Cancer drug-tolerant persister cells: from biological questions to clinical opportunities

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Abstract

The emergence of drug resistance is the most substantial challenge to the effectiveness of anticancer therapies. Orthogonal approaches have revealed that a subset of cells, known as drug-tolerant 'persister' (DTP) cells, play a prominent role in drug resistance. While long recognized in bacterial populations which have acquired resistance to antibiotics, the presence of DTPs in various cancer types has come to light only in the last two decades, yet several aspects of their biology remain enigmatic. Here we delve into the biological characteristics of DTPs and explore potential strategies for tracking and targeting them. Recent findings suggest that DTPs exhibit remarkable plasticity, being capable of transitioning between different cellular states, resulting in distinct DTP phenotypes within a single tumor. However, defining the biological features of DTPs has been challenging, partly due to the complex interplay between clonal dynamics and tissue-specific factors influencing their phenotype. Moreover, the interactions between DTPs and the tumor microenvironment, including their potential to evade immune surveillance, remain to be discovered. Lastly, the mechanisms underlying DTP-derived drug resistance and their correlation with clinical outcomes remain poorly understood. This Roadmap aims to provide a comprehensive overview of the field of DTPs, encompassing past achievements and current endeavors in elucidating their biology. We also discuss the prospect of future advancements in technologies in helping to unveil the features of DTPs and propose novel therapeutic strategies that could lead to their eradication.

[H1] Introduction

There is an increasing appreciation that targeting genetic mutations does not lead to durable cures for the majority of patients with cancer. Despite the fact that some tumors initially demonstrate impressive responses to chemotherapy or targeted agents, achieving a minimal residual disease (MRD) state, the majority of tumors eventually recur often without known resistance mutations. This has led to an increasing interest in the role of non-genetic phenotypic heterogeneity in driving tumor recurrence.

In 2010, the pioneering study by Sharma and colleagues[1] described drug-tolerant persister (DTP) cells as the subpopulation of cancer cells that acquire a reversible drug-refractory phenotype. DTPs have since been identified across a wide range of tumor types in response to various chemotherapies and targeted agents, underlying MRD and accounting for tumor relapse[2,3].

Persistence derives from the bacterial literature and was first proposed in the 1940s to be mediated by dormant, non-dividing cell subpopulations that survive high concentrations of penicillin that otherwise kill the majority of bacteria[4,5]. Indeed, the slowing or arrest of bacterial proliferation has since been shown to widely promote tolerance to antibiotics[6,7]. While persistence has been extensively characterized in bacteria[8], defining and identifying cancer DTPs, their origin, biomarkers, survival mechanisms, as well as phenotypic plasticity, heterogeneity and vulnerabilities, is an active field of investigation. Nevertheless, both bacterial and cancer DTPs can be broadly defined by these features: i) non-genetically driven phenotype; ii) quiescence; iii) ability to tolerate the lethal effects of drugs, which may be intrinsic or drug-induced; iv) reversible entry into and exit from the DTP state.

Although the term 'persisters' in cancer is broadly used to describe quiescent, drug-tolerant cells, tumors are comprised of a spectrum of cell states or phenotypes including dormant cells, senescent cells, stem-like cells, and cells exhibiting characteristics of epithelial to mesenchymal transition (EMT), which may all inherently resist therapy[9] (Table 1). The relationship between the DTP phenotype and these alternative cell states is currently unclear and under investigation. For example, while dormancy is characterized by cancer cells that remain quiescent for long periods even in the absence of drug treatment, growing evidence confirms that at least a fraction of DTPs slowly replicates[10,11]. Gene expression features associated with senescence and EMT, as well as positivity for β-galactosidase activity (a biomarker of senescence), have been observed in non-small-cell lung cancer DTPs emerging upon dual inhibition of epidermal growth factor receptor (EGFR) and MEK[12]. Notably the same features were not observed under EGFR monotherapy, thus highlighting the heterogeneous nature of DTPs. Moreover, evidence of shared features between slow replicating DTPs and cancer stem cells (CSCs) is conflicting, probably owing to differences in the tissue of origin and therapeutic regimens[9] (Table 1).

However, it is widely acknowledged that during drug exposure, tumor cells may co-opt transcriptomic and epigenetic pathways associated with drug tolerance, transiently upregulating alternative survival mechanisms and drug resistance genes, thereby inducing the DTP state. Whether these mechanisms are tumor- and/or treatment-dependent remains to be determined. Importantly, upon prolonged drug exposure, both bacterial and cancer DTPs undergo stress-induced mutagenesis (SIM), eventually fostering a spectrum of heterogeneous mechanisms leading to irreversible, inheritable, drug resistance[13-17]. Hence, persistence represents a highly plastic state enabling cancer cells to tolerate cytotoxic stress, establish MRD, and seed subsequent outgrowth of resistant cells. Relatedly,

approaches to target DTP cells prior to the development of irreversible genetic clonal resistance could enhance the durability of responses to cancer treatments.

In this Roadmap, we focus on recent advances in our understanding of cancer DTPs, including mechanisms underlying DTP formation, plasticity and heterogeneity as well as the interaction between DTPs and the tumor microenvironment (TME). We also highlight potential biomarkers and actionable vulnerabilities. Additionally, we provide recommendations to move the field forward, including the necessity to extend our framework beyond the current dependence on 2D cell culture and immunocompromised animal models, and harnessing novel technologies to bring about new insights into the cell biology of DTPs. We also address the challenges in designing clinical trials focused on DTPs and propose strategies for translating DTP biology into the clinic by identifying tumors in the DTP state in the context of patients on treatment.

[H1] The fundamentals of DTP cells

To facilitate comparison across different cell states, cancer types and treatments, and to avoid discrepancies in definitions, it is important to establish clear criteria for characterizing cancer DTPs.

[H2[*Learning lessons from bacteria*

Originally identified by Joseph Bigger, bacterial antibiotic persisters are defined as a sub-population of bacteria, within a clonal population, that become highly tolerant of antibiotics without undergoing genetic change[18,19]. These persisters remain viable and repopulate when the level of antibiotic decreases, highlighting the non-genetic determinants of bacterial tolerance to antibiotics [6,8].

Conceptually, tolerance differs from resistance. Resistance reflects the ability of cells to replicate in the presence of drug treatment[8]. Tolerance reflects the ability of cells to mitigate the killing efficacy of the drug, even at high concentrations[8]. Pre-exposure to starvation, heat, pH and many other stresses may increase the persistent population, mainly by increasing the fraction of non-growing bacteria within the growing culture[20]. A prominent example of tolerance is the ability of growth-arrested bacteria to survive high concentrations of penicillin[21]. Unlike resistant cells, bacterial persister cells do not grow in the presence of antibiotics, but rather are mostly linked to disrupted growth[22] that may be induced or stochastic[6,23], or triggered by the drug itself [24].

Mechanisms suggested to increase persisters in a bacterial population include the toxin–antitoxin system **[G]**, the ppGpp biochemical network that alters RNA transcription and DNA replication,

decreased protein synthesis and reduced metabolic activity[24-31]. Genetic screens, as well as studying the evolution of increased antibiotic tolerance in bacteria, have revealed that the 'tolerome', i.e., the genes linked to increased tolerance, is extremely large[32,33]. Interestingly, persistence and tolerance have been shown to favour the acquisition of resistance mutations, indicating a constant interplay between the different survival modes[34].

[H2] ***Experimental isolation of DTP cells***

The essential lack of reproducible markers of DTPs across different tumor types poses a challenge to their detection and characterization, as further discussed below. Therefore, special considerations should be taken into account when designing experiments aimed at isolating DTPs.

In the bacterial field, DTPs represent a fraction of the originally sensitive population. As a consequence, when antibiotic treatment is applied, the majority of the population initially dies, while the DTP subpopulation emerges and determines a reduction in the cell number decline. This creates a biphasic killing curve, which is regarded as a hallmark of bacterial DTPs[8]. Interestingly, cancer cells under treatment in vitro have been shown to undergo similar population dynamics, and therefore, biphasic killing represents a feature of cancer DTPs as well[10,35]. Usually, following an initial decline, the cell number reaches a plateau consisting of DTPs[1,10,36]. However, during some treatments (such as anti-estrogen receptor therapy), an initial population expansion could be observed before cell death becomes significant and tolerant cells arise[37]. Importantly, a constant exposure to anti-cancer agents should be maintained by periodic renewal to avoid experimental biases.

The establishment of the DTP phenotype should be confirmed by testing drug sensitivity of DTPs compared to the parental counterpart with a drug screening approach[1,17]. In this regard, DTPs are fragile cells to manipulate, and additional precautions (such as gentle trypsinizing agents and increased cell densities when plating for experimental assays) are recommended.

One key aspect involves distinguishing DTPs from genetically resistant cells which may already be present in the original population. Single-cell cloning of cancer cells is advisable since sensitive and pre-existing resistant cells can coexist in bulk populations before treatment. In this framework, pre-existing and DTP-derived resistant cells can be distinguished based on their time of appearance during a prolonged drug treatment[10,13]. As per their definition and different from resistant cells, DTPs expand to reconstitute a drug sensitive population when treatment is discontinued, and this should be tested within the experimental framework of choice[17]. Moreover, after prolonged treatment, DTPs convert

to stably resistant cells. At this stage, the reversibility of the drug-tolerant phenotype when halting treatment is completely lost. Importantly, observing these different stages of evolution in vivo can be challenging, due to treatment toxicities and the incompatibility of prolonged therapy with the mouse lifespan. Therefore, the choice of experimental models and treatment schedules should be carefully optimized to reproduce the results obtained in vitro[38,39].

[H1] Origins of Persistence

Understanding how DTPs arise is critical for the development and selection of appropriate therapeutic strategies aimed at eradicating MRD and preventing disease relapse. Studies have reported that DTPs may originate from rare pre-existing cells expressing high levels of aldehyde dehydrogenase (ALDH) or histone lysine demethylases, such as lysine-specific demethylase 5B (KDM5B), in response to anti-cancer therapy[40,41], arguing in favor of a Darwinian selection of a pre-existing persister phenotype. Similarly, the selection of pre-existing cells expressing high levels of either the receptor tyrosine kinase AXL or the nerve growth factor receptor (NGFR) has been observed in melanoma DTPs[42,43]. Lineage tracing in colorectal cancer (CRC) organoids and in vivo mouse models of CRC metastasis demonstrated that relapse after chemotherapy originates from a small subset of pre-existing quiescent leucine-rich repeat-containing G-protein coupled receptor 5 $(LGR5)^{+}$ stem cell-like tumor cells[38].

Yet, the combination of experimental characterization and mathematical modeling of melanoma and CRC cells upon treatment with targeted therapies has highlighted that the persister phenotype is primarily induced by drug treatment[10,44]. Moreover, additional studies that used barcoded cells for lineage tracing demonstrated a lack of enrichment or selection of specific barcodes in DTPs in the context of both targeted therapies and chemotherapies in multiple tumour types[11,39,45-48], suggesting a stochastic switch to the DTP phenotype. Interestingly, while treatment of PC9 cells with the EGFR inhibitor erlotinib mainly selected pre-existing DTP clones, the combinatorial inhibition of EGFR and MEK resulted in stochastic persister formation without barcode enrichment[12]. Taken together, these findings suggest that both Darwinian selection of pre-existing non-genetically resistant clones and Lamarckian induction of drug-tolerant mechanisms can occur to form DTPs.

If DTPs emerge as a result of drug exposure, it is possible that transition into a persister phenotype occurs in a subset of somehow primed cancer cells. Experimental evidence suggests that transcriptional heterogeneity driven by stochastic induction of survival genes and pathways may exist within a cancer cell population. When exposed to a lethal treatment, this heterogeneity may lead to

the determination or selection of cells with a survival advantage, transitioning them into a DTP state[42,49]. In line with this, Marsolier and colleagues[50], exploiting a high-complexity barcode library for lineage tracing coupled with single-cell RNA sequencing (scRNA-seq), inferred that a fraction of lineages present in treatment-naïve breast cancer cell populations display bivalent chromatin landscapes which prime the DTP phenotype to tolerate chemotherapy.

Regardless of whether DTPs are selected from a pre-existing cell population or emerge during drug exposure, adaptive changes to drug-induced stress occur shortly after drug exposure and last throughout treatment. Within hours of drug exposure, molecular rewirings occur[51-54] followed by weeks of 'strengthening" of epigenetic changes necessary to stabilize adaptive changes to the drug[42]. Therefore, though DTPs were formally defined as arising after 9+ days of treatment[1], an at least partial switch to a DTP phenotype may be present at earlier timepoints. Eventually, certain DTPs escape or 'awaken' following typically weeks-to-months of quiescence and, in the absence of resistance mutations, this can be driven by further epigenetic and transcriptional changes which appear to be heterogeneous and reflect many routes to non-genetic resistance[37]. Moreover, while bacterial persister cells are mostly defined as non-cycling cells, there are reports of 'cycling persisters', 'idling' and 'awakening' cells during treatment which exit quiescence yet are not considered fully resistant[11,37,55]. There are also drug-tolerant expanded persister (DTEP) colonies which emerge weeks to months into treatment yet proliferate slowly and can re-enter quiescence, as discussed below.

These cell states, which vary in abundance, duration and dynamics by tumour type, drug, and drug dosage, represent a continuum of tumour cell adaptation to drug stress[56]. Indeed, increased duration and concentrations of poly(ADP ribose) polymerase (PARP) inhibitor treatment in a high grade serous ovarian cancer cell line showed progressive adaptation of cancer cells to the treatment over a course of 311 days, while surviving cells reverted back to a treatment sensitive state following recovery from drug treatment[56]. Importantly, further work is needed to identify functional distinctions, including vulnerabilities, between these cell states representing different degrees of drug adaptation.

[H1] Mechanisms of adaptation

Emerging evidence indicates that drug tolerance can manifest in the absence of specific genetic alterations[3]. Some of the 'non-genetic' regulatory mechanisms reported to underlie the formation of

DTP cells[1,57-61] are described below, but this remains an evolving field and overall much remains to be understood.

[H2] Epigenetic rewiring.

Using scRNA-seq on paired patient samples of drug-naïve and resistant acute myeloid leukemia (AML), it was demonstrated that transcriptional plasticity can drive enduring epigenetic resistance[62] (Fig.1). Therefore, it is reasonable to assume that similar mechanisms may also contribute to the emergence of DTP cells, as shown by a recent functional genetic screen in DTP cell line models[63]. Multiple lines of evidence reported the upregulation of histone lysine demethylases (KDMs) as driving mechanisms of DTP survival in response to both targeted therapy and chemotherapy[1,41] (Fig.1). Indeed, KDM5 inhibitors or knockdown of KDM6A and KDM6B proved effective in decreasing the fraction of surviving DTPs in lung cancer, melanoma, breast cancer and glioblastoma cells[1,64,65]. In addition, an increase in the histone H3 lysine 9 trimethylatation (H3K9me3)-induced repressive chromatin state, particularly at long interspersed repeat element 1 (LINE1) retrotransposons **[G]**[57], characterized by increased expression of H3K9 and H3K27 methyltransferases, promotes the DTP phenotype and survival. Derepression of LINE1 through inhibition of methyltransferases induced cell death and significantly delayed regrowth from DTPs[57] (Fig.1).

[H2] Transcriptomic adaptation and gene regulatory network rewiring.

Distinct drug-tolerant transcriptional states were identified by using scRNA-seq on malignant cells isolated from a cohort of BRAF mutant melanoma patient-derived xenografts (PDXs) subjected to concurrent RAF and MEK inhibition[43]. Among these states, one exhibited a neural crest stem cell (NCSC) transcriptional program largely driven by the nuclear retinoid acid receptor RXRG. The use of an RXR antagonist successfully reduced the accumulation of NCSCs and slowed down the development of resistance[43]. These findings underscore the potential therapeutic benefits of reprogramming gene regulatory network (GRN) architecture to restrict transcriptional adaptation to treatment.

Along the same lines, scRNA-seq and live imaging of primary, residual, and recurrent disease in a zebrafish model of melanoma confirmed the heterogeneous nature of DTP transcriptional states[66]. Similar to the PDX models, a microphthalmia-associated transcription factor (MITF)-independent mesenchymal-like or NCSC state emerged on-treatment from a pre-existing G0-like state present in drug-naïve lesions, as well as from de novo reprogramming. Furthermore, using lineage tracing

coupled with multiplex immunohistochemistry, it was shown that these MITF-independent cells could directly contribute to melanoma recurrence and regain MITF activity, demonstrating plasticity[67] (Fig.1). A notable feature found in both zebrafish and PDX models in the mesenchymal like or NCSC state is specific expression of the neural crest transcription factor TFAP2B[68] and the TFAP2B regulon[43]. This TFAP2B-expressing NCSC state emerges in metabolically active ALDH1A3-high cells through acetaldehyde metabolism creating an acetyl donor for histone H3 modification to promote neural crest lineage gene expression[69] (Fig.1). This mechanism may link DTP metabolic states to transcriptional states because ALDH activity is a phenotypic CSC marker[70,71] required for the maintenance of an epigenetically determined reversible DTP subpopulation in cancer cells[40]. Together, these data underscore the potential to use ALDH inhibitors together with targeted therapies to target residual disease and to delay or even prevent recurrent disease[40,72].

Additionally, feedback loop upregulation of kinase receptors and pathways have been reported to promote the slow-cycling DTP state that allows cancer cells to tolerate drug-induced insults. Specifically, AXL activation[73,74], NOTCH3-dependent activation of the β-catenin signaling pathway[75], activation of insulin-like growth factor 1 receptor (IGF1R) through KDM5 or forkhead box protein A1 (FOXA1)[1,76], Janus kinase (JAK)-mediated activation of signal transducer and activator of transcription 3 (STAT3)[77], and entry into or establishment of a dormant state through epigenetic activation of the signaling pathway orchestrated by Yes-associated protein (YAP) and TEA domain family member (TEAD) transcription factors[12], have all been reported to induce the DTP state in multiple tumor types thus impairing drug-induced apoptosis (Fig.1).

Finally, transcriptional adaptation of DTPs can also involve DNA damage response (DDR) pathways, such as ATM and ATR signaling axes, which can promote DTP survival[78-80]. In particular, pharmacological or genetic targeting of DNA repair pathways sensitize cancer cells to DNA-damaging agents, impeding DTP formation and improving therapeutic outcomes in experimental models[78-80]. Oncogenes such as MYC can also enhance the DDR by upregulating checkpoint kinase 1 (CHK1) and CHK2 and protecting tumor cells from ionizing radiation[81]. The knockdown of MYC or CHK1 and CHK2 resensitizes tumor cells to this treatment in models of nasopharyngeal carcinoma[81].

[H2] Translational rewiring

Residual DTPs may also emerge through translational reprogramming, often characterized by a reduction of global protein neosynthesis and an increase in the translation of proteins that promote

adaptation and survival. For instance, in a subset of BRAF mutated melanoma cells tolerant to BRAF and MEK inhibitors, a reversible remodelling of mRNA translation occurs concurrently with changes in drug sensitivity[59]. Although this process leads to a global decrease in protein synthesis, specific mRNAs demonstrate enhanced translation efficiency. Targeting the eukaryotic initiation factor 4A (eIF4A) RNA helicase, a component of the eIF4F translation initiation complex[82], abrogates this selectively increased translation and proves lethal to DTPs[59]. Translation remodelling in DTPs also correlates with an increase in the presence of the N^6-methyladenosine (m6A) modification in the 5'-untranslated region of some highly translated mRNAs. An m6A modification near the stop codon or within the 3′-UTR of mRNA promotes its degradation. Conversely, an m6A modification near the 5′-UTR recruits translation initiation factors[83], such as eIF4A, enhancing the translation of a specific subset of mRNAs that may associate with the survival of DTPs upon combined BRAF and MEK inhibition. Combining an eIF4A inhibitor with BRAF and MEK inhibitors effectively hinders the emergence of DTPs, offering a promising therapeutic strategy to forestall acquired drug resistance[59] (Fig.1).

Although the origin of this translational regulation remains to be established, the integrated stress response (ISR) is a likely driver of such an adaptive mechanism in response to different chemotherapies and targeted agents, across several cancer types[84-89] (Fig.1). The ISR, which can be triggered by a variety of intracellular and extracellular stressors such as amino acid deprivation, inflammation and endoplasmic reticulum (ER) stress, can in fact inhibit translation initiation and suppress 90% of the cell's translatome[90]. Simultaneously, this pathway initiates a cytoprotective transcriptional and translational program, orchestrated by activating transcription factor 4 (ATF4)[91], which initiates a gene expression program that enhances nutrient uptake, promotes autophagy, and reduces oxidative stress[91,92]. In melanoma, ATF4 represses MITF to promote an MITF-low-AXL-high DTP phenotype[87]. Accordingly, the ISR was shown to be a hallmark shared by all dedifferentiated melanoma DTP subpopulations, and to promote oxidative phosphorylation **[G]** (OXPHOS)[88] (Fig.1).

As a part of the ISR, the unfolded protein response (UPR) is triggered by the accumulation of misfolded proteins within the ER and helps maintain cellular homeostasis and survival under stress conditions. It was found that hypoxia activates the UPR pathway[93], which facilitates resistance to irradiation by shielding tumor cells from reactive oxygen species (ROS)[94]. A direct link between the UPR and resistance to pharmacological treatment was also revealed by a multi-omics approach[95]. In this study, tumor cells became resistant to the folate-based antimetabolites methotrexate and

pemetrexed by means of a UPR-mediated translational control of enzymes involved in a pathway that diverts metabolites from glycolysis to fuel mitochondrial one-carbon metabolism[95]. Additionally, a set of molecular chaperones known as heat shock proteins (HSPs) can protect tumor cells from the detrimental effects of protein denaturation and aggregation under stressful conditions generated by drug treatment[92,96].

A recent study also provided evidence that the ISR promotes the recruitment of a specific long non-coding RNA (lncRNA), LISR, to a subset of ribosomes to enhance translation of proteins involved in immune evasion, such programmed cell death protein 1 ligand 1 (PDL1) and the glycocalyx **[G]** [97]. Given the broad impact of ISR activation on the DTP proteome, it will be important to distinguish which of the epigenetic alterations observed in DTPs are in fact downstream of ISR activation from those that are ISR-independent.

[H2] Metabolic rewiring

Mounting evidence reveals that cancer cells can display distinct metabolic profiles. For instance, increased glucose consumption and lipid anabolism are associated with proliferation, whereas a reliance on exogenous fatty acids and an oxidative state are linked to invasion, metastatic dissemination and drug tolerance[98]. Findings in NSCLC, breast cancer, gastric cancer and melanoma[41,99,100] revealed that DTPs are less dependent on glycolysis and rely more on OXPHOS for energy production[44]. This shift towards the mitochondrial respiratory chain makes them highly dependent on antioxidant pathways to mitigate elevated ROS.

Expression of ALDHs, which detoxify ROS-generated reactive aldehyde products, is essential for DTPs which are, accordingly, sensitive to treatment with the ALDH inhibitor disulfiram[40] or the ALDH1 suicide inhibitor, nifuroxazide[69]. Similarly, DTPs are sensitized to cell death by ferroptosis, for example by inhibiting the phospholipid hydroperoxidase glutathione peroxidase 4 (GPX4)[101,102] (Fig.1). However, the mechanism by which DTPs become sensitized to oxidative cell death remains poorly understood. While ROS production caused by increased mitochondrial respiration undoubtably contributes, ROS are only modestly elevated in DTPs. Instead, DTPs dramatically disable antioxidant programs by reducing levels of the GPX4 cofactor glutathione (GSH) and of the cofactor NADPH, as well as by downregulating GSH and NADPH biosynthetic genes, iron sequestration genes and various antioxidant genes[101].

Beyond sensitivity to oxidative stress, the altered metabolism of DTPs results in additional dependencies. In a recent study in BRAF-mutated melanoma, it was demonstrated that DTPs are sustained by peroxisomal fatty acid β-oxidation (FAO)[103], as a result of a metabolic shift from glycolysis to oxidative respiration. Knockdown of the key peroxisomal FAO enzyme acyl-coenzyme A oxidase 1 (ACOX1) or treatment with the FAO inhibitor thioridazine, specifically hampers the oxidative respiration of DTPs and significantly diminishes their emergence (Fig. 2). Utilizing scRNA-seq, it was found that FAO inhibitors, including thioridazine, can reduce the prevalence of therapy-induced NGFR-high NCSC-like resilient melanoma cells[104]. In another study, it was also shown that different FAO inhibitors, including ranolazine, thioridazine and etomoxir, can significantly reduce the establishment of colonies resistant to the BRAF inhibitor vemurafenib [105]. Interestingly, FAO is a conserved metabolic pathway that has also been implicated in bacterial DTPs and thioridazine treatment was found to be highly effective against a diverse array of DTPs obtained from various bacterial species or subjected to different antibiotic treatments[106].

[H1] Phenotypic plasticity and heterogeneity

The survival strategies of DTPs include phenotype switching, whereby they dynamically assume cell identities unrelated to the drug-targeted pathway[49,107]. The ability to adaptively reprogram and acquire diverse phenotypes, transcending genetically determined lineages, enables tumor DTPs to concomitantly adopt distinct survival strategies. In this context, we highlight key characteristics of phenotypic plasticity and heterogeneity of DTPs, which may inform the development of novel and efficacious therapeutic strategies.

[H2] Hybrid cell states and transdifferentiation

Numerous studies have described the ability of DTPs to undergo EMT (Fig.1), a process historically linked to cancer progression and invasiveness[101,108-111]. The activation of EMT appears to be related to specific transcriptional programs induced by DTPs, with certain transcription factors, such as ZEB1, playing a substantial role[102]. In estrogen receptor $(ER)^{+}$ breast cancer, simulation of a network consisting of regulators of EMT and resistance to tamoxifen, a selective estrogen receptor modulator revealed 6 possible phenotypes that co-occur and switch between one another to drive resistance to targeted therapy. In addition, simulated data inferred that inducers of a mesenchymal-to-epithelial transition (MET) promote a phenotypic switch from a more drug-resistant mesenchymal state to a more drug-sensitive epithelial state[112].

Moreover, DTPs can convert to different cell lineages, thus changing cell identity, to adapt and survive under treatment, in a process called transdifferentiation. For example, residual CRC cells after EGFR blockade acquire a Paneth cell-like phenotype by non-genetically inactivating YAP signaling[113]. Similarly, drug-induced neuroendocrine transdifferentiation has been observed in both prostate tumors, with the acquisition of an androgen independent phenotype[114], and lung adenocarcinomas, showing a transition to small-cell lung cancer, a process integral to the acquisition of resistance to EGFR inhibitors[115]. Lastly, basal cell carcinoma cells can transcriptionally reprogram to switch between different cell identities corresponding to different stem cell compartments present in the normal hair follicle[116].

The molecular switches governing these cell state transitions are currently under active investigation. Certain genetically inherited traits, such as mutations in *RB1* and *TP53*, can promote specific transdifferentiation events, as observed in neuroendocrine transition in prostate and lung cancer[115]. However, studies have also shown that loss of *RB1* and *TP53* alone was not sufficient for adenocarcinoma to undergo neuroendocrine transdifferentiation, indicating that these transitions are likely to be drug-induced and also the result of non-genetic adaptations. Specifically, the involvement of several transcriptional regulators, such as MITF, SRY-box 2 (SOX2) and enhancer of zeste homolog 2 (EZH2), was shown to be critical for neuroendocrine transdifferentiation in prostate and lung cancer[49]. While some regulators can direct non-genetic adaptations subsequent to genetic alterations (for instance, SOX2 is upregulated in TP53-deficient and RB1-deficient prostate and lung adenocarcinomas), others such as MITF and EZH2 control the emergence of distinct cell states in response to treatment to mediate drug tolerance.

Owing to advances in tumor profiling through single-cell sequencing, recent studies have highlighted the heterogeneity within a single tumor population and tumor plasticity across various cancer types including CRC, prostate, breast, lung and pancreatic cancer[50,54,117-122]. ScRNA-seq revealed that DTPs with mesenchymal- or luminal-like transcriptional phenotypes can coexist in breast cancers[123]. Similarly, multiple cell phenotypes can coexist in melanomas under treatment, and different patients can have distinct ‘compositions’ of DTP cell lineages at relapse[43]. Furthermore, comprehensive single-cell molecular profiling together with barcoding technology for lineage tracing has revealed the diversity of clonal fates post-treatment in genetically similar cancer cell lines[124]. However, elucidating the biology of how cancer cells transition from a treatment-naive state to DTP states is a key challenge, particularly with regards to linking and tracking tumor genotypes to their phenotypes longitudinally.

[H2] Cycling vs non-cycling

Sustained proliferation is recognized as a key feature of cancer and current standard of care treatments for most tumors involve chemotherapeutic agents, which are effective against rapidly dividing cancer cells[125,126]. Emerging evidence indicates that cancer cells in the DTP state not only reside in a senescent or quiescent state[11,38,39,41,45,127,128], but may also be actively cycling under continuous drug treatment[11,51,129] (Fig.1). The ability of tumor cells to dynamically transition between cycling and non-cycling proliferative cell states likely contributes to the maintenance of MRD and potentially to the emergence of early growing clones, known as DTEPs.

For instance, in lung cancer cell models, the ability to re-enter the cell cycle is specific to a distinct fraction of DTPs, with cycling and non-cycling DTPs displaying distinct transcriptional and metabolic programs that cluster in specific cell lineages[11]. Likewise, in melanoma, proliferating DTPs were found to rely on distinct stress response signaling pathways owing to the increased replication stress associated with cell proliferation, which in turn primes these cells for acquiring mutations and potentially enhances their capacity to drive resistance and tumor relapse[51]. Importantly, cycling DTPs arise through non-mutational cell state transitions and can drive disease recurrence in the absence of any additional genetic alteration[130,131]. These findings are in line with recent observations in bacteria that challenge the traditional view of DTPs as largely dormant[132].

[H2] De-differentiation and stem-like phenotype

In some tumor types, the phenotypic plasticity of DTPs has been associated with the acquisition of embryonic stem cell features (such as self-renewal and the presence of stem cell markers[1]) (Fig.1). For example, NOTCH signalling along with either increased expression of stemness markers in glioblastoma DTPs[58] or increased expression of ALDH in gastric DTPs[40] have been linked to plasticity. NSCLC tumor cells, which recovered after acute apoptotic stress were also recently shown to have an increased capacity to form DTPs and also an increased tumor initiating ability[133]. Interestingly, primary AML cells were shown to enter a transient DTP state with a senescence-like phenotype following chemotherapy, irrespective of stem cell state, followed by emergence of relapsed AMLs with increased stem cell potential[127].

Several studies have revealed that therapy-induced damage in CRC triggers an adaptive state reminiscent of fetal-intestinal progenitor cells, which enhances resistance and helps regenerate the disease after treatment. Specifically, it was shown that a subset of stem-like cells with low

proliferative rates and marked by the expression of the RNA binding protein MEX3A exhibit resistance to chemotherapy. Lineage tracing experiments in vitro and in vivo demonstrated that the descendants of MEX3A-positive DTPs regenerate the disease post-treatment[38]. Mechanistically, MEX3A-positive cells deactivate the WNT pathway and adopt a fetal intestinal-like program driven by the transcription factor YAP[38]. MEX3A-null CRC cells were unable to undergo the stem cell-to-fetal progenitor transition and died after chemotherapy, although the reason for such a dependency remains elusive. The induction of a YAP-driven fetal-like chemoresistant program in DTPs was also found to be dependent on p53 in a subset of CRCs[134]. Cancer-associated fibroblasts (CAFs) facilitate the co-option of the fetal intestinal-like state, thereby shielding CRC patient-derived organoids (PDOs) from therapy[135]. Furthermore, metastatic lesions in patients with CRC are enriched with tumor cells expressing the fetal-intestinal transcriptional program and exhibiting increased plasticity. Notably, chemotherapy exacerbates this phenotype in the metastases[119].

[H2] Diapause-like status

At the intersection of developmental biology and cancer, an embryonic survival phenotype, diapause, was identified as a defining feature of DTPs in multiple cancer types[39,45,123,127]. Diapause is a reversible physiological state of suspended development of an embryo (in insects, invertebrates, and certain mammals) triggered by adverse conditions[136-138]. While experimental evidence is only just emerging for the potential of human embryos to enter diapause[139], human cancer cells (breast, prostate and colon cancer, and AML) in the DTP state were shown to acquire this transient diapause-like gene expression profile to survive chemotherapy and targeted therapy[39,45,123,127] (Fig.1). Phenotypically, chemotherapy-induced CRC DTPs were found to be slow cycling and not in cell cycle arrest, similar to embryonic diapause[39,140]. Upon drug withdrawal, DTPs resumed rapid growth and recapitulated the drug sensitivity profile of treatment-naive cells[39,45,46]. Elegant barcode studies demonstrated no discernable alterations in genetic heterogeneity highlighting the equipotent nature of tumor cells to enter the DTP state, akin to embryonic stem cells entering and exiting diapause in response to environmental conditions[39,45-47]. Mechanistically, tumor cells in the DTP state exhibit a significant decrease in total RNA abundance, whilst selectively activating pathways to enter the DTP state and survive chemotherapy, which were found to be part of the same developmentally conserved program of survival employed by embryonic stem cells in diapause[39,45,123,127]. CRC DTPs were found to decrease mTOR signaling[39] resulting in selective activation of the downstream autophagy pathway for survival, akin to embryos exhibiting diapause[39,140]. Similarly, loss of MYC signaling in DTPs from across various cancer types induced a reversible, diapause-like state[45,127,141]. These observations support the possibility that the use of evolutionarily conserved embryonic survival strategies might

underlie the ability of cancer cells to survive the hostile environment created by exposure to chemotherapy and/or targeted agents.

[H1] The microenvironment and immune evasion

The ability of DTPs to persist for months or even years in patients with cancer suggests that they have the capacity to evade immune surveillance. In fact, scRNA-seq of MRD after targeted therapy of lung cancer shows an inflammatory phenotype including T cell infiltration and a more immune-suppressive TME upon disease progression[142]. Therefore, the relationship between DTPs and the TME raises several critical questions. Of these, one of the foremost questions is how do DTPs evade eradication by the immune system? Two options are that they persist 'passively', possibly by residing within immune sanctuaries, or that they possess the ability to induce specific immune tolerance or resistance mechanisms.

A common occurrence observed in patients with metastatic melanoma or lung cancer is relapse localized exclusively in the brain and/or the leptomeningeal areas, often associated with a very poor prognosis[143,144]. This phenomenon may be attributed to the limited bioavailability of certain drugs, meaning they are unable to penetrate the blood–brain or the blood–leptomeningeal barrier to reach these residual tumour cells. However, the relative increased frequency of relapses in the brain could also stem from the central nervous system being an immune-privileged environment compared to extra-cerebral organs. For example, a subpopulation of $STAT3^+$ reactive astrocytes, which are brain-resident cells surrounding metastatic lesions, triggers an immunosuppressive environment by decreasing the number of $CD25^+CD44^+$ $CD8^+$ T cells and increasing the presence of $CD74^+$ microglia in the metastatic lesions[145]. This result suggests that blocking STAT3 could reduce metastatic outgrowth in the brain of different tumour types.

Another related question is whether tolerance can also arise from anticancer drugs directly affecting the immune environment. Indeed, evidence suggests that several immunotherapeutic approaches may promote the emergence or selection of DTPs through various mechanisms. Nevertheless, the interplay between cancer DTPs, the TME and the immune system remains a complex subject of active investigation, and while we outline key findings below, several aspects remain to be elucidated.

[H2] Microenvironmental cues promoting the DTP phenotype

Several studies have shown that the acidic, hypoxic, and nutrient-depleted tumor niche favors the emergence of DTPs (reviewed in[146]). Hypoxia induces EMT[147], a main feature of DTPs as we have discussed above. Furthermore, melanoma cells under hypoxic conditions or nutrient deprivation acquire a DTP cell state, leading to resistance to both BRAF inhibitors and platinum-based chemotherapy[148]. These DTPs exhibit extensive chromatin remodeling, characterized by loss and gain of epigenetic marks (such as H3K9me3). Another study showed that lung cancer cells enter a dormant state in hypoxic regions through induction of mitogen-inducible gene 6 protein (MIG6), a negative regulator of ERBB signaling, and become resistant to anti-EGFR treatment[149]. In head and neck squamous cell carcinoma and breast cancer, hypoxic microenvironments in the primary tumor promote a long-term dormancy-like program[150]. Once disseminated, the dormant state persisted even in the absence of hypoxia, which helped isolated tumor cells to resist treatment[150]. It was also shown that quiescent breast cancer cells localized within hypoxic areas coordinate an immune suppressive program that evades T cell attack[151], suggesting that this population may also represent a source of relapse after immunotherapy treatment.

In addition, nutrient deprivation triggers the ISR, suppresses mTOR signaling and promotes autophagy[152]. Autophagy enables the degradation and recycling of damaged cellular components, providing energy but also protecting from proteotoxicity and suppressing cell death signals[153]. It was shown that dormant breast cancer cells rely on autophagy and pharmacological blockade of autophagy targets selectively disseminated dormant cells[154]. Inhibition of autophagy also synergizes with chemotherapy to reduce relapse after treatment[39].

Additionally, a therapy-induced secretome (TIS) in tumors has been described in response to stress induced by targeted inhibition of EGFR, ALK and BRAF oncogenes[155]. This complex network of secreted mediators sustains the survival of drug-sensitive cells and stimulates the outgrowth and migration of the drug-refractory subpopulation of cells, through the activation of multiple signaling pathways, including nuclear factor-κB (NF-κB) and PI3K–AKT, thus suggesting combinatorial therapeutic strategies that might be effective[155] (Fig.2). Importantly, key secreted mediators were consistently observed irrespective of the tumor histology or the oncogenic driver. Relatedly, a recent in vitro screen showed that multiple pro-persistence environmental signals, such as fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF1) and interferon γ (IFNγ), can promote intrinsic tolerance of DTPs to drug treatment in lung cancer and melanoma cells[155,156] (Fig.2). Whether these environmental factors are part of the TIS that promotes the immune evasion of DTPs requires further investigation.

An interesting example of cell cooperation was discovered during chemotherapy responses in CRC PDO models[157]. It was shown that chemotherapy-induced tumor cell death causes ATP release, which signals to adjacent cells through the purinergic P2X4 receptor. P2X4 activation triggers an mTOR-dependent prosurvival program in DTPs under chemotherapy[157]. Blockade of mTOR or P2X4 eliminates DTPs and synergizes with chemotherapy[157]. However, whether this interplay exists in the context of organized population survival strategies (akin to bacterial 'quorum sensing' **[G]**) remains unknown.

[H2] ***Immune evasion factors***

There are examples where the biological processes involved in drug tolerance are also the ones that drive immune evasion. For instance, NSCLC DTPs emerging upon treatment with the EGFR inhibitor osimertinib show epigenetic upregulation of specific immunosuppressive factors such as the CD70 receptor[158] (Fig.2). In this model, CD70 controls the survival and invasiveness of cells that have undergone EMT-associated osimertinib resistance. Additionally, CD70 expression is known to facilitate immune evasion by binding to CD27 on immune cells[159], potentially promoting the survival of CD70 expressing DTPs. Interestingly, since CD70 is a cell surface receptor, it can be targeted by chimeric antigen receptor (CAR)-T cells or antibodies, which have been found to target DTPs *in vitro* and have demonstrated a therapeutic antitumor effect in mouse models[159].

Reciprocal interactions between TME cells and DTPs can facilitate the survival and immune tolerance of DTPs. In certain instances, drugs that induce the selection and/or the emergence of DTPs may directly affect the TME, thereby enhancing cancer cell stemness and enabling DTP survival. For example, in mouse models, BRAF mutated melanoma cells survive in stroma-rich areas due to the paradoxical activation of the MAPK-pathway in melanoma-associated fibroblasts by the specific BRAF inhibitor PLX4720. The resulting matrix remodeling subsequently reactivates the MAPK pathway in melanoma cells via β1 integrin–focal adhesion kinase (FAK)–SRC signaling[160] (Fig.2).

In multiple cancer types, e.g., melanoma, NSCLC, CRC and glioblastoma, CAFs promote drug resistance through secretion of factors which could modulate multiple cancer cell phenotypes, like EMT and quiescence[161], as well as cancer cell signalling pathways[161,162]. For example, the secretion of HGF from CAFs, which binds to MET on cancer cells, activates both MAPK and PI3K pathways and bypasses the therapeutic inhibition of mutant EGFR or BRAF[156,162-164] (Fig.2). However, the mechanisms underlying CAF activation have not been elucidated.

On the other hand, secreted factors from tumor cells in MRD may promote persistence and tumor regrowth by acting on the extracellular matrix (ECM). Taking advantage from CRC PDOs, Ohta and colleagues[165] found that the ECM, specifically the collagen XVII cell adhesion molecule and component of hemidesmosomes, has a crucial role in maintaining the DTP state of a chemorefractory $LGR5^{+}p27^{+}$ subpopulation of CRC cells. Chemotherapy-induced remodelling on the ECM induces loss of collagen XVII, leading to $LGR5^{+}p27^{+}$ DTPs re-entering the cell cycle through the FAK–YAP signaling pathway[165,166].

Other cells within the TME, such as monocytes and macrophages, can also play a role. In the context of human epidermal growth factor receptor 2 (HER2)-positive breast cancer DTPs, a direct effect of DTPs on the TME has been demonstrated. HER2 blockade induces an inflammatory gene program driven by tumor necrosis factor (TNF)–NF-κB signaling, which leads to immune cell infiltration. Specifically, CC-chemokine ligand 5 (CCL5) secretion as part of this inflammatory gene program causes the recruitment of CC-chemokine receptor 5 (CCR5)-expressing tumor associated macrophages (TAMs). These TAMs, in turn, promote collagen secretion and DTP survival[167].

[H2] DTPs under immunotherapy treatment

Cancer cell intrinsic mechanisms of immune evasion are adopted by DTPs once they are challenged by immune cells. Some of these mechanisms have been recently described through the characterization of immunotherapy-associated DTPs, and include the upregulation of immune checkpoint molecules, the shielding of membrane receptors, the downregulation of antigens and antigen presentation machinery and the secretion of molecules with immune modulatory functions. In line with this, the autocrine and paracrine secretion of midkine by melanoma cells has been shown to educate macrophages towards a tolerogenic phenotype and induce dysfunction in effector T-cells[168]. Immune evasion can also be achieved by dedifferentiation, which causes the loss of tumor-associated antigens. This was shown to be the result of TNF secreted by the inflamed TME following adoptive cell transfer (ACT) in genetically engineered mouse models of melanoma and in patients[169,170].

Furthermore, silencing of the antigen presentation machinery is also a common immune escape mechanism across cancer types[171,172]. Interestingly, a genome-wide CRISPR-Cas9 screen for regulators of major histocompatibility complex class I (MHCI) expression in leukaemia K-562 cells, identified polycomb repressive complex 2 (PRC2) as a reversible inactivator of MHCI expression

and potentially druggable[173]. Examples of the upregulation of immune checkpoint molecules include the expression of cytotoxic T lymphocyte-associated antigen (CTLA4), a negative regulator of T cell activation on squamous cell carcinoma DTPs in a mouse model of ACT against CD80[174]. In addition, the dampening of cancer cell immunogenicity via the upregulation of the inhibitory ligand PDL1 at the transcriptional and translational level has been shown in several cancer types[175-177] (Fig. 2).

ScRNA-seq analysis of early responses to immune checkpoint blockade in melanoma led to the identification of novel transcriptional mechanisms of immune evasion. The upregulation of the transcription factor TCF4 was recently shown to coordinate multiple gene programs leading to a dedifferentiated mesenchymal-like state associated with resistance to targeted and immune therapy *in vitro* and *in vivo*[178]. Similarly, in a mouse-derived organotypic spheroid model, a subpopulation of DTPs emerged under programmed cell death protein 1 (PD1) blockade, which expressed SNAI1 and stem cell antigen 1 (SCA1) in association with EMT characteristics, and were able to resist $CD8^+$ T cell-mediated cell death via the expression of anti-apoptotic proteins baculoviral IAP repeat-containing protein 2 (BIRC2) and BIRC3. Combining PD1 inhibition with BIRC2 and BIRC3 inhibition enhanced the anti-tumor effect of anti-PD1 *in vivo*[179].

[H1] Tolerance to resistance

The transition from MRD to tumor recurrence is a critical step in acquired resistance. A mechanistic understanding of this process may reveal novel therapeutic approaches to prolong the duration of treatment responses. Here, we address key advances and open questions.

[H2] Adaptive mutability in cancers: how bacterial biology translates to cancer.

The concepts of mutability and evolution are intrinsically connected because mutations create the heterogeneous populations from which new and fitter phenotypes are selected. While the prevalent view was that mutations occurred randomly at a constant rate, this concept has been challenged by the past three decades of discoveries of various molecular mechanisms of mutagenesis upregulated by SIM[180]. These mechanisms allow cells to increase their mutation rate and their ability to evolve preferentially when they are poorly adapted to their environment, i.e., when stressed[15,180,181]. The emergence and selection of SIM is supported by studies of bacterial populations, in which reversible hypermutable phenotypes, sometimes in small cell subpopulations, have the advantage of increasing population diversity without the drawback of continued accumulation of potentially dangerous

mutations once cells adapt and exit stress[182,183]. Moreover, mathematical modeling reveals that SIM mechanisms are selected for in changing environments[184].

Intriguingly, bacteria activate the SIM response when their genome integrity is threatened by accumulating DNA double-strand breaks (DSBs), switching repair of DSBs from high-fidelity homology-directed repair **[G]** to mutagenic processes[180,185]. In this instance, the upregulation of error-prone DNA polymerases (Pol) IV, V, and II through the SOS response, and the downregulation of mismatch repair **[G]** (MMR) and high-fidelity replicative DNA Pol III through the general σS stress response, allow the accumulation of single-base substitutions, small insertions and deletions (INDELs) and genomic rearrangements around DSBs. These represent regulated responses in which bacterial populations increase their mutability transiently, in designated 'gambler' subpopulations, thus hampering their eradication and leading to antibiotic resistance[15,180,185].

Similar to bacteria, cancer DTPs surviving under treatment with targeted therapies experience increased levels of DNA damage and actively switch to a mutagenic form of DNA replication mediated by error-prone DNA polymerases (e.g., POLH, POLI, POLK, and REV1[186]), while downregulating MMR and homologous recombination. Hence, DSBs are repaired by non-homologous end joining or microhomology-mediated break-induced replication[187], causing genome rearrangements[36]. This in turn leads to increased genomic instability and a temporary increase of the mutation rate in DTPs, thus momentarily increasing the likelihood of resistance mechanisms being acquired[10,17,188] (Fig.1). This response seems to involve modulation of the mTOR signaling pathway[188], although the requirement of mTOR, to activate *per se* the response, and its therapeutic relevance remain unclear.

Lung cancer DTPs challenged with EGFR inhibitors activate growth arrest-specific protein 6 (GAS6)–AXL signaling, which in turn promotes error-prone translesion synthesis **[G]** (TLS) through the regulation of RAD18 and adaptively impairs nucleotide metabolism, leading to nucleotide imbalance and purine mutational bias. The result of these concerted responses is an accumulation of mutations which fuel the emergence of resistance[36]. Mathematical modeling estimates that when challenged with targeted therapies, CRC cells increase their mutation rate by 7-50 fold and promote the emergence of resistant colonies[10], while lung cancer cells acquiring resistance to EGFR inhibitors in vivo show a 2-fold increase in mutational load compared to the parental population of origin[36]. In several patients with NSCLC that underwent sequential treatments with tyrosine kinase inhibitors, whole exome sequencing showed an increase in the tumor mutational burden after treatment[189]. The

acquired mutations were enriched for the mutational signatures (SBS2) and SBS13 that have been attributed to apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) cytidine deaminase activity. Subsequent experiments in pre-clinical models linked those genetic perturbations mainly with the induction of APOBEC3A expression in DTP cells [189] (Fig.1).

[H2] Co-occurrence and co-operation.

Given the high level of intra-tumoral heterogeneity, a prevalent view holds that drug resistance is driven by Darwinian selection of pre-existing resistant mutated clones present in the tumor lesion, although at low frequency, before drug administration[190,191]. Once administered, anti-cancer agents eradicate sensitive cells, thus selecting resistant sub-clones that become fitter and expand, leading to tumor relapse. From this perspective, treatment failure is a *fait accompli*[190]. Clearly this view does not apply to all drugs and all clinical cases of tumor relapse. When drug resistance develops after a prolonged clinical response and a time window in which MRD is undetectable by radiological imaging, it is widely accepted that cell plasticity and non-genetic drug tolerance may play a crucial role in causing drug resistance. The phenotypic diversity generated from genetically identical cancer cells may stochastically provide multiple phenotypes able to cope with changing hostile environments (e.g., drug-induced altered environments)[192].

Interestingly, genetic and non-genetic resistance trajectories may co-exist and give rise to different recurrence dynamics[10,13,130]. Notably, one major effect of persistence observed in experimental evolution in bacteria is the increase in the surviving fraction's effective population size. This enables mutations conferring partial resistance to become fixed in the population, providing leverage for further secondary resistance mutations[193]. Analogously, in cancer, the Darwinian selection of pre-existing resistant cells and Lamarckian induction of the DTP state can synergize to produce a transient drug-refractory phenotype, providing a fitter cell state for the acquisition of reversible and irreversible drug resistance[188,194].

A dedicated experimental setup is required to dissect the role of DTPs in the acquisition of drug resistance. First, single-cell cloning of cancer cells is advisable since sensitive and pre-existing resistant cells can coexist in bulk populations before treatment. In this framework, pre-existing and DTP-derived resistant cells can be distinguished based on their time of appearance during a prolonged drug treatment[10]. Importantly, Hata and colleagues[13] observed that DTPs can give rise to mixed clonal populations of resistant cells. Indeed, while EGFR-T790M-positive cells were found in both the early-emerging clones that derived from pre-existing resistant cells and in a fraction of the late-emerging

resistant clones deriving from DTPs, several late-emerging clones remained EGFR-T790M-negative but acquired genetic alterations in NRAS, KRAS, BRAF and RET oncogenes[13]. Similar findings were reported by independent investigators that observed the emergence of a variety of resistance mechanisms when erlotinib-resistant persister-derived colonies arose from a single clonal cell population that was obtained from the PC9 cell line. Importantly, these results suggest that multiple strategies may evolve to escape drug pressure despite the evolutionary bottleneck that occurs during the DTP phase when a bulk population is challenged with anticancer therapies. From a clinical perspective, this increased diversity complicates the identification of therapeutic strategies to eradicate cancer cells. However, which mutations are acquired after an initial response to drug treatment may not be completely random due to the activation of adaptive mutability mechanisms in DTP cells, and mutational signature analysis can be exploited to dissect which mutagenic processes are activated[189,195].

The DTP phenotype is associated with a transient transcriptional and epigenetic rewiring whose main features in theory should not be present when cells eventually gain resistance and fully adapt to the new environment. However, DTP-derived resistant cells can instead maintain features of the drug-tolerant state[13]. The transcriptional profile of DTP-derived EGFR-T790M mutated PC9 cells was indeed found to be more similar to that of DTPs than to that of either parental or pre-existing resistant cells. This included the upregulation of genes related to EMT and to reduced activation of apoptosis[13]. Moreover, it was shown that the differential serine/threonine phosphorylation of the insulin receptor substrate 1 (IRS1) determines the inherited probability of lung and head and neck cancer cells to persist when treated with an EGFR inhibitor[196].

These findings indicate that, similar to other transiently induced cell states[197,198], DTPs might retain information about their previous drug exposures. Such drug-induced cellular ‘memory’ has important clinical implications and raises multiple questions. Would short-term exposure of cancer cells to therapy-induced stress push cancer cells a step closer to the acquisition of permanent resistance? Does the percentage of surviving DTPs increase with subsequent exposure to the same anti-cancer drug? And linked to this, do DTPs evolve faster after repeated exposure to the same therapy? Essentially, does the period of drug holiday between subsequent pulses of treatment simply allow any acquired features or alterations to become fixed? If so, would it be better to apply a ‘double punch’ approach by alternating drugs targeting different cancer cell features, rather than exploiting pulses of the same anti-cancer agent, or would constant exposure to lethal doses of a therapeutic regimen exert a stronger

pressure on DTP plasticity, thus resulting in a prolonged effect? This, of course, warrants additional exploration.

[H1] Innovative technologies

One of the key challenges in studying the DTP state is that, by definition, it is a transient reversible phenotype involving relatively rare cells. Molecular tools to barcode, record, and trace cellular lineages and fates[124,199,200] at single-cell resolution are becoming critical tools in developing a more comprehensive profile of heterogeneous DTPs, including their interaction with the surrounding microenvironment across relevant contexts[201,202]. In line with this, the European PERSIST-SEQ consortium, a coalition of field-leading researchers and medical oncologists, aims to exploit scRNA-seq to capture DTP heterogeneity and unveil potential vulnerabilities. Of note, emerging technologies with multi-modal readouts[203] hold the potential to reveal hybrid cellular states that cannot be measured using a single modality alone.

In recent years, single-cell lineage tracing and sequencing techniques have emerged as powerful tools that are enabling this rare cellular state to be captured and the molecular mechanisms sustaining it to be pinpointed. Such studies typically involve longitudinal sampling of cells before and during the course of treatment and then using the lineage information obtained to reconstruct the landscape of cellular trajectories of DTPs. These trajectories can then be interrogated to address clinically important questions such as the predictability of the DTP fate. In this context, barcoding technologies like single-cell profiling and lineage tracing (SPLINTR)[204] and CRISPR activation tracing of clones in heterogeneous cell populations (CaTCH)[205] have emerged as powerful tools, enabling the tracking of individual cells in complex biological systems.

For instance, Harmange et al.[206] used lineage tracing in a BRAF-driven melanoma model to identify a subpopulation of cells that are primed to become DTPs. Transcriptional priming was also identified in the context of AML treated with chemotherapy[204]. In addition to transcriptional priming, a study focusing on chemotolerance in triple-negative breast cancer revealed epigenetic priming, in which the repressive histone mark H3K27me3 dictates cell fate trajectory upon treatment[50]. Of note, epigenetic and transcriptional priming are unlikely to be mutually exclusive, as changes in histone modifications can lead to transcriptional activation. In all the aforementioned studies, the molecular states associated with priming were heritable and seemed to arise from cell-intrinsic properties that could be sustained over multiple cell divisions both *in vitro* and *in vivo*.

Another important trait that was exposed by single-cell lineage studies is the extent of DTP heterogeneity and the molecular processes that underlie it. For instance, cycling and non-cycling DTPs that co-occur were shown to arise from separate cell lineages[11]. Correspondingly, it was shown that DTP clones emerging from single-cell-derived cancer cells can assume multiple morphological and functional phenotypes[124]. Distinct DTP states were also identified in *in vivo* models of melanoma and patient biopsies, highlighting the complexity of the non-genetic DTP landscape[43].

Recent advancements in single-cell multi-omics techniques[207], which can simultaneously profile the (epi)genome, transcriptome and other molecular layers, have been instrumental in inferring the intricate interplay between chromatin, transcription factors and genes generating a complex regulatory circuit represented as GRNs. This includes the regulatory circuits governing cellular identity and function[208]. Notably, such approaches identified GRNs orchestrating the transition of melanoma cells into a neural-crest-like DTP state[43]. Moreover, the usage of synthetic locus control regions **[G]** and artificial intelligence (AI)-designed enhancers **[G]** will greatly facilitate the monitoring and manipulation of specific DTP states, offering a deeper understanding of their biology and the mechanisms underlying their dynamic emergence[209,210].

Advancements in single-cell technologies extend beyond genomics and transcriptomics, and now also encompass proteomics[211]. Despite being in its infancy, single-cell proteomics has witnessed considerable methodological progress, including miniaturized sample preparation, multi-omics analyses, and the integration of mass spectrometry and microscopy techniques. While proteins cannot be amplified like transcripts, recent improvement in instrumentations and algorithmic development[212] are beginning to overcome this limitation, and the anticipation is that single-cell proteomics, complementing single-cell transcriptomics, will provide invaluable insights into the biology of DTPs. The pursuit of a scalable and high-throughput single-cell multi-omics platform is therefore a promising future direction.

Visualization of cellular heterogeneity and spatial architecture in the TME is increasingly important for understanding disease progression and therapeutic response, particularly in the era of cancer immunotherapy. Emerging spatial technologies, capable of measuring the epigenome, transcriptome, proteome, and even the metabolome at single-cell resolution[213], holds tremendous value for achieving a holistic understanding of the TME, identifying specific DTP niches, and more. The field of single-cell and spatial multi-omics technologies is evolving rapidly, with computational strategies playing a

crucial role in integrating information across molecular layers[214]. Deep learning techniques have emerged as powerful tools in this integration, showcasing better performance over classical computational methods. However, as the field grows exponentially, navigating the vast landscape of tools and analysis steps becomes increasingly challenging. Continuous efforts towards independent benchmarking studies and comprehensive best-practice workflows are essential[215]. Given that cell–cell interactions instruct cell fate and function, the integration of single-cell and spatial-omics holds promise in unraveling interactions contributing to the acquisition of drug tolerance and/or resistance phenotypes[216] and computational tools for inferring cell–cell interactions are becoming increasingly accurate[217].

Combining technologies such as cell state reporter methods with live-cell imaging or intravital microscopy will provide spatial, molecular, and morphological data over time of DTPs. This will contribute to a deeper understanding of the cellular dynamics of DTPs in response to treatment[216]. Upon deciphering the dynamics and spatial distribution of DTPs, it will be crucial to dissect the cellular and molecular intrinsic and extrinsic mechanisms underlying their emergence and maintenance. Functional genomics approaches using the most clinically-relevant model systems will be required (Box 1). Perturbation at the single-cell level can be achieved by programmable genome-engineering technologies, such as CRISPR screens, connecting genes to disease-relevant phenotypes[63,218,219]. These technologies, combined with high-dimensional profiling of single-cell types, enable the impact of genetic perturbations on the cellular transcriptome, proteome, and epigenome to be measured. Such approaches will likely substantially increase our understanding of DTP biology, identifying intrinsic vulnerabilities[63], and innovative approaches for targeting intercellular communication nodes required for establishing drug tolerance.

[H1] Actionable vulnerabilities

The development of drug resistance is a stepwise dynamic process involving cancer cell intrinsic adaptive mechanisms and extrinsic interactions with the TME (Fig. 3). In this section, we illustrate the therapeutic potential of MRD-directed therapy to limit tumor recurrence (Table 2).

[H2] Targeting cancer cell intrinsic vulnerabilities of DTPs

The diversity of biological features of DTPs results in a multitude of potential therapeutic vulnerabilities with which to eradicate them. However, the tolerability of therapies against such vulnerabilities is a potential bottleneck in translating these findings to the clinic.

Metabolic rewiring is one of the most studied features of DTPs, and its targeting has been explored therapeutically in animal models. For example, a FAO inhibitor has been reported to delay the onset of resistance *in vivo* by targeting melanoma DTPs expressing elevated FAO[103]. Another consequence of deregulated metabolism is accumulation of ROS in cancer cells[11,220,221]. A dependence on the antioxidant response to neutralize the toxicity of ROS in DTPs is generally reported across cancer types. This sensitivity to oxidative stress is thought to underlie the sensitivity of DTPs to ALDH inhibition using disulfiram[40]. Recently, an unbiased CRISPR screen in lung cancer DTPs identified bromodomain and extraterminal (BET) inhibition as a vulnerability of DTPs through downregulation of anti-oxidative genes, thereby further increasing ROS to toxic levels. Importantly, treatment of EGFR mutant lung cancer cells with the EGFR inhibitor osimertinib followed by a switch to BET inhibition when tumors had completely regressed resulted in a delay in tumor recurrence[63].

Fatty acid synthase (FASN) expression was consistently increased upon the onset of therapy resistance in melanoma and was associated with decreased lipid poly-unsaturation[222]. Indeed, the exploitation of this vulnerability by combining MAPK and/or FASN inhibitors with the clinical ROS-inducing compound arsenic trioxide delayed the onset of therapy resistance and dramatically increased the survival of melanoma PDX models[222]. Additionally, it was reported that BRAF inhibition in therapy-sensitive cells induced downregulation of the lipogenic regulator sterol regulatory element-binding protein 1 (SREBP1) and thereby lipogenesis. Irrespective of the escape mechanism, resistant cells invariably restore this process to promote lipid saturation and protect melanoma cells from ROS-induced damage and lipid peroxidation. Pharmacological inhibition of SREBP1 restores sensitivity of resistant melanoma cells to BRAF inhibitors both in vitro and in a PDX model[223].

Another DTP vulnerability is sensitivity to inactivation of GPX4, which leads to selective ferroptotic death of DTPs in multiple cell line models in culture and in immunodeficient mice[101,220]. The development of bioavailable GPX4 inhibitors and inhibitors of other ferroptosis suppressor proteins such as ferroptosis suppressor protein 1 (FSP1)[224] is currently being pursued. However, it remains to be determined whether toxicity to normal tissues from ferroptosis induction can be avoided. Furthermore, opposing roles for ferroptosis have emerged in the context of tumour immunity. While $CD8^+$ T-cells sensitize target cancer cells to ferroptosis[225], in syngeneic tumor models, it was found that ferroptosis-blocking antioxidant treatment improves responses to immunotherapy by preventing the immunosuppressive death of tumour-associated neutrophils[226]. Therefore, while ferroptosis represents a promising approach to target DTPs, more work is needed to determine the optimal approach to modulate ferroptosis as a potential clinical strategy. Additional characteristics of DTPs

such as the EMT transcriptional state[102], decreased cell–cell contact[227], and cell cycle arrest[228], each of which have been demonstrated to promote ferroptosis in other contexts, may also contribute to the sensitivity of DTPs to ferroptosis.

Other inhibitors targeting acquired vulnerabilities of DTPs include ABT263 targeting apoptosis resistance, eIF4A inhibitors for altered mRNA translation and AXL and ULK1 inhibitors for disrupting autophagic flux **[G]** [36,39,59]. Moreover, combinatorial inhibition of WNT signaling, upregulated in LGR5-expressing DTPs, in two genetically engineered mouse models of basal cell carcinoma treated with the Hedgehog pathway inhibitor vismodegib led to tumor eradication[229].

[H2] Targeting mechanisms of adaptation and plasticity

Sharma and colleagues[1] were first in describing the selective sensitivity of NSCLC DTPs to inhibition of the histone demethylase KDM5. Since then, targeting epigenetic enzymes has been further explored in the context of DTPs and altered histone landscapes have been identified through chromatin immunoprecipitation followed by sequencing (ChIP-seq)[50]. For instance, a recent publication reported that treatment-naïve DTP precursors, which were isolated from a triple negative breast cancer PDX model treated with chemotherapy, are primed with the histone marks H3K27me3 and H3K4me3[50]. However, only the repressive mark H3K27me3 determined the cell fate upon chemotherapeutic challenge. Intriguingly, depletion of the H3K27me3 mark prior to capecitabine treatment enhanced chemo-tolerance while simultaneous treatment with both capecitabine and a KDM6 inhibitor (to increase global levels of H3K4me3) delayed tumor recurrence[50].

In addition, histone deacetylase (HDAC) inhibitors, such as vorinostat and etinostat, lead to eradication of DTPs through the derepression of LINE1 repetitive element expression[57]. Similarly, the inhibition of EZH2, the histone methyltransferase subunit of PRC2, a master transcriptional regulator altered in multiple cancer types[230], significantly impairs the survival of DTPs[49,57]. Lastly, the inhibition of cyclin-dependent kinase 7 (CDK7) and CDK12 disrupts the transcriptional rewiring and enhancer remodeling induced by targeted therapy and required for cancer cell survival, thus hampering the emergence of DTPs in both *in vitro* and *in vivo* cancer models[231].

The data above indicate that: 1) altered histone landscapes in DTP precursors could be used as biomarkers to predict the response of patients to therapy; 2) not all differentially distributed chromatin markers are meaningful for the DTP phenotype; and 3) the order of drug administration might become a key aspect to consider with respect to DTP-driven MRD (Box 2).

Moreover, targeting the later phases of DTPs in which they evolve to genetically resistant cells is of potential importance. Indeed, increased mutation rates have been observed in DTPs[10,36,188], suggesting that strategies to restrain DTP progression to genetic resistance could be achived through targeting DNA damage repair pathways. Indeed, inhibition of REV1, an error-prone DNA polymerase involved in TLS, a mutagenic form of DNA replication, sensitizes cancer cells to cisplatin by reducing chemotherapy-induced mutagenicity[232] and significantly delays the development of secondary resistance to targeted therapy[10]. Along the same lines, interfering with sustained mutagenesis induced by the activation of the cytidine deaminase APOBEC3A in lung DTPs in response to targeted therapy might potentially delay the acquisition of drug resistance[189]. In addition to an increase in the mutation rate, it has been shown that inhibitors of EGFR, ALK, KRAS and BRAF signaling induce DNA double-strand breaks[233]. DTPs withstand this deleterious effect of these drugs by activating ATM-dependent DNA repair. ATM inhibition sensitized DTPs to gefitinib, an EGFR inhibitor, despite ATM inhibition not being associated with single-agent toxicity at the same concentrations[233].

Finally, the concept of 'stealth' drugs has been proposed in the bacterial field. Stealth drugs are drugs that reduce evolvability without any inhibition of growth rate or fitness. The idea is that if the drugged cells proliferate as usual, there is no advantage to or selection for mutants resistant to the evolution-slowing drug. Those mutants might appear, but they will not overtake the cell population. Two stealth evolution-slowing drugs have been shown to reduce the development of new mutations conferring resistance to antibiotics, without imposing a selection for resistance to the inhibitors[15,234]. Similarly, stealth evolvability inhibitors might boost the efficacy of standard-of-care anti-cancer agents by slowing the evolution of resistance.

[H2] Targeting the stromal and immune niche of DTPs

As outlined above, response to treatment is a dynamic process involving interactions between tumor cells and their TME, from which DTPs can derive survival benefit[161] (Fig. 2). A general obstacle in studying these interactions is that most studies on DTPs are performed in 2D in vitro systems where the potential interactions between the microenvironment and DTPs cannot be assessed (Box 1). At the same time, in vivo analyses are complicated by the lack of gold standard biomarkers to identify DTPs. While there are very limited studies investigating the interplay between DTPs and the TME, those available show that the TME could affect drug resistance mechanisms of DTPs[155,158,160,167]. For instance, DTPs are reported to exhibit a senescence-associated secretory phenotype (SASP), which is generally believed to reshape the immune microenvironment[12]. A 'one-two punch' therapy

consisting of a senescence inducer and a senolytic drug to selectively eliminate senescent cells has shown remarkable success in preclinical models[235] (Fig. 3, Box 2).

Moreover, disrupting the complex interactions between DTPs and their microenvironment can substantially impact their survival. For example, interfering with the CAF-mediated induction of the HGF–MET axis in BRAF-mutant melanoma cells restores sensitivity to BRAF inhibition[162]. Additionally, blocking YAP activation prevents $LGR5^{+}p27^{+}$ CRC chemoresistant surviving cells from exiting the ECM-induced persistent dormant state, thus delaying tumor regrowth[165] (Fig.1).

Cytokines involved in the communication between cancer and TME cells could also be a promising target. Transforming growth factor β (TGFβ)–dependent interleukin 6 (IL-6) secretion during inflammation has been shown to be critical for the survival of NSCLC cells, characterized by EMT features and resistant to erlotinib. These cells are under long-term selection with erlotinib treatment, resembling a population of DTEPs. Blocking IL-6 restores sensitivity to targeted therapy[236] (Fig.1). Furthermore, a recent single cell analysis of a patient-derived EGFR-mutated lung adenocarcinoma model showed that DTP-secreted TGFβ promotes an IL-6 enriched microenviroment that may improve the survival of residual DTPs upon erlotinib treatment[237]. In another study, Sun and colleagues [156] observed a significant impairment of DTP survival upon inhibition of IGF1-mediated activation of IGF1R with the inhibitor AEW541, and upon disruption of the ability of IFNγ–STAT1 signalling to promote persistence through the inhibition of type I protein arginine methyltransferase (PRMT1).

Finally, another strategy centres around the activation of immunostimulatory pathways to promote the eradication of residual DTPs by the immune system. In a lung cancer mouse model, stimulator of interferon genes (STING) agonists eliminate disseminated dormant cancer cells and suppress tumor relapse in a natural killer (NK) cell, $CD4^{+}$ T-cell and $CD8^{+}$ T-cell dependent manner[238]. Moreover, newer immunotherapy approaches, such as adoptive T-cell transfer and CAR-T cells, may represent a promising avenue to eradicate DTPs[172]. Clearly, more work is needed to study the complex interplay between DTPs and the TME to develop appropriate targeting strategies.

[H1] DTPs and MRD in clinical settings

Amongst the clinical challenges, akin to the persistence conundrum observed in bacterial cells, the assessment of therapeutic strategies involving DTPs is complicated by the difficulty in detecting and

monitoring this rare, heterogeneous and plastic cell population as widely discussed in previous sections[2]. Furthermore, the DTP phenotype exhibits substantial diversity across different cancer histologies and in response to various treatments, posing a considerable challenge in identifying broadly applicable and clinically relevant biomarkers for DTPs (Box 3).

The current landscape of research investigating the role of these cells in cancer predominantly relies on *in vitro* or ex vivo models (Box 1), which makes it difficult to define clinical biomarkers[35]. Mouse models[88,239], PDXs[240], and patient samples[131] have been used to study MRD. These in vivo models can recapitulate some transcriptional features of DTPs observed in vitro. However, due to tumor heterogeneity, identifying universal biomarkers for DTPs in in vivo models or patient samples remains challenging. Unlike primary resistance, where tumor progression is primarily due to early treatment failure rather than an initial favorable response[241], DTP-involved resistance is typified by a latency of tumor recurrence in the clinical setting. In this sense, a particularly important challenge is the analysis of DTPs in patients with locally advanced disease undergoing treatment after surgery of the primary tumor, aiming to eliminate MRD and prevent relapse, which typically occurs in the form of metastasis. The molecular and biological characteristics of the residual disseminated tumor cells lodged in various organs remain largely uncharacterized, making it unclear which mechanisms they employ to resist therapy and whether current *in vitro* models are useful for investigating them. Recent advances in modeling metastatic relapse in mouse models could help bridge this gap[242] (Box 3).

Another important consideration is the need to systematically develop biomarkers, which include extrinsic factors that may impact on DTP dynamics, often overlooked in studies to date. Experiments conducted *in vitro* and *in vivo* indicate that residual DTPs might elevate the expression of innate tumor immunity pathways, encompassing interferon, TNF and damage-associated molecular pattern (DAMP)-associated signaling[239]. These preclinical observations suggest potential interactions between residual tumor cells and the TME. For example, depletion studies using mice clearly show that cytotoxic T-cells are of critical importance in shaping the response dynamics[243], however, whether they are the only relevant cells for immune control of the tumor remains unclear at the clinical level. Other studies have shown that distinct cell types in the TME may be positively or negatively involved in the effector response, including regulatory T-cells[244], macrophages[245], T-helper cells [246] and myeloid-derived suppressor cells[247]. How systemic treatment may reshape these immune cells in the context of killing residual DTPs remains unexplored (Box 3). Therefore, clinically relevant DTP biomarkers should consider immune-associated markers relevant to DTP phenotypes or dynamics.

In the clinical setting, the most advanced biomarkers of tumor response to systemic treatment include factors ranging from specific mutations (e.g., EGFR-L858R mutation and BRAF-V600E mutation), to gene sets involved in transcriptional regulation, or PDL1 expression in immunoncology. However, these biomarkers and others were all obtained from pre-treatment patient samples and the underlying assumption is that the patient's likelihood of responding is dependent on these pre-existing tumor settings. For instance, pre-treatment evaluation of PDL1 expression, tumor mutational burden (TMB), tumor-infiltrating lymphocytes (TILs), or tumor-intrinsic microsatellite instability status can identify patients likely to respond to immune checkpoint blockade. This is exemplified in NSCLC, where a PDL1 cutoff of >50% has been successfully used to determine the usage of atezolizumab monotherapy[248,249] or cemiplimab therapy[250].

Systemic treatment initially induces rapid regression in responsive patients. However, residual tumor cells survive, leading to a deceleration of the killing effect, accounting for the biphasic killing dynamics of tumor cells. This almost dichotomous therapeutic response in distinct tumor cells points to a critical state transition, akin to those observed in several complex systems such as ecology, economics and biology. At a critical state transition, even a minor perturbation could trigger a dramatic change in system behavior, resulting in predisposed conditions or biomarkers failing to predict the consequences of the state transition. Hence, the need arises for longitudinal or dynamic biomarkers to anticipate the critical state transition occurring in DTPs. While this concept has not been conclusively demonstrated in clinical settings, we can draw insight from bacterial DTPs: the dynamic recovery rate of bacterial phenotypes under various stress conditions can differentiate between the bacterial DTP states[22].

Accordingly, the kinetics of DTPs during treatment can be clinically determined by leveraging metabolic imaging to quantify the fraction of residual cancer cell populations during clinical response. Additionally, the evaluation of DTPs can be enriched by analyzing sequentially obtained blood samples[251] for the presence of circulating DTPs (cDTPs) and DTP-derived circulating tumor DNA (ctDNA), guided by their distinct functional characteristics. However, the detection of measurable positron emission tomography (PET)–computed tomography (CT) signals is challenging due to the minute fraction of viable DTPs likely surviving in most tumors. Furthermore, given their scarcity and the absence of DTP-specific biomarkers, current liquid biopsy-based technologies cannot reliably detect cDTPs and differentiate them from other treatment-resistant residual tumor cells, such as disseminated tumor cells and micrometastatic cell aggregates. To define the sensitivity of each approach, future comparative studies evaluating the minimum fractions of detectable DTPs using

these various methodologies will be important[252]. The intricate and elusive nature of DTP cells in the context of cancer therapy requires innovative and practical solutions for their clinical assessment (Box 3). Nevertheless, the proposed approaches utilizing metabolic imaging and sequential blood sample analysis could be valuable in deciphering the kinetics of DTP cells during treatment.

[H1] Conclusions

MRD refers to the persistence of low-level disease in patients after therapy, which is invisible using conventional image-based practices, but can lead to relapse[43,253,254]. While it is widely believed that DTPs are central to MRD-derived relapse, many issues remain to be addressed before DTPs can be targeted for clinical purposes. First, identifying DTPs is still controversial, as they are often misidentified as CSCs, senescent cells or dormant tumor cells. Until now, with the possible exception of CSCs, generally accepted markers to unambiguously identify all of these cell states have been lacking, making it difficult to study them in vivo. Second, the transitory nature and scarcity of DTPs renders them quite challenging to study. Last but not least, most of the available studies have used *in vitro* preclinical models. Hence, how DTPs interact with the TME and how these cells are capable of hiding from the immune system to survive for months, or even years, after therapy has been largely overlooked.

Despite the challenges, technologies to detect and monitor DTPs in patients with cancer could lead to more effective therapeutic strategies in the ever-evolving landscape of cancer treatment. Knowledge of the mechanisms driving DTP survival and dynamic plasticity might affect the choice and success of therapeutic strategies aimed at preventing tumor recurrence. Importantly, dissecting the mechanisms underlying the emergence of DTPs may dictate the schedule and timing of combinatorial regimen administration. Future efforts should prioritize the development of robust experimental models, both *in vitro* and *in vivo*, taking advantage of immunocompetent models and single-cell and spatial multiomics technologies, alongside innovative tissue and liquid biopsies to monitor MRD, to accelerate the dynamic tracking and therapeutic exploitation of the DTP phenotype, with the ultimate goal of inducing long-lasting clinical responses in patients with cancer.

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DISPLAY ITEMS

Table 1. Drug-tolerant persister cells in the context of quiescence. Cancer drug-tolerant persisters (DTPs) share multiple characteristics with bacterial DTPs and other quiescent cell types, such as cancer stem cells, diseminated dormant cells and senescent cells, and in several instances the terms persistence, stemness, dormancy and senescence are used synonymously. Nevertheless, DTPs also display distinctive features, such as the ability to genetically evolve and display phenotypic plasticity, which play crucial roles in driving tumor relapse under treatment[2,8,35]. FAO, fatty acid β-oxidation; OXPHOS, oxidative phosphorylation.

	Bacterial persisters	Drug-tolerant persister cells	Cancer stem cells	Tumor dormant cells	Senescent cells
Quiescence	Spontaneous or triggered by stress	Acquired under treatment	Intrinsic trait	Acquired during dissemination	Induced by specific stressors
Markers	No consensus	Stem- and senescence-like	CD44, CD133, ALDH, CD24, CD26, CD166, EPCAM	Largely unknown	B-galactosidase, SASP
Proliferation	Yes, but slow and only in a subset	Yes, but slow and only in a subset	Yes, with asymmetric division	No	No
Metabolism	Switch to FAO but no consensus	OXPHOS, FAO and oxidative stress	Both glycolysis and OXPHOS	Increased autophagy	Dysregulated lipid metabolism and autophagy
Microenvironment	May trigger persistence	Rewired for survival and immune evasion	Immune evasion	Rewired for metastatic outgrowth	Can mediate exit from senescence
Heterogeneity	Yes, phenotypic	Yes, phenotypic	Hierarchy of stemness and differentiation	Largely unknown	Variable phenotypes
Genetic evolvability	Yes, acquisition of resistance	Yes, acquisition of resistance	No	No	No

Table 2. Therapeutic vulnerabilities of cancer drug-tolerant persister cells. ADCs, antibody–drug conjugates; CAFs, cancer-associated fibroblasts; CAR, chimeric antigen receptor; ChIP-seq, chromatin immunoprecipitation followed by sequencing; CRC, colorectal cancer; BCC, basal-cell carcinoma; FAO, fatty acid β-oxidation; SCC, squamous-cell carcinoma; DTP, drug-tolerant phenotype; ER, oestrogen receptor; MAFs, melanoma-associated fibroblasts; KO, knockout; TKI, tyrosine kinase inhibitors; TME, tumor microenvironment; EMT, expithelial-to-mesenchimal transition; ECM, extra cellular matrix; CAF, cancer associated fibroblasts; MAF; melanoma associated fibroblasts; TIS, therapy-induced secretome; HDAC, histone deacetylase; EGFR, epidermal growth factor receptor; scRNA-seq, single cell RNA sequencing; ATAC-seq, assay for transposase-accessible chromatin with sequencing; PRMT1, protein arginine methyltransferase 1; GAS6, growth arrest-specific protein 6; WGS, whole-genome sequencing.

Cancer Histology	Experimantal models	Treatment	Technology used to reveal DTP phenotype	Persistence mechanism(s)	DTP vulnerabilities	Tested inhibitory strategies	Refs
ng cancer	In vitro preclinical models	Erlotinib (EGFR inhibitor)	Gene expression analysis	Epigenetic and chromatin rewiring	KDM5	Trichostatin A	1
					IGF1R	AEW541	
Lung cancer	In vitro and in vivo preclinical models	Erlotinib	Gene expression analysis	DTP–TME interaction	TGFβ– IL-6	IL-6 neutralizing antibody	236
Lung cancer	In vivo preclinical models and patient-derived samples	Erlotinib	Microarray expression profiling	Transcriptional rewiring and EMT	AXL	XL-880 and MP-470	74
Lung cancer	In vitro preclinical models	Erlotinib	Mass spectrometry	Chromatin rewiring	HDACs	MS275 and TCA	57

Lung cancer	In vitro and in vivo preclinical models and patient-derived samples	Erlotinib	Gene expression analysis	Transcriptional rewiring	WNT–β-catenin	ICG-001 and XAV939	75
Lung cancer	In vitro preclinical models	Osimertinib (EGFR inhibitor)	Tyrosine kinase phosphorylation array	Transcriptional rewiring	AXL	NPS1034	73
Lung cancer	In vitro preclinical models	Osimertinib	Receptor-tyrosine kinase array	Transcriptional rewiring	IGF1R	Linsitinib	76
Lung cancer	In vitro and in vivo preclinical models	Osimertinib + trametinib (MEK inhibitor)	Barcoding library + scRNA-seq + ATAC-seq	Trascriptional rewiring and senescence	YAP and TEAD	XAV939 and MYF-01-37	12
Lung cancer	In vitro and in vivo preclinical models	Erlotinib	In vitro analysis of secreted signals + drug screening	DTP–TME interaction	PRMT1	MS023	156
Lung cancer	In vitro and in vivo preclinical models and patient-derived samples	TKIs	Gene expression analysis	Transcriptional and metabolic rewiring	GAS6–AXL axis	Anti-AXL654	36
Lung cancer	In vitro and in vivo preclinical models and patient-derived samples	Erlotinib	Gene expression analysis	Transcriptional rewiring and EMT	CD70	CD70 ADCs and CD70-targeting CAR T cells and CAR NK cells	158
Lung cancer	In vitro and in vivo preclinical models and patient-derived samples	TKIs	WGS + barcoding library	Chromatin and transcriptional rewiring	APOBEC3A	A3A KO	189
Melanoma	Co-culture system of cancer cells and constituents of the TME	Vemurafenib (BRAF inhibitor)	Proteome analysis	DTP–TME interaction (CAFs)	HGF–MET pathway	Crizotinib	162
Melanoma	In vitro and in vivo preclinical models	Vemurafenib and Cisplatin chemotherapy	Proteome profiling	Metabolic rewiring (OXPHOS)	ATP-synthetase	Oligomycin and Bz-423	41

					NADH dehydrogenase (complex I)	Rotenone and phenformin	
Melanoma	In vitro and in vivo preclinical models and patient-derived samples	Vemurafenib	EKAREV biosensor, co-cultures and microarray analysis	DTP–TME interaction (MAFs)	FAK	PF562271 and PF573228	160
Melanoma	In vivo preclinical models and patient-derived samples	Dabrafenib (BRAF inhibitor) + trametinib	Single-cell transcriptomic analysis	Transcriptional heterogeneity and transdifferentiation	RXRG	XHX531 and bexarotene	43
Melanoma	In vitro preclinical models	Vemurafenib + cobimetinib (MEK inhibitor)	Translational analysis by puromycin incorporation into nascent proteins	Translational rewiring	eIF4A	Silvestrol	59
Melanoma	In vitro and in vivo preclinical models and patient-derived samples	Vemurafenib + cobimetinib	Bulk and single cell gene expression analysis	Metabolic rewiring (OXPHOS)	FAO	thioridazine	103
CRC	In vivo preclinical models and patient-derived samples	5-fluorouracil, oxaliplatin and irinotecan chemotherapies	Barcoding library, gene expression analysis and mathematical modeling	Diapause-like status	Autophagy	SBI-0206965 (ULK inhibitor) and CPT-11	39
CRC	In vitro and in vivo preclinical models	5-fluorouracil, oxaliplatin and irinotecan	LGR5-CreER/Cre-activatable Rainbow reporter and gene expression analysis	DTP–ECM interaction	Collagen XVII–FAK–YAP axis	YAP or TAZ knockdown	165
CRC	In vitro and in vivo preclinical models and patient-derived samples	Dabrafenib, cetuximab (EGFR inhibitor)	Mathematical modeling	Slow cycling	Error-prone DNA polymerases	JH-RE-06 (REV1 inhibitor)	10
Breast cancer	In vitro preclinical models	Trametinib and BEZ235 (Dual pan-class PI3K and mTOR inhibitor)	Gene expression analysis	Epigenetic and chromatin rewiring	BRD4	JQ1	111
Breast cancer	In vitro and in vivo preclinical models and patient-	5-fluorouracil	Single cell gene expression analysis, barcoding library and	Slow cycling, epigenetic and transcriptional rewiring and EMT	H3K27me3	UNC1999 (EZH2 inhibitor)	50

	derived samples		single cell ChIP-seq				
Breast cancer	In vitro preclinical models	TKIs	Barcoding library, gene expression analysis and ChIP-seq	Transcriptional rewiring, diapause-like status and senescence	ER–-SGK3–mTORC1	Fulvestrant (ER antagonist) and SGK3 inhibition	123
BCC	In vivo preclinical models	Vismodegib (Hedgehog pathway inhibitor)	Gene expression analysis	Chromatin rewiring and cell identity switch	WNT pathway	Anti-LRP6	116
BCC	In vivo preclinical models	Vismodegib	In situ hybridization and microarray analysis	Transcriptional rewiring and differentiation	WNT pathway	LGK-974	229
SCC	In vivo preclinical models	Adoptive T cell transfer immunotherapy	Single-cell transcriptomic analysis and barcoding library	DTP–TME interaction	TGFβ– CD80	Blocking antibodies	174
AML	In vitro and in vivo preclinical models	Ara-C chemotherapy	Gene expression analysis	Senescence, inflammation and diapause-like status	MYC and ATR	ATR inhibition	127
Lung, breast and gastric cancer	In vivo preclinical models	TKIs	Analysis of secreted growth factors and cytokines and gene expression analysis	Transcriptional rewiring	FGFR–STAT3 axis	Ponatinib	77
					JAK1–STAT3 axis	Ruxolitinib	
Lung and gastric cancer	In vitro and in vivo preclinical models	TKIs	Gene expression analysis	Transcriptional rewiring	ALDH	Disulfiram	40
Lung and breast cancer, melanomaand CRC	In vitro preclinical models	TK1s	Drug screening	Epigenetic and chromatin rewiring	KDM5A	CPI-455	64

Glioblastoma	In vitro and in vivo preclinical models	Dasatinib (TKI)	Single-cell transcriptomic analysis	Epigenetic and chromatin rewiring	Notch	compound-E and GSKJ4	58
Lung, breast and ovarian cancer and melanoma	In vitro preclinical models	TKIs	Gene expression analysis	Metabolic rewiring (lipid peroxidation)	GPX4	RSL3	101
Lung and bladder cancer	In vitro and in vivo preclinical models	TKIs	Gene expression analysis	Epigenetic and transcriptional rewiring	CDK7 and CDK12	THZ1	231
Breast and prostate cancer	In vitro and in vivo preclinical models	Docetaxel and vinblastine chemotherapies and afatinib (TKI)	Barcoding library, gene expression analysis and proteomic analysis	Diapause-like status	MYC	CDK9 inhibition	45
Lung and breast cancer and melanoma	In vitro and in vivo preclinical models	TKIs	DTP-derived single clones, proteomic analysis, gene expression analysis and drug screening	Transcriptional rewiring	IRS1	NT219	196
Lung and gastric cancer and melanoma	In vitro and in vivo preclinical models	TKIs	CRISPR-based genetic screening, drug screening and single cell gene expression analysis	Transcriptional and metabolic rewiring and senescence	BRD2	NEO2734, ARV-771 and CC-90010	63

Figure 1. The key features of drug-tolerant persister cells. Numerous studies have shown that drug-tolerant persisters (DTPs) can rewire multiple cellular functions (from the epigenome to transcription to translation to metabolism) and manipulate the surrounding microenvironment to promote their survival. Moreover, the phenotypic and genetic adaptability of DTPs are increasingly regarded as key drivers of tumor relapse under cancer treatment[1,10,11,17,36,38-39,40-47,50-51,54,57-141,188,189]. ALDH, aldehyde dehydrogenase; APOBEC, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like; CAF, cancer-associated fibroblast; ECM, extracellular matrix; eIF4A, eukaryotic initiation factor 4A; FAO, fatty acid β-oxidation; GPX4, glutathione peroxidase 4; IGF1R, insulin-like growth factor 1 receptor; KDM5/6, lysine demethylase 5/6; LINE1, long interspersed repeat element 1; m6A, N^6-methyladenosine; MITF, microphthalmia-associated transcription factor; OXPHOS, oxidative phosphorylation; PuMB, purine mutational bias; TAM, tumor-associated

macrophage; TCR, T cell receptor; TEAD, TEA domain family member; YAP, yes-associated protein.

Figure 2. Drug-tolerant persister cells rewire their microenvironment to escape immunity and survive. A complex secretory interplay between drug-tolerant persisters (DTPs), the extracellular matrix (ECM) and the other elements of the tumor microenvironment (encompassing macrophages, fibroblasts and inflammatory cells) promote the dormancy and survival of DTPs under treatment and, ultimately, enhances their escape from the immune system[146-179, 236-238]. CAF, cancer-associated fibroblast; FAK, focal adhesion kinase; FGF2, fibroblast growth factor 2; HGF, hepatocyte growth factor; IFNγ, interferon γ; IGF1, insulin-like growth factor 1; IL-6, interleukin 6; MHC, major histocompatibility complex; PDL1, programmed cell death protein 1 ligand 1; TAM, tumor-associated macrophage; TCR, T cell receptor; TGFβ, transforming growth factor β.

Figure 3. Therapeutic strategies to prevent tumor relapse from drug-tolerant persister cells. **(A)** Drug-tolerant persisters (DTPs) emerging under treatment (possibly from predestined 'pre-DTP' cells) activate different strategies to adapt their phenotype in a plastic manner and evolve their genotype. The resulting adapted cells with (epi)genetic mechanisms of resistance are then further selected. **(B)** The identification of a vulnerability of DTPs can drive the design of innovative therapeutic approaches to eradicate them, possibly preventing tumor relapse. Treatments targeting pre-DTP cells would be administered concomitantly with standard-of-care treatment, while drugs tackling specific vulnerabilities and/or adaptability of DTPs could be administered as part of a sequential strategy. Finally, pulsatory regimens could be designed to maintain a stable reservoir of sensitive cancer cells throughout treatment. CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage.

[b1] Box 1: Modelling cancer drug-tolerant persister cell states

No single model can capture all aspects of drug-tolerant persisters (DTPs) in every cancer type. However, experimental designs that maximize the strength of each model, combined with understanding its limitations can help identify the key biological features of DTP cells.

Cellular models - including 2D cultures and 3D co-cultures, organoids and assembloids - are often employed to identify and isolate the small fraction of DTPs surviving drug treatments[1,13,17,38,59].

Likely reflecting cell autonomous mechanisms in culture, they capture, at least in part, cell heterogeneity, drug-induced reprogramming and adaptive mutability at the single cell level[38]. Key advantages are the ability to use high content imaging and multi-omics to capture cell state changes in real time, which can be combined with barcodes to track cell lineages[42,255]. Chemical and genetic screens can be performed to identify DTP mechanisms, which can be functionally interrogated with perturbation experiments[64,156,256].

Animal models, including mice and zebrafish, capture DTPs in situ enabling the tracking of plasticity states overtime and in response to therapy through multispectral and intravital live cell imaging, fate mapping and barcodes, and single-cell technologies[38,39,66,67,229,257]. Key advantages include studying DTPs within the context of the microenvironment, including stromal and immune cells, and spatiotemporal resolution of DTP heterogeneity in vivo throughout disease progression. In parallel, in an immunodeficient context, patient-derived xenograft (PDX) models make important links to the clinic by facilitating the study of DTP heterogeneity of individual patients, including patient specific disease states[43,178].

Looking forward, it will be critical to integrate multi-omic data from preclinical models with analyses on patient samples and tumor explants (ex vivo) to identify which DTPs truly represent human disease biology in response to neo-adjuvant therapy and therapy combinations, and how non-genetic and genetic mechanisms of DTPs evolve over time[58,130,242,258]. This will be especially important to combine with clinical data and dynamic measurements of samples including liquid biopsies, microdevices, medical imaging, and artificial intelligence (AI) in digital pathology. Models may become predictive of patient responses, helping clinicians discern the optimal treatment combinations for patients.

[b2] Box 2: The grand challenges and therapeutic opportunities of cancer drug-tolerant persister cells

The characterization of cancer drug-tolerant persisters (DTPs) is challenging for multiple reasons. A lack of biomarkers and shared vulnerabilities represent a major obstacle on the way to eradicating minimal residual disease (MRD). Whether mechanisms driving drug-tolerance vary across different tumor types, are dependent on the specific oncogenic dependency or are treatment specific is still unknown. The transitory nature of DTP phenotype and the high levels of cellular plasticity represent not only a scientific but a technical challenge. Even if barcoded libraries and single-cell multiomics

technologies are emerging as fundamental tools for the identification, characterization and monitoring of heterogenous DTPs, these features still pose challenges for the identification of therapeutic strategies and the design of clinical trials. There are essentially five main therapeutic strategies:

i) preventing the drug sensitive-to-DTP phenotypic switch by interfering with pathways activated upon inhibition of oncogenic drivers or those governing switches of cell identity, to decrease the number of residual DTPs[1,57,196,231,259].

ii) preventing DTPs from exiting dormancy or a slow cycle and thereby sustaining the DTP state[235,260].

iii) targeting vulnerabilities of DTPs to eradicate MRD[36,101].

iv) preventing DTP evolution and acquisition of non-reversible mechanisms of drug resistance[10,189].

A successful approach will have to combine standard-of-care therapy acting on sensitive cells with agents targeting the phenotype and vulnerabilities of DTPs. Importantly, knowing how DTPs arise has important therapeutic implications. An upfront combinatorial strategy targeting an oncogenic dependency and DTP phenotype might be the preferred approach in the event that there are pre-existing DTPs. In contrast, a sequential or alternating administration of standard-of-care therapy and anti-cancer agents aimed at eradicating DTPs (exploiting a 'one-two punch' approach) or preventing their complete adaptation (before acquisition of de novo genetic alterations), may result in a more efficient and prolonged clinical response (FIG. 4).

[b3] Box 3: Outstanding questions in the field of cancer drug-tolerant persister cells

Multiple lines of research have considerably improved our understanding of cancer drug-tolerant persister cells (DTPs) and their role in tumor recurrence after treatment. From these milestones, new translationally and clinically relevant questions arise.

- If the origin of DTP cells is not genetically determined, is it purely stochastic or are there key environmental cues which prime cells to be DTPs and which could be potentially predicted?
- Are there genetic or epigenetic markers of DTPs which are shared across multiple tumor types and which could be exploited for disease monitoring in the clinic?
- Do population dynamics such as quorum sensing or lineage hierarchy play a role in the emergence of DTPs?
- A comprehensive characterization of the survival strategies of DTPs, in the context of other cellular stress response pathways, is lacking.

- The molecular switches governing the transition to the persistence state and subsequent re-expansion of cancer cells during relapse require further elucidation.
- Do DTPs communicate with each other to benefit survival or regrowth?
- How does phenotypic heterogeneity arise in DTPs? Does it reflect the presence of 'gambler' cells which probe different survival strategies, or can it be linked to specific genetic traits or environmental stimuli?
- Precise elucidation of how phenotypic heterogeneity and lineage plasticity impact the strategies of survival and adaptation of DTPs is required.
- How do DTPs escape eradication from the immune system? Are they intrinsically immune refractory, or do they escape following transient selective pressure exerted by immunity? Does anticancer treatment itself suppress the activity of immune cells against DTPs?
- The detection and monitoring of DTPs in the context of their microenvironment remains challenging, and new models and technologies are required to overcome this obstacle.
- Stress-induced mutagenesis has been mainly described in preclinical models, and its impact on tumor recurrence in patients demands additional validation.
- Could treatment-induced mutagenesis in DTPs be exploited to enhance their immunogenicity?
- If DTPs are able to adaptively increase genome mutability to promote evolution, are there also treatment-induced mechanisms which enhance epigenetic diversity?
- Innovative strategies are required to understand when resistance occurs through selection of pre-existing resistant cells and when it emerges from adaptation of DTPs in patients.
- As more vulnerabilities of DTPs are discovered, how can we prioritize targets for subsequent therapeutic implementations? How can the schedule of standard anti-cancer treatments be optimized to achieve DTP eradication?
- What is the timescale of memory acquisition and retention in persister cells? If DTPs maintain this cellular 'memory' when they acquire stable resistant mechanisms, what impact does it have on subsequent lines of treatment? What are the optimal therapeutic strategies and dosing schedules which interfere with it?
- Multimodal and interdisciplinary approaches, coupled with integrated single cell multiomics, will be instrumental to comprehensively characterize cancer DTPs, both at the preclinical level and in patients.

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Table of Contents summary

Resistance to therapy remains the biggest challenge to achieving cures in patients with cancer. In this Roadmap article, Russo et al. overview the field of cancer drug-tolerant persister cells providing paths to advance our understanding of their biology with innovative technologies and recommend strategies to therapeutically target them to ensure more prolonged responses are achieved in patients with cancer.

Glossary

Artificial intelligence (AI)-designed enhancers: Starting from a collection of random sequences, deep learning models are used to design synthetic sequences that act as cell type-specific enhancers in order to better understand the regulatory logic of enhancers and, ultimately, how they can be altered to manipulate cell states.

Autophagic flux: The process measuring autophagosome formation, fusion with lysosomes, and degradation of autophagic cargo.

Glycocalyx: A carbohydrate layer on cell surfaces, aiding cellular protection and communication.

Homology-directed repair: An error-free mechanism by which cells repair DNA double-stranded breaks using a sister DNA molecule as a template.

Mismatch repair: a DNA repair pathway that allows cells to detect and correct the insertion, deletion and mis-incorporation of nucleotides that can occur in the newly synthesised strand during DNA replication.

Oxidative phosphorylation: (OXPHOS). The process of ATP production by the mitochondrial electron transport chain.

Quorum sensing: A process of cell–cell communication that allows bacteria to share information about cell density and adjust gene expression accordingly.

Retrotransposons: Repetitive DNA sequences that can self-propagate in the human genome by using a ‘copy-and-paste’ mechanism in which an intermediate RNA molecule is reverse-transcribed to make a new genomic insertion.

Synthetic locus control regions: reporter systems that can be designed to reflect which transcriptional programmes and signalling pathways are active in cancer cells.

The toxin–antitoxin system: A gene pair ubiquitous in prokaryotes which may mediate growth arrest when the toxin is expressed in excess of its cognate antitoxin, leading to tolerance.

Translesion synthesis: (TLS). A DNA damage tolerance mechanism that employs error-prone DNA polymerases to proceed with DNA replication despite the presence of unrepaired DNA lesions.

REFERENCES

1 Sharma, S. V. *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69-80, doi:10.1016/j.cell.2010.02.027 (2010).

2 Shen, S., Vagner, S. & Robert, C. Persistent Cancer Cells: The Deadly Survivors. *Cell* **183**, 860-874, doi:10.1016/j.cell.2020.10.027 (2020).

3 Marine, J. C., Dawson, S. J. & Dawson, M. A. Non-genetic mechanisms of therapeutic resistance in cancer. *Nat Rev Cancer* **20**, 743-756, doi:10.1038/s41568-020-00302-4 (2020).

4 Bigger, J. TREATMENT OF STAPHYLOCOCCAL INFECTIONS WITH PENICILLIN BY INTERMITTENT STERILISATION. *The Lancet* **244**, 497-500, doi:10.1016/S0140-6736(00)74210-3 (1944).

5 Hobby, G. L., Meyer, K. & Chaffee, E. Observations on the Mechanism of Action of Penicillin. *Proceedings of the Society for Experimental Biology and Medicine* **50**, 281-285, doi:10.3181/00379727-50-13773 (1942).

6 Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L. & Leibler, S. Bacterial persistence as a phenotypic switch. *Science* **305**, 1622-1625, doi:10.1126/science.1099390 (2004).

7 Pontes, M. H. & Groisman, E. A. Slow growth determines nonheritable antibiotic resistance in. *Sci Signal* **12**, doi:10.1126/scisignal.aax3938 (2019).

8 Balaban, N. Q. *et al.* Definitions and guidelines for research on antibiotic persistence. *Nat Rev Microbiol* **17**, 441-448, doi:10.1038/s41579-019-0196-3 (2019).

9 Tape, C. J. Plastic persisters: revival stem cells in colorectal cancer. *Trends Cancer* **10**, 185-195, doi:10.1016/j.trecan.2023.11.003 (2024).

10 Russo, M. *et al.* A modified fluctuation-test framework characterizes the population dynamics and mutation rate of colorectal cancer persister cells. *Nat Genet* **54**, 976-984, doi:10.1038/s41588-022-01105-z (2022).

11 Oren, Y. *et al.* Cycling cancer persister cells arise from lineages with distinct programs. *Nature* **596**, 576-582, doi:10.1038/s41586-021-03796-6 (2021).

12 Kurppa, K. J. *et al.* Treatment-Induced Tumor Dormancy through YAP-Mediated Transcriptional Reprogramming of the Apoptotic Pathway. *Cancer Cell* **37**, 104-122.e112, doi:10.1016/j.ccell.2019.12.006 (2020).

13 Hata, A. N. *et al.* Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med* **22**, 262-269, doi:10.1038/nm.4040 (2016).

14 Ramirez, M. *et al.* Diverse drug-resistance mechanisms can emerge from drug-tolerant cancer persister cells. *Nat Commun* **7**, 10690, doi:10.1038/ncomms10690 (2016).

15 Pribis, J. P., Zhai, Y., Hastings, P. J. & Rosenberg, S. M. Stress-Induced Mutagenesis, Gambler Cells, and Stealth Targeting Antibiotic-Induced Evolution. *mBio* **13**, e0107422, doi:10.1128/mbio.01074-22 (2022).

16 Galhardo, R. S., Hastings, P. J. & Rosenberg, S. M. Mutation as a stress response and the regulation of evolvability. *Crit Rev Biochem Mol Biol* **42**, 399-435, doi:10.1080/10409230701648502 (2007).

17 Russo, M. *et al.* Adaptive mutability of colorectal cancers in response to targeted therapies. *Science* **366**, 1473-1480, doi:10.1126/science.aav4474 (2019).

18 Brauner, A., Fridman, O., Gefen, O. & Balaban, N. Q. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat Rev Microbiol* **14**, 320-330, doi:10.1038/nrmicro.2016.34 (2016).

19 Bigger, J. W. The bactericidal action of penicillin on Staphylococcus pyogenes. *Irish Journal of Medical Science (1926-1967)* **19**, 553-568, doi:10.1007/BF02948386 (1944).

20 Lewis, K. Persister cells, dormancy and infectious disease. *Nat Rev Microbiol* **5**, 48-56, doi:10.1038/nrmicro1557 (2007).

21 Tuomanen, E. Phenotypic tolerance: the search for beta-lactam antibiotics that kill nongrowing bacteria. *Rev Infect Dis* **8 Suppl 3**, S279-291, doi:10.1093/clinids/8.supplement_3.s279 (1986).

22 Kaplan, Y. *et al.* Observation of universal ageing dynamics in antibiotic persistence. *Nature* **600**, 290-294, doi:10.1038/s41586-021-04114-w (2021).

23 Manuse, S. *et al.* Bacterial persisters are a stochastically formed subpopulation of low-energy cells. *PLoS Biol* **19**, e3001194, doi:10.1371/journal.pbio.3001194 (2021).

24 Dörr, T., Vulić, M. & Lewis, K. Ciprofloxacin causes persister formation by inducing the TisB toxin in Escherichia coli. *PLoS Biol* **8**, e1000317, doi:10.1371/journal.pbio.1000317 (2010).

25 Schuster, C. F. & Bertram, R. Toxin-antitoxin systems are ubiquitous and versatile modulators of prokaryotic cell fate. *FEMS Microbiol Lett* **340**, 73-85, doi:10.1111/1574-6968.12074 (2013).

26 Keren, I., Shah, D., Spoering, A., Kaldalu, N. & Lewis, K. Specialized persister cells and the mechanism of multidrug tolerance in Escherichia coli. *J Bacteriol* **186**, 8172-8180, doi:10.1128/JB.186.24.8172-8180.2004 (2004).

27 Amato, S. M., Orman, M. A. & Brynildsen, M. P. Metabolic control of persister formation in Escherichia coli. *Mol Cell* **50**, 475-487, doi:10.1016/j.molcel.2013.04.002 (2013).

28 Korch, S. B., Henderson, T. A. & Hill, T. M. Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis. *Mol Microbiol* **50**, 1199-1213, doi:10.1046/j.1365-2958.2003.03779.x (2003).

29 Kwan, B. W., Valenta, J. A., Benedik, M. J. & Wood, T. K. Arrested protein synthesis increases persister-like cell formation. *Antimicrob Agents Chemother* **57**, 1468-1473, doi:10.1128/AAC.02135-12 (2013).

30 Li, Y. & Zhang, Y. PhoU is a persistence switch involved in persister formation and tolerance to multiple antibiotics and stresses in Escherichia coli. *Antimicrob Agents Chemother* **51**, 2092-2099, doi:10.1128/AAC.00052-07 (2007).

31 Zhang, L. *et al.* The catabolite repression control protein Crc plays a role in the development of antimicrobial-tolerant subpopulations in Pseudomonas aeruginosa biofilms. *Microbiology (Reading)* **158**, 3014-3019, doi:10.1099/mic.0.061192-0 (2012).

32 Girgis, H. S., Harris, K. & Tavazoie, S. Large mutational target size for rapid emergence of bacterial persistence. *Proc Natl Acad Sci U S A* **109**, 12740-12745, doi:10.1073/pnas.1205124109 (2012).

33 Fridman, O., Goldberg, A., Ronin, I., Shoresh, N. & Balaban, N. Q. Optimization of lag time underlies antibiotic tolerance in evolved bacterial populations. *Nature* **513**, 418-421, doi:10.1038/nature13469 (2014).

34 Sulaiman, J. E. & Lam, H. Evolution of Bacterial Tolerance Under Antibiotic Treatment and Its Implications on the Development of Resistance. *Front Microbiol* **12**, 617412, doi:10.3389/fmicb.2021.617412 (2021).

35 Pu, Y. *et al.* Drug-tolerant persister cells in cancer: the cutting edges and future directions. *Nat Rev Clin Oncol* **20**, 799-813, doi:10.1038/s41571-023-00815-5 (2023).

36 Noronha, A. *et al.* AXL and Error-Prone DNA Replication Confer Drug Resistance and Offer Strategies to Treat EGFR-Mutant Lung Cancer. *Cancer Discov* **12**, 2666-2683, doi:10.1158/2159-8290.CD-22-0111 (2022).

37 Rosano, D. *et al.* Long-term Multimodal Recording Reveals Epigenetic Adaptation Routes in Dormant Breast Cancer Cells. *Cancer Discov* **14**, 866-889, doi:10.1158/2159-8290.CD-23-1161 (2024).

38 Álvarez-Varela, A. *et al.* Mex3a marks drug-tolerant persister colorectal cancer cells that mediate relapse after chemotherapy. *Nat Cancer* **3**, 1052-1070, doi:10.1038/s43018-022-00402-0 (2022).

39 Rehman, S. K. *et al.* Colorectal Cancer Cells Enter a Diapause-like DTP State to Survive Chemotherapy. *Cell* **184**, 226-242.e221, doi:10.1016/j.cell.2020.11.018 (2021).

40 Raha, D. *et al.* The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. *Cancer Res* **74**, 3579-3590, doi:10.1158/0008-5472.can-13-3456 (2014).

41 Roesch, A. *et al.* Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell* **23**, 811-825, doi:10.1016/j.ccr.2013.05.003 (2013).

42 Shaffer, S. M. *et al.* Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. *Nature* **546**, 431-435, doi:10.1038/nature22794 (2017).

43 Rambow, F. *et al.* Toward Minimal Residual Disease-Directed Therapy in Melanoma. *Cell* **174**, 843-855.e819, doi:10.1016/j.cell.2018.06.025 (2018).

44 Karki, P., Angardi, V., Mier, J. C. & Orman, M. A. A Transient Metabolic State in Melanoma Persister Cells Mediated by Chemotherapeutic Treatments. *Front Mol Biosci* **8**, 780192, doi:10.3389/fmolb.2021.780192 (2021).

45 Dhimolea, E. *et al.* An Embryonic Diapause-like Adaptation with Suppressed Myc Activity Enables Tumor Treatment Persistence. *Cancer Cell* **39**, 240-256.e211, doi:10.1016/j.ccell.2020.12.002 (2021).

46 Echeverria, G. V. *et al.* Resistance to neoadjuvant chemotherapy in triple-negative breast cancer mediated by a reversible drug-tolerant state. *Sci Transl Med* **11**, doi:10.1126/scitranslmed.aav0936 (2019).

47 Merino, D. *et al.* Barcoding reveals complex clonal behavior in patient-derived xenografts of metastatic triple negative breast cancer. *Nat Commun* **10**, 766, doi:10.1038/s41467-019-08595-2 (2019).

48 Kreso, A. *et al.* Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science* **339**, 543-548, doi:10.1126/science.1227670 (2013).

49 Boumahdi, S. & de Sauvage, F. J. The great escape: tumour cell plasticity in resistance to targeted therapy. *Nat Rev Drug Discov* **19**, 39-56, doi:10.1038/s41573-019-0044-1 (2020).

50 Marsolier, J. *et al.* H3K27me3 conditions chemotolerance in triple-negative breast cancer. *Nat Genet* **54**, 459-468, doi:10.1038/s41588-022-01047-6 (2022).

51 Yang, C., Tian, C., Hoffman, T. E., Jacobsen, N. K. & Spencer, S. L. Melanoma subpopulations that rapidly escape MAPK pathway inhibition incur DNA damage and rely on stress signalling. *Nat Commun* **12**, 1747, doi:10.1038/s41467-021-21549-x (2021).

52 Hoffman, T. E. *et al.* Multiple cancers escape from multiple MAPK pathway inhibitors and use DNA replication stress signaling to tolerate aberrant cell cycles. *Sci Signal* **16**, eade8744, doi:10.1126/scisignal.ade8744 (2023).

53 Kinnunen, P. C., Humphries, B. A., Luker, G. D., Luker, K. E. & Linderman, J. J. Characterizing heterogeneous single-cell dose responses computationally and experimentally using threshold inhibition surfaces and dose-titration assays. *NPJ Syst Biol Appl* **10**, 42, doi:10.1038/s41540-024-00369-x (2024).

54 Xue, J. Y. *et al.* Rapid non-uniform adaptation to conformation-specific KRAS(G12C) inhibition. *Nature* **577**, 421-425, doi:10.1038/s41586-019-1884-x (2020).

55 Jia, D. *et al.* Drug-Tolerant Idling Melanoma Cells Exhibit Theory-Predicted Metabolic Low-Low Phenotype. *Front Oncol* **10**, 1426, doi:10.3389/fonc.2020.01426 (2020).

56 França, G. S. *et al.* Cellular adaptation to cancer therapy along a resistance continuum. *Nature*, doi:10.1038/s41586-024-07690-9 (2024).

57 Guler, G. D. *et al.* Repression of Stress-Induced LINE-1 Expression Protects Cancer Cell Subpopulations from Lethal Drug Exposure. *Cancer Cell* **32**, 221-237.e213, doi:10.1016/j.ccell.2017.07.002 (2017).

58 Liau, B. B. *et al.* Adaptive Chromatin Remodeling Drives Glioblastoma Stem Cell Plasticity and Drug Tolerance. *Cell Stem Cell* **20**, 233-246.e237, doi:10.1016/j.stem.2016.11.003 (2017).

59 Shen, S. *et al.* An epitranscriptomic mechanism underlies selective mRNA translation remodelling in melanoma persister cells. *Nat Commun* **10**, 5713, doi:10.1038/s41467-019-13360-6 (2019).

60 Zhao, X. *et al.* BCL2 Amplicon Loss and Transcriptional Remodeling Drives ABT-199 Resistance in B Cell Lymphoma Models. *Cancer Cell* **35**, 752-766.e759, doi:10.1016/j.ccell.2019.04.005 (2019).

61 Aissa, A. F. *et al.* Single-cell transcriptional changes associated with drug tolerance and response to combination therapies in cancer. *Nat Commun* **12**, 1628, doi:10.1038/s41467-021-21884-z (2021).

62 Bell, C. C. *et al.* Targeting enhancer switching overcomes non-genetic drug resistance in acute myeloid leukaemia. *Nat Commun* **10**, 2723, doi:10.1038/s41467-019-10652-9 (2019).

63 Chen, M. *et al.* Targeting of vulnerabilities of drug-tolerant persisters identified through functional genetics delays tumor relapse. *Cell Rep Med* **5**, 101471, doi:10.1016/j.xcrm.2024.101471 (2024).

64 Vinogradova, M. *et al.* An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. *Nat Chem Biol* **12**, 531-538, doi:10.1038/nchembio.2085 (2016).

65 Banelli, B. *et al.* The histone demethylase KDM5A is a key factor for the resistance to temozolomide in glioblastoma. *Cell Cycle* **14**, 3418-3429, doi:10.1080/15384101.2015.1090063 (2015).

66 Travnickova, J. *et al.* Zebrafish MITF-Low Melanoma Subtype Models Reveal Transcriptional Subclusters and MITF-Independent Residual Disease. *Cancer Res* **79**, 5769-5784, doi:10.1158/0008-5472.can-19-0037 (2019).
67 Travnickova, J. *et al.* Fate mapping melanoma persister cells through regression and into recurrent disease in adult zebrafish. *Dis Model Mech* **15**, doi:10.1242/dmm.049566 (2022).
68 Brombin, A. *et al.* Tfap2b specifies an embryonic melanocyte stem cell that retains adult multifate potential. *Cell Rep* **38**, 110234, doi:10.1016/j.celrep.2021.110234 (2022).
69 Lu, Y. *et al.* ALDH1A3-acetaldehyde metabolism potentiates transcriptional heterogeneity in melanoma. *Cell Rep* **43**, 114406, doi:10.1016/j.celrep.2024.114406 (2024).
70 Ginestier, C. *et al.* ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* **1**, 555-567, doi:10.1016/j.stem.2007.08.014 (2007).
71 Luo, Y. *et al.* ALDH1A isozymes are markers of human melanoma stem cells and potential therapeutic targets. *Stem Cells* **30**, 2100-2113, doi:10.1002/stem.1193 (2012).
72 Sarvi, S. *et al.* ALDH1 Bio-activates Nifuroxazide to Eradicate ALDH. *Cell Chem Biol* **25**, 1456-1469.e1456, doi:10.1016/j.chembiol.2018.09.005 (2018).
73 Taniguchi, H. *et al.* AXL confers intrinsic resistance to osimertinib and advances the emergence of tolerant cells. *Nat Commun* **10**, 259, doi:10.1038/s41467-018-08074-0 (2019).
74 Zhang, Z. *et al.* Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* **44**, 852-860, doi:10.1038/ng.2330 (2012).
75 Arasada, R. R. *et al.* Notch3-dependent β-catenin signaling mediates EGFR TKI drug persistence in EGFR mutant NSCLC. *Nat Commun* **9**, 3198, doi:10.1038/s41467-018-05626-2 (2018).
76 Wang, R. *et al.* Transient IGF-1R inhibition combined with osimertinib eradicates AXL-low expressing EGFR mutated lung cancer. *Nat Commun* **11**, 4607, doi:10.1038/s41467-020-18442-4 (2020).
77 Lee, H. J. *et al.* Drug resistance via feedback activation of Stat3 in oncogene-addicted cancer cells. *Cancer Cell* **26**, 207-221, doi:10.1016/j.ccr.2014.05.019 (2014).
78 Priya, B., Ravi, S. & Kirubakaran, S. Targeting ATM and ATR for cancer therapeutics: Inhibitors in clinic. *Drug Discov Today* **28**, 103662, doi:10.1016/j.drudis.2023.103662 (2023).
79 Prevo, R. *et al.* The novel ATR inhibitor VE-821 increases sensitivity of pancreatic cancer cells to radiation and chemotherapy. *Cancer Biol Ther* **13**, 1072-1081, doi:10.4161/cbt.21093 (2012).
80 Huntoon, C. J. *et al.* ATR inhibition broadly sensitizes ovarian cancer cells to chemotherapy independent of BRCA status. *Cancer Res* **73**, 3683-3691, doi:10.1158/0008-5472.CAN-13-0110 (2013).
81 Wang, W. J. *et al.* MYC regulation of CHK1 and CHK2 promotes radioresistance in a stem cell-like population of nasopharyngeal carcinoma cells. *Cancer Res* **73**, 1219-1231, doi:10.1158/0008-5472.CAN-12-1408 (2013).
82 Cerezo, M., Robert, C., Liu, L. & Shen, S. The Role of mRNA Translational Control in Tumor Immune Escape and Immunotherapy Resistance. *Cancer Res* **81**, 5596-5604, doi:10.1158/0008-5472.can-21-1466 (2021).
83 Fabbri, L., Chakraborty, A., Robert, C. & Vagner, S. The plasticity of mRNA translation during cancer progression and therapy resistance. *Nat Rev Cancer* **21**, 558-577, doi:10.1038/s41568-021-00380-y (2021).
84 Song, K. A. *et al.* Increased Synthesis of MCL-1 Protein Underlies Initial Survival of. *Clin Cancer Res* **24**, 5658-5672, doi:10.1158/1078-0432.ccr-18-0304 (2018).
85 Calvo, V. *et al.* A PERK-Specific Inhibitor Blocks Metastatic Progression by Limiting Integrated Stress Response-Dependent Survival of Quiescent Cancer Cells. *Clin Cancer Res* **29**, 5155-5172, doi:10.1158/1078-0432.ccr-23-1427 (2023).
86 Sannino, S. *et al.* Non-Essential Amino Acid Availability Influences Proteostasis and Breast Cancer Cell Survival During Proteotoxic Stress. *Mol Cancer Res* **21**, 675-690, doi:10.1158/1541-7786.mcr-22-0843 (2023).
87 Falletta, P. *et al.* Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev* **31**, 18-33, doi:10.1101/gad.290940.116 (2017).

88 Vendramin, R. *et al.* Activation of the integrated stress response confers vulnerability to mitoribosome-targeting antibiotics in melanoma. *J Exp Med* **218**, doi:10.1084/jem.20210571 (2021).
89 Palam, L. R., Gore, J., Craven, K. E., Wilson, J. L. & Korc, M. Integrated stress response is critical for gemcitabine resistance in pancreatic ductal adenocarcinoma. *Cell Death Dis* **6**, e1913, doi:10.1038/cddis.2015.264 (2015).
90 Guan, B. J. *et al.* A Unique ISR Program Determines Cellular Responses to Chronic Stress. *Mol Cell* **68**, 885-900.e886, doi:10.1016/j.molcel.2017.11.007 (2017).
91 Costa-Mattioli, M. & Walter, P. The integrated stress response: From mechanism to disease. *Science* **368**, doi:10.1126/science.aat5314 (2020).
92 Pakos-Zebrucka, K. *et al.* The integrated stress response. *EMBO Rep* **17**, 1374-1395, doi:10.15252/embr.201642195 (2016).
93 Rouschop, K. M. *et al.* The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* **120**, 127-141, doi:10.1172/JCI40027 (2010).
94 Rouschop, K. M. *et al.* PERK/eIF2α signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. *Proc Natl Acad Sci U S A* **110**, 4622-4627, doi:10.1073/pnas.1210633110 (2013).
95 Reich, S. *et al.* A multi-omics analysis reveals the unfolded protein response regulon and stress-induced resistance to folate-based antimetabolites. *Nat Commun* **11**, 2936, doi:10.1038/s41467-020-16747-y (2020).
96 Hu, C. *et al.* Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. *MedComm (2020)* **3**, e161, doi:10.1002/mco2.161 (2022).
97 Sonia, C. *et al.* The cancer-specific lncRNA LISR customizes ribosomes to suppress anti-tumour immunity. *bioRxiv*, 2023.2001.2006.523012, doi:10.1101/2023.01.06.523012 (2023).
98 Falletta, P., Goding, C. R. & Vivas-García, Y. Connecting Metabolic Rewiring With Phenotype Switching in Melanoma. *Front Cell Dev Biol* **10**, 930250, doi:10.3389/fcell.2022.930250 (2022).
99 Zhang, W. C. *et al.* miR-147b-mediated TCA cycle dysfunction and pseudohypoxia initiate drug tolerance to EGFR inhibitors in lung adenocarcinoma. *Nat Metab* **1**, 460-474, doi:10.1038/s42255-019-0052-9 (2019).
100 Goldman, A. *et al.* Targeting tumor phenotypic plasticity and metabolic remodeling in adaptive cross-drug tolerance. *Sci Signal* **12**, doi:10.1126/scisignal.aas8779 (2019).
101 Hangauer, M. J. *et al.* Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* **551**, 247-250, doi:10.1038/nature24297 (2017).
102 Viswanathan, V. S. *et al.* Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* **547**, 453-457, doi:10.1038/nature23007 (2017).
103 Shen, S. *et al.* Melanoma Persister Cells Are Tolerant to BRAF/MEK Inhibitors via ACOX1-Mediated Fatty Acid Oxidation. *Cell Rep* **33**, 108421, doi:10.1016/j.celrep.2020.108421 (2020).
104 Liu, Z. *et al.* CPT1A-mediated fatty acid oxidation confers cancer cell resistance to immune-mediated cytolytic killing. *Proc Natl Acad Sci U S A* **120**, e2302878120, doi:10.1073/pnas.2302878120 (2023).
105 Redondo-Muñoz, M. *et al.* Metabolic rewiring induced by ranolazine improves melanoma responses to targeted therapy and immunotherapy. *Nat Metab* **5**, 1544-1562, doi:10.1038/s42255-023-00861-4 (2023).
106 Mohiuddin, S. G., Nguyen, T. V. & Orman, M. A. Pleiotropic actions of phenothiazine drugs are detrimental to Gram-negative bacterial persister cells. *Commun Biol* **5**, 217, doi:10.1038/s42003-022-03172-8 (2022).
107 McDonald, P. C. & Dedhar, S. Persister cell plasticity in tumour drug resistance. *Semin Cell Dev Biol* **156**, 1-10, doi:10.1016/j.semcdb.2023.11.003 (2024).
108 Shibue, T. & Weinberg, R. A. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol* **14**, 611-629, doi:10.1038/nrclinonc.2017.44 (2017).
109 Weng, C. H. *et al.* Epithelial-mesenchymal transition (EMT) beyond EGFR mutations per se is a common mechanism for acquired resistance to EGFR TKI. *Oncogene* **38**, 455-468, doi:10.1038/s41388-018-0454-2 (2019).

110 Chung, J. H. *et al.* Clinical and molecular evidences of epithelial to mesenchymal transition in acquired resistance to EGFR-TKIs. *Lung Cancer* **73**, 176-182, doi:10.1016/j.lungcan.2010.11.011 (2011).
111 Risom, T. *et al.* Differentiation-state plasticity is a targetable resistance mechanism in basal-like breast cancer. *Nat Commun* **9**, 3815, doi:10.1038/s41467-018-05729-w (2018).
112 Sahoo, S. *et al.* A mechanistic model captures the emergence and implications of non-genetic heterogeneity and reversible drug resistance in ER+ breast cancer cells. *NAR Cancer* **3**, zcab027, doi:10.1093/narcan/zcab027 (2021).
113 Lupo, B. *et al.* Colorectal cancer residual disease at maximal response to EGFR blockade displays a druggable Paneth cell-like phenotype. *Sci Transl Med* **12**, doi:10.1126/scitranslmed.aax8313 (2020).
114 Davies, A. H., Beltran, H. & Zoubeidi, A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. *Nat Rev Urol* **15**, 271-286, doi:10.1038/nrurol.2018.22 (2018).
115 Lee, J. K. *et al.* Clonal History and Genetic Predictors of Transformation Into Small-Cell Carcinomas From Lung Adenocarcinomas. *J Clin Oncol* **35**, 3065-3074, doi:10.1200/jco.2016.71.9096 (2017).
116 Biehs, B. *et al.* A cell identity switch allows residual BCC to survive Hedgehog pathway inhibition. *Nature* **562**, 429-433, doi:10.1038/s41586-018-0596-y (2018).
117 Chan, J. M. *et al.* Lineage plasticity in prostate cancer depends on JAK/STAT inflammatory signaling. *Science* **377**, 1180-1191, doi:10.1126/science.abn0478 (2022).
118 Deng, S. *et al.* Ectopic JAK-STAT activation enables the transition to a stem-like and multilineage state conferring AR-targeted therapy resistance. *Nat Cancer* **3**, 1071-1087, doi:10.1038/s43018-022-00431-9 (2022).
119 Moorman, A. R. *et al.* Progressive plasticity during colorectal cancer metastasis. *bioRxiv*, doi:10.1101/2023.08.18.553925 (2023).
120 Chan, J. M. *et al.* Signatures of plasticity, metastasis, and immunosuppression in an atlas of human small cell lung cancer. *Cancer Cell* **39**, 1479-1496.e1418, doi:10.1016/j.ccell.2021.09.008 (2021).
121 Raghavan, S. *et al.* Microenvironment drives cell state, plasticity, and drug response in pancreatic cancer. *Cell* **184**, 6119-6137.e6126, doi:10.1016/j.cell.2021.11.017 (2021).
122 Burdziak, C. *et al.* Epigenetic plasticity cooperates with cell-cell interactions to direct pancreatic tumorigenesis. *Science* **380**, eadd5327, doi:10.1126/science.add5327 (2023).
123 Chang, C. A. *et al.* Ontogeny and Vulnerabilities of Drug-Tolerant Persisters in HER2+ Breast Cancer. *Cancer Discov* **12**, 1022-1045, doi:10.1158/2159-8290.CD-20-1265 (2022).
124 Goyal, Y. *et al.* Diverse clonal fates emerge upon drug treatment of homogeneous cancer cells. *Nature* **620**, 651-659, doi:10.1038/s41586-023-06342-8 (2023).
125 Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov* **12**, 31-46, doi:10.1158/2159-8290.CD-21-1059 (2022).
126 Anand, U. *et al.* Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis* **10**, 1367-1401, doi:10.1016/j.gendis.2022.02.007 (2023).
127 Duy, C. *et al.* Chemotherapy Induces Senescence-Like Resilient Cells Capable of Initiating AML Recurrence. *Cancer Discov* **11**, 1542-1561, doi:10.1158/2159-8290.cd-20-1375 (2021).
128 Chen, J. *et al.* A restricted cell population propagates glioblastoma growth after chemotherapy. *Nature* **488**, 522-526, doi:10.1038/nature11287 (2012).
129 Paudel, B. B. *et al.* A Nonquiescent "Idling" Population State in Drug-Treated, BRAF-Mutated Melanoma. *Biophys J* **114**, 1499-1511, doi:10.1016/j.bpj.2018.01.016 (2018).
130 Marin-Bejar, O. *et al.* Evolutionary predictability of genetic versus nongenetic resistance to anticancer drugs in melanoma. *Cancer Cell* **39**, 1135-1149.e1138, doi:10.1016/j.ccell.2021.05.015 (2021).
131 Zhou, X. *et al.* Persister cell phenotypes contribute to poor patient outcomes after neoadjuvant chemotherapy in PDAC. *Nat Cancer* **4**, 1362-1381, doi:10.1038/s43018-023-00628-6 (2023).
132 Zou, J., Peng, B., Qu, J. & Zheng, J. Are Bacterial Persisters Dormant Cells Only? *Front Microbiol* **12**, 708580, doi:10.3389/fmicb.2021.708580 (2021).
133 Kalkavan, H. *et al.* Sublethal cytochrome c release generates drug-tolerant persister cells. *Cell* **185**, 3356-3374.e3322, doi:10.1016/j.cell.2022.07.025 (2022).

134 Solé, L. *et al.* p53 wild-type colorectal cancer cells that express a fetal gene signature are associated with metastasis and poor prognosis. *Nat Commun* **13**, 2866, doi:10.1038/s41467-022-30382-9 (2022).
135 Ramos Zapatero, M. *et al.* Trellis tree-based analysis reveals stromal regulation of patient-derived organoid drug responses. *Cell* **186**, 5606-5619.e5624, doi:10.1016/j.cell.2023.11.005 (2023).
136 Fenelon, J. C., Banerjee, A. & Murphy, B. D. Embryonic diapause: development on hold. *Int J Dev Biol* **58**, 163-174, doi:10.1387/ijdb.140074bm (2014).
137 Fenelon, J. C. & Renfree, M. B. The history of the discovery of embryonic diapause in mammals. *Biol Reprod* **99**, 242-251, doi:10.1093/biolre/ioy112 (2018).
138 Boroviak, T. *et al.* Lineage-Specific Profiling Delineates the Emergence and Progression of Naive Pluripotency in Mammalian Embryogenesis. *Dev Cell* **35**, 366-382, doi:10.1016/j.devcel.2015.10.011 (2015).
139 Iyer, D. P. *et al.* Delay of human early development via in vitro diapause. *bioRxiv*, 2023.2005.2029.541316, doi:10.1101/2023.05.29.541316 (2023).
140 Bulut-Karslioglu, A. *et al.* Inhibition of mTOR induces a paused pluripotent state. *Nature* **540**, 119-123, doi:10.1038/nature20578 (2016).
141 Scognamiglio, R. *et al.* Myc Depletion Induces a Pluripotent Dormant State Mimicking Diapause. *Cell* **164**, 668-680, doi:10.1016/j.cell.2015.12.033 (2016).
142 Maynard, A. *et al.* Therapy-Induced Evolution of Human Lung Cancer Revealed by Single-Cell RNA Sequencing. *Cell* **182**, 1232-1251.e1222, doi:10.1016/j.cell.2020.07.017 (2020).
143 Omuro, A. M. *et al.* High incidence of disease recurrence in the brain and leptomeninges in patients with nonsmall cell lung carcinoma after response to gefitinib. *Cancer* **103**, 2344-2348, doi:10.1002/cncr.21033 (2005).
144 Smalley, I. *et al.* Single-Cell Characterization of the Immune Microenvironment of Melanoma Brain and Leptomeningeal Metastases. *Clin Cancer Res* **27**, 4109-4125, doi:10.1158/1078-0432.ccr-21-1694 (2021).
145 Priego, N. *et al.* STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. *Nat Med* **24**, 1024-1035, doi:10.1038/s41591-018-0044-4 (2018).
146 Mancini, C., Lori, G., Pranzini, E. & Taddei, M. L. Metabolic challengers selecting tumor-persistent cells. *Trends Endocrinol Metab* **35**, 263-276, doi:10.1016/j.tem.2023.11.005 (2024).
147 Wicks, E. E. & Semenza, G. L. Hypoxia-inducible factors: cancer progression and clinical translation. *J Clin Invest* **132**, doi:10.1172/JCI159839 (2022).
148 Ravindran Menon, D. *et al.* A stress-induced early innate response causes multidrug tolerance in melanoma. *Oncogene* **34**, 4448-4459, doi:10.1038/onc.2014.372 (2015).
149 Endo, H. *et al.* The induction of MIG6 under hypoxic conditions is critical for dormancy in primary cultured lung cancer cells with activating EGFR mutations. *Oncogene* **36**, 2824-2834, doi:10.1038/onc.2016.431 (2017).
150 Fluegen, G. *et al.* Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments. *Nat Cell Biol* **19**, 120-132, doi:10.1038/ncb3465 (2017).
151 Baldominos, P. *et al.* Quiescent cancer cells resist T cell attack by forming an immunosuppressive niche. *Cell* **185**, 1694-1708.e1619, doi:10.1016/j.cell.2022.03.033 (2022).
152 Poillet-Perez, L., Sarry, J. E. & Joffre, C. Autophagy is a major metabolic regulator involved in cancer therapy resistance. *Cell Rep* **36**, 109528, doi:10.1016/j.celrep.2021.109528 (2021).
153 Russell, R. C. & Guan, K. L. The multifaceted role of autophagy in cancer. *EMBO J* **41**, e110031, doi:10.15252/embj.2021110031 (2022).
154 Vera-Ramirez, L., Vodnala, S. K., Nini, R., Hunter, K. W. & Green, J. E. Autophagy promotes the survival of dormant breast cancer cells and metastatic tumour recurrence. *Nat Commun* **9**, 1944, doi:10.1038/s41467-018-04070-6 (2018).
155 Obenauf, A. C. *et al.* Therapy-induced tumour secretomes promote resistance and tumour progression. *Nature* **520**, 368-372, doi:10.1038/nature14336 (2015).
156 Sun, X. *et al.* Modulating environmental signals to reveal mechanisms and vulnerabilities of cancer persisters. *Sci Adv* **8**, eabi7711, doi:10.1126/sciadv.abi7711 (2022).

157 Schmitt, M. *et al.* Colon tumour cell death causes mTOR dependence by paracrine P2X4 stimulation. *Nature* **612**, 347-353, doi:10.1038/s41586-022-05426-1 (2022).
158 Nilsson, M. B. *et al.* CD70 is a therapeutic target upregulated in EMT-associated EGFR tyrosine kinase inhibitor resistance. *Cancer Cell* **41**, 340-355.e346, doi:10.1016/j.ccell.2023.01.007 (2023).
159 Flieswasser, T. *et al.* The CD70-CD27 axis in oncology: the new kids on the block. *J Exp Clin Cancer Res* **41**, 12, doi:10.1186/s13046-021-02215-y (2022).
160 Hirata, E. *et al.* Intravital imaging reveals how BRAF inhibition generates drug-tolerant microenvironments with high integrin β1/FAK signaling. *Cancer Cell* **27**, 574-588, doi:10.1016/j.ccell.2015.03.008 (2015).
161 Mikubo, M., Inoue, Y., Liu, G. & Tsao, M. S. Mechanism of Drug Tolerant Persister Cancer Cells: The Landscape and Clinical Implication for Therapy. *J Thorac Oncol* **16**, 1798-1809, doi:10.1016/j.jtho.2021.07.017 (2021).
162 Straussman, R. *et al.* Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* **487**, 500-504, doi:10.1038/nature11183 (2012).
163 Heynen, G. J., Fonfara, A. & Bernards, R. Resistance to targeted cancer drugs through hepatocyte growth factor signaling. *Cell Cycle* **13**, 3808-3817, doi:10.4161/15384101.2014.988033 (2014).
164 Yano, S. *et al.* Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res* **68**, 9479-9487, doi:10.1158/0008-5472.can-08-1643 (2008).
165 Ohta, Y. *et al.* Cell-matrix interface regulates dormancy in human colon cancer stem cells. *Nature* **608**, 784-794, doi:10.1038/s41586-022-05043-y (2022).
166 Rehman, S. K. & O'Brien, C. A. Persister cells that survive chemotherapy are pinpointed. *Nature* **608**, 675-676, doi:10.1038/d41586-022-01866-x (2022).
167 Walens, A. *et al.* CCL5 promotes breast cancer recurrence through macrophage recruitment in residual tumors. *Elife* **8**, doi:10.7554/eLife.43653 (2019).
168 Cerezo-Wallis, D. *et al.* Midkine rewires the melanoma microenvironment toward a tolerogenic and immune-resistant state. *Nat Med* **26**, 1865-1877, doi:10.1038/s41591-020-1073-3 (2020).
169 Landsberg, J. *et al.* Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature* **490**, 412-416, doi:10.1038/nature11538 (2012).
170 Mehta, A. *et al.* Immunotherapy Resistance by Inflammation-Induced Dedifferentiation. *Cancer Discov* **8**, 935-943, doi:10.1158/2159-8290.cd-17-1178 (2018).
171 van Weverwijk, A. & de Visser, K. E. Mechanisms driving the immunoregulatory function of cancer cells. *Nat Rev Cancer* **23**, 193-215, doi:10.1038/s41568-022-00544-4 (2023).
172 Goddard, E. T. *et al.* Immune evasion of dormant disseminated tumor cells is due to their scarcity and can be overcome by T cell immunotherapies. *Cancer Cell* **42**, 119-134.e112, doi:10.1016/j.ccell.2023.12.011 (2024).
173 Burr, M. L. *et al.* An Evolutionarily Conserved Function of Polycomb Silences the MHC Class I Antigen Presentation Pathway and Enables Immune Evasion in Cancer. *Cancer Cell* **36**, 385-401.e388, doi:10.1016/j.ccell.2019.08.008 (2019).
174 Miao, Y. *et al.* Adaptive Immune Resistance Emerges from Tumor-Initiating Stem Cells. *Cell* **177**, 1172-1186.e1114, doi:10.1016/j.cell.2019.03.025 (2019).
175 Cerezo, M. *et al.* Translational control of tumor immune escape via the eIF4F-STAT1-PD-L1 axis in melanoma. *Nat Med* **24**, 1877-1886, doi:10.1038/s41591-018-0217-1 (2018).
176 Xu, Y. *et al.* Translation control of the immune checkpoint in cancer and its therapeutic targeting. *Nat Med* **25**, 301-311, doi:10.1038/s41591-018-0321-2 (2019).
177 Suresh, S. *et al.* eIF5B drives integrated stress response-dependent translation of PD-L1 in lung cancer. *Nat Cancer* **1**, 533-545, doi:10.1038/s43018-020-0056-0 (2020).
178 Pozniak, J. *et al.* A TCF4-dependent gene regulatory network confers resistance to immunotherapy in melanoma. *Cell* **187**, 166-183.e125, doi:10.1016/j.cell.2023.11.037 (2024).
179 Sehgal, K. *et al.* Dynamic single-cell RNA sequencing identifies immunotherapy persister cells following PD-1 blockade. *J Clin Invest* **131**, doi:10.1172/jci135038 (2021).

180 Fitzgerald, D. M., Hastings, P. J. & Rosenberg, S. M. Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance. *Annu Rev Cancer Biol* **1**, 119-140, doi:10.1146/annurev-cancerbio-050216-121919 (2017).
181 Bhandari, V. *et al.* Molecular landmarks of tumor hypoxia across cancer types. *Nat Genet* **51**, 308-318, doi:10.1038/s41588-018-0318-2 (2019).
182 Torkelson, J. *et al.* Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J* **16**, 3303-3311, doi:10.1093/emboj/16.11.3303 (1997).
183 Pribis, J. P. *et al.* Gamblers: An Antibiotic-Induced Evolvable Cell Subpopulation Differentiated by Reactive-Oxygen-Induced General Stress Response. *Mol Cell* **74**, 785-800.e787, doi:10.1016/j.molcel.2019.02.037 (2019).
184 Ram, Y. & Hadany, L. Stress-induced mutagenesis and complex adaptation. *Proc Biol Sci* **281**, doi:10.1098/rspb.2014.1025 (2014).
185 Ponder, R. G., Fonville, N. C. & Rosenberg, S. M. A switch from high-fidelity to error-prone DNA double-strand break repair underlies stress-induced mutation. *Mol Cell* **19**, 791-804, doi:10.1016/j.molcel.2005.07.025 (2005).
186 Paniagua, I. & Jacobs, J. J. L. Freedom to err: The expanding cellular functions of translesion DNA polymerases. *Mol Cell* **83**, 3608-3621, doi:10.1016/j.molcel.2023.07.008 (2023).
187 Hastings, P. J., Ira, G. & Lupski, J. R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet* **5**, e1000327, doi:10.1371/journal.pgen.1000327 (2009).
188 Cipponi, A. *et al.* MTOR signaling orchestrates stress-induced mutagenesis, facilitating adaptive evolution in cancer. *Science* **368**, 1127-1131, doi:10.1126/science.aau8768 (2020).
189 Isozaki, H. *et al.* Therapy-induced APOBEC3A drives evolution of persistent cancer cells. *Nature* **620**, 393-401, doi:10.1038/s41586-023-06303-1 (2023).
190 Diaz, L. A. *et al.* The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* **486**, 537-540, doi:10.1038/nature11219 (2012).
191 Misale, S. *et al.* Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* **486**, 532-536, doi:10.1038/nature11156 (2012).
192 Salgia, R. & Kulkarni, P. The Genetic/Non-genetic Duality of Drug 'Resistance' in Cancer. *Trends Cancer* **4**, 110-118, doi:10.1016/j.trecan.2018.01.001 (2018).
193 Levin-Reisman, I. *et al.* Antibiotic tolerance facilitates the evolution of resistance. *Science* **355**, 826-830, doi:10.1126/science.aaj2191 (2017).
194 Russo, M. Genetic and non-genetic drug resistance: Darwin or Lamarck? *Mol Oncol* **18**, 241-244, doi:10.1002/1878-0261.13601 (2024).
195 Koh, G., Degasperi, A., Zou, X., Momen, S. & Nik-Zainal, S. Mutational signatures: emerging concepts, caveats and clinical applications. *Nat Rev Cancer* **21**, 619-637, doi:10.1038/s41568-021-00377-7 (2021).
196 Jacob Berger, A. *et al.* IRS1 phosphorylation underlies the non-stochastic probability of cancer cells to persist during EGFR inhibition therapy. *Nat Cancer* **2**, 1055-1070, doi:10.1038/s43018-021-00261-1 (2021).
197 Price, C. C., Mathur, J., Boerckel, J. D., Pathak, A. & Shenoy, V. B. Dynamic self-reinforcement of gene expression determines acquisition of cellular mechanical memory. *Biophys J* **120**, 5074-5089, doi:10.1016/j.bpj.2021.10.006 (2021).
198 Jain, P. *et al.* Epigenetic memory acquired during long-term EMT induction governs the recovery to the epithelial state. *J R Soc Interface* **20**, 20220627, doi:10.1098/rsif.2022.0627 (2023).
199 Choi, J. *et al.* A time-resolved, multi-symbol molecular recorder via sequential genome editing. *Nature* **608**, 98-107, doi:10.1038/s41586-022-04922-8 (2022).
200 Wei, C. *et al.* Multiplex genomic recording of enhancer and signal transduction activity in mammalian cells. *bioRxiv*, 2021.2011.2005.467434, doi:10.1101/2021.11.05.467434 (2021).
201 Yang, D. *et al.* Lineage tracing reveals the phylodynamics, plasticity, and paths of tumor evolution. *Cell* **185**, 1905-1923.e1925, doi:10.1016/j.cell.2022.04.015 (2022).

202 Quinn, J. J. *et al.* Single-cell lineages reveal the rates, routes, and drivers of metastasis in cancer xenografts. *Science* **371**, doi:10.1126/science.abc1944 (2021).
203 Weng, C. *et al.* Deciphering cell states and genealogies of human haematopoiesis. *Nature* **627**, 389-398, doi:10.1038/s41586-024-07066-z (2024).
204 Fennell, K. A. *et al.* Non-genetic determinants of malignant clonal fitness at single-cell resolution. *Nature* **601**, 125-131, doi:10.1038/s41586-021-04206-7 (2022).
205 Umkehrer, C. *et al.* Isolating live cell clones from barcoded populations using CRISPRa-inducible reporters. *Nat Biotechnol* **39**, 174-178, doi:10.1038/s41587-020-0614-0 (2021).
206 Harmange, G. *et al.* Disrupting cellular memory to overcome drug resistance. *Nat Commun* **14**, 7130, doi:10.1038/s41467-023-41811-8 (2023).
207 Baysoy, A., Bai, Z., Satija, R. & Fan, R. The technological landscape and applications of single-cell multi-omics. *Nat Rev Mol Cell Biol* **24**, 695-713, doi:10.1038/s41580-023-00615-w (2023).
208 Badia-I-Mompel, P. *et al.* Gene regulatory network inference in the era of single-cell multi-omics. *Nat Rev Genet* **24**, 739-754, doi:10.1038/s41576-023-00618-5 (2023).
209 Schmitt, M. J. *et al.* Phenotypic Mapping of Pathologic Cross-Talk between Glioblastoma and Innate Immune Cells by Synthetic Genetic Tracing. *Cancer Discov* **11**, 754-777, doi:10.1158/2159-8290.cd-20-0219 (2021).
210 Taskiran, I. I. *et al.* Cell-type-directed design of synthetic enhancers. *Nature* **626**, 212-220, doi:10.1038/s41586-023-06936-2 (2024).
211 Kim, S., Kamarulzaman, L. & Taniguchi, Y. Recent methodological advances towards single-cell proteomics. *Proc Jpn Acad Ser B Phys Biol Sci* **99**, 306-327, doi:10.2183/pjab.99.021 (2023).
212 Rosenberger, F. A., Thielert, M. & Mann, M. Making single-cell proteomics biologically relevant. *Nat Methods* **20**, 320-323, doi:10.1038/s41592-023-01771-9 (2023).
213 Planque, M., Igelmann, S., Ferreira Campos, A. M. & Fendt, S. M. Spatial metabolomics principles and application to cancer research. *Curr Opin Chem Biol* **76**, 102362, doi:10.1016/j.cbpa.2023.102362 (2023).
214 Vandereyken, K., Sifrim, A., Thienpont, B. & Voet, T. Methods and applications for single-cell and spatial multi-omics. *Nat Rev Genet* **24**, 494-515, doi:10.1038/s41576-023-00580-2 (2023).
215 Heumos, L. *et al.* Best practices for single-cell analysis across modalities. *Nat Rev Genet* **24**, 550-572, doi:10.1038/s41576-023-00586-w (2023).
216 Alieva, M., Wezenaar, A. K. L., Wehrens, E. J. & Rios, A. C. Bridging live-cell imaging and next-generation cancer treatment. *Nat Rev Cancer* **23**, 731-745, doi:10.1038/s41568-023-00610-5 (2023).
217 Browaeys, R., Saelens, W. & Saeys, Y. NicheNet: modeling intercellular communication by linking ligands to target genes. *Nat Methods* **17**, 159-162, doi:10.1038/s41592-019-0667-5 (2020).
218 Morris, J. A., Sun, J. S. & Sanjana, N. E. Next-generation forward genetic screens: uniting high-throughput perturbations with single-cell analysis. *Trends Genet* **40**, 118-133, doi:10.1016/j.tig.2023.10.012 (2024).
219 Frangieh, C. J. *et al.* Multimodal pooled Perturb-CITE-seq screens in patient models define mechanisms of cancer immune evasion. *Nat Genet* **53**, 332-341, doi:10.1038/s41588-021-00779-1 (2021).
220 Rodriguez, R., Schreiber, S. L. & Conrad, M. Persister cancer cells: Iron addiction and vulnerability to ferroptosis. *Mol Cell* **82**, 728-740, doi:10.1016/j.molcel.2021.12.001 (2022).
221 Zhang, Z., Tan, Y., Huang, C. & Wei, X. Redox signaling in drug-tolerant persister cells as an emerging therapeutic target. *EBioMedicine* **89**, 104483, doi:10.1016/j.ebiom.2023.104483 (2023).
222 Talebi, A. *et al.* Pharmacological induction of membrane lipid poly-unsaturation sensitizes melanoma to ROS inducers and overcomes acquired resistance to targeted therapy. *J Exp Clin Cancer Res* **42**, 92, doi:10.1186/s13046-023-02664-7 (2023).
223 Talebi, A. *et al.* Sustained SREBP-1-dependent lipogenesis as a key mediator of resistance to BRAF-targeted therapy. *Nat Commun* **9**, 2500, doi:10.1038/s41467-018-04664-0 (2018).
224 Nakamura, T. *et al.* Phase separation of FSP1 promotes ferroptosis. *Nature* **619**, 371-377, doi:10.1038/s41586-023-06255-6 (2023).
225 Wang, W. *et al.* CD8. *Nature* **569**, 270-274, doi:10.1038/s41586-019-1170-y (2019).

226 Kim, R. *et al.* Ferroptosis of tumour neutrophils causes immune suppression in cancer. *Nature* **612**, 338-346, doi:10.1038/s41586-022-05443-0 (2022).
227 Wu, J. *et al.* Intercellular interaction dictates cancer cell ferroptosis via NF2-YAP signalling. *Nature* **572**, 402-406, doi:10.1038/s41586-019-1426-6 (2019).
228 Rodencal, J. *et al.* Sensitization of cancer cells to ferroptosis coincident with cell cycle arrest. *Cell Chem Biol* **31**, 234-248.e213, doi:10.1016/j.chembiol.2023.10.011 (2024).
229 Sánchez-Danés, A. *et al.* A slow-cycling LGR5 tumour population mediates basal cell carcinoma relapse after therapy. *Nature* **562**, 434-438, doi:10.1038/s41586-018-0603-3 (2018).
230 Kim, K. H. & Roberts, C. W. Targeting EZH2 in cancer. *Nat Med* **22**, 128-134, doi:10.1038/nm.4036 (2016).
231 Rusan, M. *et al.* Suppression of Adaptive Responses to Targeted Cancer Therapy by Transcriptional Repression. *Cancer Discov* **8**, 59-73, doi:10.1158/2159-8290.cd-17-0461 (2018).
232 Wojtaszek, J. L. *et al.* A Small Molecule Targeting Mutagenic Translesion Synthesis Improves Chemotherapy. *Cell* **178**, 152-159.e111, doi:10.1016/j.cell.2019.05.028 (2019).
233 Ali, M. *et al.* Small-molecule targeted therapies induce dependence on DNA double-strand break repair in residual tumor cells. *Sci Transl Med* **14**, eabc7480, doi:10.1126/scitranslmed.abc7480 (2022).
234 Zhai, Y. *et al.* Drugging evolution of antibiotic resistance at a regulatory network hub. *Sci Adv* **9**, eadg0188, doi:10.1126/sciadv.adg0188 (2023).
235 Wang, L. *et al.* cFLIP suppression and DR5 activation sensitize senescent cancer cells to senolysis. *Nat Cancer* **3**, 1284-1299, doi:10.1038/s43018-022-00462-2 (2022).
236 Yao, Z. *et al.* TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. *Proc Natl Acad Sci U S A* **107**, 15535-15540, doi:10.1073/pnas.1009472107 (2010).
237 Moghal, N. *et al.* Single-Cell Analysis Reveals Transcriptomic Features of Drug-Tolerant Persisters and Stromal Adaptation in a Patient-Derived EGFR-Mutated Lung Adenocarcinoma Xenograft Model. *J Thorac Oncol* **18**, 499-515, doi:10.1016/j.jtho.2022.12.003 (2023).
238 Hu, J. *et al.* STING inhibits the reactivation of dormant metastasis in lung adenocarcinoma. *Nature* **616**, 806-813, doi:10.1038/s41586-023-05880-5 (2023).
239 Song, C. *et al.* Recurrent Tumor Cell-Intrinsic and -Extrinsic Alterations during MAPKi-Induced Melanoma Regression and Early Adaptation. *Cancer Discov* **7**, 1248-1265, doi:10.1158/2159-8290.CD-17-0401 (2017).
240 Haderk, F. *et al.* Focal adhesion kinase-YAP signaling axis drives drug-tolerant persister cells and residual disease in lung cancer. *Nat Commun* **15**, 3741, doi:10.1038/s41467-024-47423-0 (2024).
241 Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **168**, 707-723, doi:10.1016/j.cell.2017.01.017 (2017).
242 Cañellas-Socias, A. *et al.* Metastatic recurrence in colorectal cancer arises from residual EMP1. *Nature* **611**, 603-613, doi:10.1038/s41586-022-05402-9 (2022).
243 Sutmuller, R. P. *et al.* Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* **194**, 823-832, doi:10.1084/jem.194.6.823 (2001).
244 Simpson, T. R. *et al.* Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* **210**, 1695-1710, doi:10.1084/jem.20130579 (2013).
245 Zhu, Y. *et al.* CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res* **74**, 5057-5069, doi:10.1158/0008-5472.can-13-3723 (2014).
246 Santegoets, S. J. *et al.* T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. *Cancer Immunol Immunother* **62**, 245-256, doi:10.1007/s00262-012-1330-5 (2013).

247 Pico de Coaña, Y. *et al.* Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. *Cancer Immunol Res* **1**, 158-162, doi:10.1158/2326-6066.cir-13-0016 (2013).
248 Rizvi, N. A. *et al.* Durvalumab With or Without Tremelimumab vs Standard Chemotherapy in First-line Treatment of Metastatic Non-Small Cell Lung Cancer: The MYSTIC Phase 3 Randomized Clinical Trial. *JAMA Oncol* **6**, 661-674, doi:10.1001/jamaoncol.2020.0237 (2020).
249 Gandara, D. R. *et al.* Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med* **24**, 1441-1448, doi:10.1038/s41591-018-0134-3 (2018).
250 Sezer, A. *et al.* Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. *Lancet* **397**, 592-604, doi:10.1016/S0140-6736(21)00228-2 (2021).
251 Kruger, S. *et al.* Repeated mutKRAS ctDNA measurements represent a novel and promising tool for early response prediction and therapy monitoring in advanced pancreatic cancer. *Ann Oncol* **29**, 2348-2355, doi:10.1093/annonc/mdy417 (2018).
252 He, J., Tan, W., Tang, X. & Ma, J. Variations in EGFR ctDNA Correlates to the Clinical Efficacy of Afatinib in Non Small Cell Lung Cancer with Acquired Resistance. *Pathol Oncol Res* **23**, 307-315, doi:10.1007/s12253-016-0097-y (2017).
253 Fürstenau, M., De Silva, N., Eichhorst, B. & Hallek, M. Minimal Residual Disease Assessment in CLL: Ready for Use in Clinical Routine? *Hemasphere* **3**, e287, doi:10.1097/hs9.0000000000000287 (2019).
254 Terpos, E. *et al.* Impact of Minimal Residual Disease Detection by Next-Generation Flow Cytometry in Multiple Myeloma Patients with Sustained Complete Remission after Frontline Therapy. *Hemasphere* **3**, e300, doi:10.1097/hs9.0000000000000300 (2019).
255 Wouters, J. *et al.* Robust gene expression programs underlie recurrent cell states and phenotype switching in melanoma. *Nat Cell Biol* **22**, 986-998, doi:10.1038/s41556-020-0547-3 (2020).
256 Emert, B. L. *et al.* Variability within rare cell states enables multiple paths toward drug resistance. *Nat Biotechnol* **39**, 865-876, doi:10.1038/s41587-021-00837-3 (2021).
257 Li, X. *et al.* Disseminated Melanoma Cells Transdifferentiate into Endothelial Cells in Intravascular Niches at Metastatic Sites. *Cell Rep* **31**, 107765, doi:10.1016/j.celrep.2020.107765 (2020).
258 Zhang, M., Yang, L., Chen, D. & Heisterkamp, N. Drug-tolerant persister B-cell precursor acute lymphoblastic leukemia cells. *bioRxiv*, doi:10.1101/2023.02.28.530540 (2023).
259 Bevill, S. M., Zawistowski, J. S. & Johnson, G. L. Enhancer remodeling regulates epigenetic adaptation and resistance to MEK1/2 inhibition in triple-negative breast cancer. *Mol Cell Oncol* **4**, e1300622, doi:10.1080/23723556.2017.1300622 (2017).
260 Wang, C. *et al.* Inducing and exploiting vulnerabilities for the treatment of liver cancer. *Nature* **574**, 268-272, doi:10.1038/s41586-019-1607-3 (2019).
261 Chen, W. *et al.* Symbolic recording of signalling and cis-regulatory element activity to DNA. *Nature*, doi:10.1038/s41586-024-07706-4 (2024).