategies against these elusive cells.

While there are notable similarities between bacterial persisters and residual cancer cells, significant differences also exist. Both rely on phenotypic switching and transcriptional reprogramming, but the nature of these changes varies considerably. Although cancer cells exhibit characteristics reminiscent of unicellular organisms and can activate ancestral survival programs under stress [112], the strategies employed by residual cancer cells to evade therapeutic effects are often unique and cannot be directly equated to bacterial mechanisms. For instance, concepts like CSCs and EMT are specific to cancer biology. Furthermore, cancer cells originate from the body's own tissues, necessitating different tactics for immune evasion compared to foreign microorganisms that also persist within host cells following phagocytosis. In our opinion, a key distinction lies in the underlying evolution of persistence (Figure 2). Bacterial persistence is a strategy for population-level survival, often involving kin selection phenomena

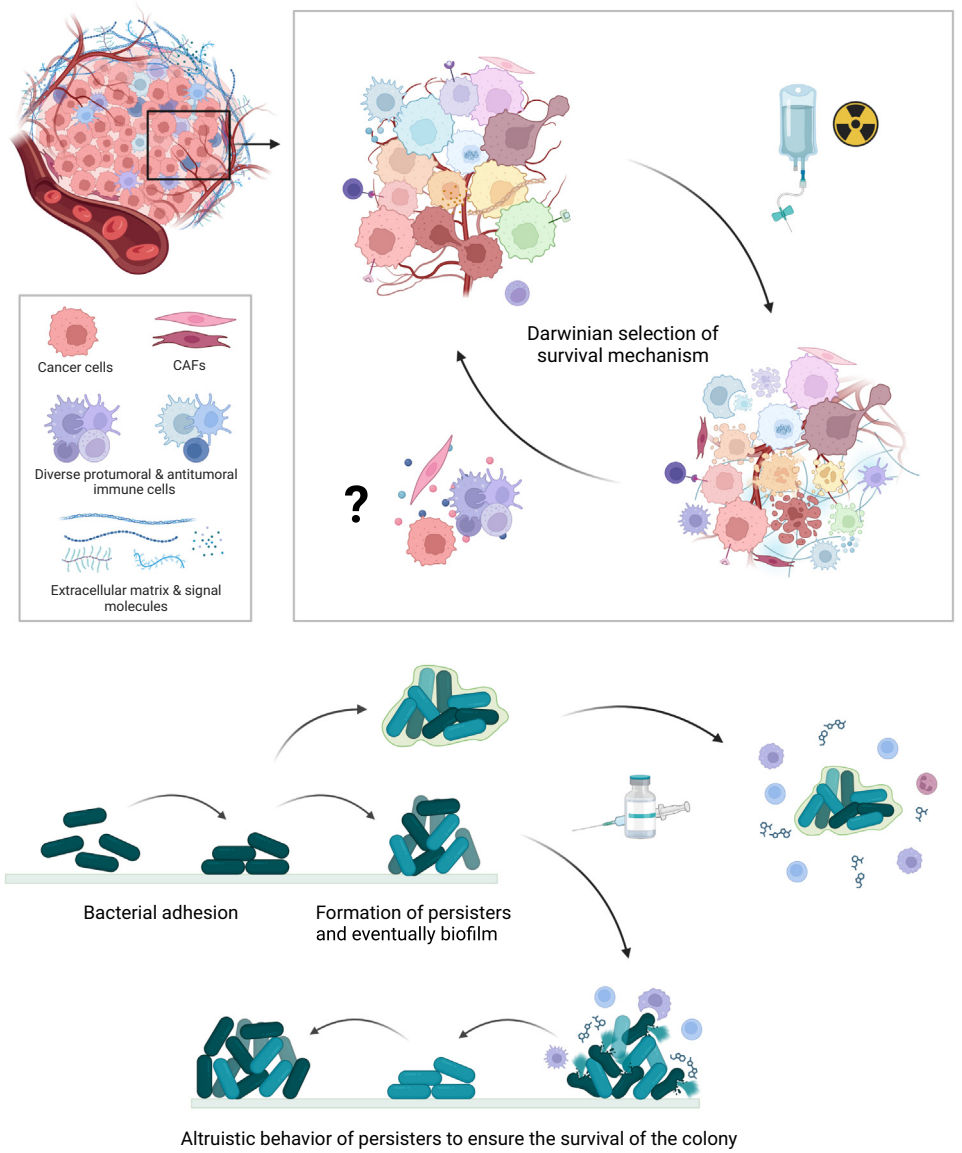

Figure 2. Persistence in cancer relies on different survival strategies compared to bacterial persistence. Stochastic processes within a tumor generate subpopulations of cancer cells with distinct phenotypes. Upon treatment, the tumor cells undergo a Darwinian selection, leaving only the ones bearing the mechanisms that allow them to fight for their own survival. The residual cancer cells can remain dormant for a while, often supported by a specific niche in the tumor microenvironment (TME). Changes in the TME eventually give the cue to awaken the residual cells, as the surrounding conditions are suitable again. In this regrowing tumor, each individual cell aspires to outcompete the others, for its own benefit. In a bacterial population, stochastic fluctuations in the transcription give rise to a subpopulation with reduced fitness. Upon antibiotic treatment, this subpopulation has yet a survival advantage, leading to recurrent infection. Therefore, the bacterial persisters can ensure the long-term survival of the colony, at the cost of their own proliferation, as an altruistic behavior. The formation of a biofilm can also support the survival of the persisters, as it shields them against the immune system and antibiotics. Abbreviation: CAFs, cancer-associated fibroblasts.

where a subset of bacteria sacrifices individual fitness for the colony's collective benefit, especially under adverse conditions like antibiotic treatment [4,113,114]. This altruistic behavior can save the whole colony when the environment changes so quickly that the bacteria would have otherwise no time to adapt. Conversely, cancer cells are typically characterized by their 'selfish' behavior at the cellular level. This shift from a host-defined fitness paradigm to a self-defined one is a feature of carcinogenesis [115]. While experimentally demonstrating this trait in residual cancer cells presents challenges, it is evident that these tumor cells represent dysregulated cells within the body. They have acquired growth advantages, favoring their own cellular fitness over the collective welfare of the organism. As a result, they engage in a competitive struggle for survival [4,115], thereby altering the balance of multicellular physiology and contributing to disease progression. An aggressive cancer cell population is genetically and phenotypically heterogeneous and residual cells persist by leveraging mechanisms that inhibit uncontrolled proliferation and evade cell death, even when damaged. This egoistic behavior contributes solely to the survival of individual cells, regardless of the fate of the others. As tumors develop, cancer cells do interact and cooperate to some extent, a trait acquired under selective pressure to enhance survival [116]. However, these interactions, detailed in further studies [116], are more relevant to the long-term evolution of cancer rather than the immediate survival of individual cells. In contrast, the drug-tolerant phenotype often represents a short-term adaptive mechanism for individual cell survival in response to sudden environmental threats. While this behavior may lead to the emergence of a resistant subclonal population, the primary aim of a residual cancer cell is self-preservation. Given these differences, it is crucial to understand both how and why these cells persist. Hence, to avoid confusion and acknowledge the distinct nature of their persistence, we suggest that persisting cancer cells should not be referred to as 'persisters' in the same context as their bacterial counterparts.

Concluding remarks

Research on residual cancer cells and bacterial persisters reveals striking similarities in their behavior. These persisting cells exhibit analogous features, prompting the utilization of parallel investigative approaches such as genetic screens, single cell studies with transcriptomics, or mathematical modeling [22], to unravel their persistence mechanisms. This convergence underscores the urgent need for enhanced therapeutic strategies targeting these drug-tolerant cells (DTCs), both in oncology and bacteriology (see Outstanding questions).

While cancer cells and bacteria diverge significantly in their biological foundations and therapeutic vulnerabilities, there exists a noteworthy overlap in treatment avenues. Indeed, certain antibiotics, notably the anticancer antibiotics such as anthracyclines and bleomycin, have demonstrated remarkable efficacy across a spectrum of cancer types in the clinic [117]. Additionally, the pursuit of inhibiting multi-drug-resistant ABC transporters, initially developed for combating cancer cells, holds promise in the context of bacterial infections. Notably, one study [118] showcased the potential of a taxane-based derivative in significantly enhancing antibiotic efficacy, even against persister populations.

However, we think that it is crucial to acknowledge that despite the cross-disciplinary insights, cancer cells and bacterial persisters employ distinct survival strategies. Brauner *et al*. [7] emphasize the necessity of distinguishing between diverse survival strategies to craft effective therapeutic interventions (Box 1). Given the limited diagnostic and therapeutic arsenal in both cancer and bacterial infection domains, the present moment beckons us to recognize that persisting cancer cells and bacterial persisters follow distinct trajectories driven by unique objectives. Only by comprehending the underlying motives for their persistence can we devise and implement strategies with the potential to eradicate them definitively.

Outstanding questions

How do DTCs arise in cancer? Are they pre-existing, induced by therapy, or a result of stochastic selection that may involve both?

What is the 'tolerome' of DTCs? Which precise mechanisms contribute to it?

Are there specific biomarkers to identify DTCs in cancer?

How can DTCs be killed? Are strategies to kill bacterial persisters useful to eradicate DTCs?

If dormancy is the mechanism of DTCs, should one rather try to wake them up or keep them dormant?

What is the mechanistic basis of bacterial persisters? Have we missed identifying their contingency genes?

Box 1. Defining tolerance, persistence, and resistance

In the comprehensive review by Brauner *et al*. [7], the distinctions among tolerance, persistence, and resistance in bacteriology are emphasized. Resistance is characterized by the fact that the microorganisms have a higher minimal inhibitory concentration, enabling continued growth despite the administration of high antibiotic doses. Tolerance is a somewhat general term and, in agreement with Kester and Fortune [119], it describes a condition that '…enables bacterial cells to survive a transient exposure to antibiotics at concentrations that would otherwise be lethal.' This may be due to an (epi-)genetic or environmental alteration. An example is slow growth of the population. There may even be dormancy, which is also referred to as 'drug indifference' when causing drug tolerance. In contrast to the aforementioned conditions, which characterize a whole population, '…"persistence" is the ability of a subpopulation of a clonal bacterial population to survive exposure to high concentrations of an antibiotic. … The slower rate of killing of the persistent subpopulation is non-heritable: when persistent bacterial cells are isolated, regrown and re-exposed to the same antibiotic treatment, the same heterogeneous response to the drug will be observed as in the original population, with the division of the population into persistent and non-persistent subpopulations' [7].

Brauner *et al*. [7] underscore the importance of classifying drug response correctly, as a misclassification can lead to therapy failure. Moreover, enhancing understanding of these mechanisms, which operate independently, can facilitate the development of more precise therapeutic strategies. Although these distinctions are well established within bacteriology, providing a solid foundation for novel treatment modalities, their delineation remains ambiguous in oncology. It thus becomes imperative to establish clear definitions within our field. To just copy the terminology from microbiology may be misleading, however. Hence, in oncology, we suggest avoiding the microbiology-derived term 'persisters' for the description of residual tumor cells that give rise to a tumor that is sensitive again when retreated. Instead, we suggest using the term drug-tolerant cells (DTCs), with the definition that drug tolerance is transient, in contrast to resistance.

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Declaration of interests

The authors declare no competing interests.

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