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## Persistent cancer cells: the deadly survivors

Shensi Shen<sup>1,\*</sup>, Stéphan Vagner<sup>3,4,5,\*</sup>, Caroline Robert<sup>1,2,6,\*</sup>

Affiliations :

<sup>1</sup>INSERM U981, Gustave Roussy Cancer Campus, Villejuif, France.

<sup>2</sup>Université Paris-Sud, Université Paris-Saclay, Kremlin-Bicêtre, France.

<sup>3</sup>Institut Curie, PSL Research University, CNRS UMR3348, INSERM U1278, Orsay, France.

<sup>4</sup>Université Paris Sud, Université Paris-Saclay, CNRS UMR3348, INSERM U1278, Orsay, France.

<sup>5</sup>Equipe Labellisée Ligue Nationale Contre le Cancer, France.

<sup>6</sup>Dermato-Oncology, Gustave Roussy Cancer Campus, Villejuif, France.

\* Correspondence to: SHENSI.SHEN@gustaveroussy.fr (S.S), Stephan.Vagner@curie.fr (S.V)  
and Caroline.Robert@gustaveroussy.fr (C.R).

**Abstract:**

Persistent cancer cells are the discrete and usually undetected cells that survive cancer drug treatment and constitute a major cause of treatment failure. These cells are characterized by their slow proliferation, highly flexible energy consumption, adaptation to their microenvironment, and phenotypic plasticity. Mechanisms that underlie their persistence offer highly coveted and sought-after therapeutic targets, and include diverse epigenetic, transcriptional and translational regulatory processes, as well as complex cell-cell interactions. Although the successful clinical targeting of persistent cancer cells remains to be realized, immense progress has been made in understanding their persistence, yielding promising preclinical results.

## INTRODUCTION

Persistent cancer cells could well be described as “...that army known as invincible in peace, invisible in war...” (William Tecumseh Sherman), (Miers, 1951). This is because undetectable residual cancer cells persist in the body after treatment and can eventually and unpredictably give rise to metastatic relapses. Most patients with metastatic cancers, who have what is described by oncologists as a partial or complete response to treatment with cancer drugs (Eisenhauer et al., 2009) (Figure 1), eventually relapse. These relapses can occur after months or even years even in the absence of detectable tumors following treatment, as assessed by using the most sophisticated image techniques, such as computerized tomography scan, positron emission tomography (PET) scan and magnetic resonance imaging. This is because of the existence of residual disease, which creates a reservoir that gives rise to drug-resistant cells.

Cancer patients respond to treatment in a variety of ways (Figure 1). One frequently noted response in patients being treated for metastatic cancers is that of *dissociated* or *mixed responses*, where some metastases respond to treatment while others do not. These so-called *dissociated responses* make the management of patients more complex because treatment interruption can result in the growth of non-responding, as well as of responding, metastases, frequently leading physicians to continue treatment beyond disease progression (Beaver et al., 2018). *Stable disease* is another response type (Eisenhauer et al., 2009). It is seen, for example, when cytostatic drugs, such as hormonal receptor blockers, are used to treat a hormone-sensitive cancers (Saad and Fizazi, 2015). Stable disease could be related to tumors in which cancer cell proliferation is very slow or is balanced by cancer cell death.

Thus, it is likely that cancer cells persist and are omnipresent in all types of clinical response. Physicians are aware of the existence and persistence of these evasive cells, and so are often reluctant to tell their patients that they are cured; instead, they refer to tumor-free periods as long remissions. This clinical reality highlights the urgent need to better understand the biology of this invisible enemy with the aim of destroying or neutralizing it.

Cancer cells are known to be genetically unstable. This genetic heterogeneity can result in variable levels of drug sensitivity, which can cause both primary resistance and dissociated responses to anti-cancer drugs (Turner and Reis-Filho, 2012) (Figure 1). However, cancer persistence is not due to the presence of cancer cell genetic variants but is instead linked to

non-genetic variations that are associated with a non- or slow-proliferation status, a response that was first observed in bacteria on their treatment with antibiotics (Balaban et al., 2019). In contrast to the concept of tolerance that involves the whole bacterial population, persistence is defined as the capacity of a subpopulation of bacteria to survive exposure to antibiotics without undergoing *de novo* genetic alternations. The presence of persistence in a bacterial population results in a biphasic pattern of death in response to antibiotic treatment. When persistent cells are isolated and re-exposed to the same antibiotic, they show the same biphasic response, with most of the bacteria being killed by the antibiotic, illustrating the reversibility of this process (Harms et al., 2016). This persistent status can occur when bacteria have a slow growth rate and a reduced metabolism, possibly induced by starvation, in the presence of certain host factors or is induced by the antibiotic itself. Similarly, persistent cancer cells have been characterized *in vitro* as being a subpopulation of cells that survives initial anti-cancer treatment, showing a lower proliferative rate and surviving without *de novo* genetic mutations (Sharma et al., 2010). In the context of cancer, the distinction between tolerance and persistence is not defined as clearly as it is for bacteria. The term “drug tolerant persisters” is frequently used to describe cancer cells with non-mutational mechanisms of “resistance”. Nevertheless, persistent cancer cells do have the capacity to mutate to produce genetically altered, resistant clones later on (Russo et al., 2019). As shown decades before with bacteria, persistent cancer cells can resume their initial behavior and drug sensitivity upon drug removal (Ramirez et al., 2016; Shen et al., 2019).

Cancer persistence might conceivably occur via two main mechanisms. Individual, persistent cells might exist prior to drug treatment, which are then selected for by the treatment, following classical Darwinian rules. Individual cancer cells might also become persistent upon exposure to a treatment, via a Lamarkian type of adaptation (Mayr, 1972). In this latter concept, the capacity of drug persistence is not pre-existing but is induced by the treatment. For example, the percentage of HL-60 leukemia cells that express high levels of multidrug resistance 1 (MDR1) increases from ~1-2% to over 25% following a 48-hour treatment period with vincristine, without developing additional genetic mutations (Pisco et al., 2013).

*In vitro* studies of cancer cells suffer from a major limitation in that the cells are not exposed to the tumor microenvironment. The vital impact of the tumor microenvironment on cancer cells has been underscored in previous studies, which have shown that only ~0.02% of

solitary disseminated cancer cells can initiate a macroscopic metastasis (Celia-Terrassa and Kang, 2016; Luzzi et al., 1998; Ubellacker et al., 2020). This could explain why some metastases in the same patient continue to grow in certain organs but disappear in others upon treatment, in a dissociated response.

Altogether persistent cancer cells are seen following the use of anti-cancer agent and exhibit a reversible and slow-cycling status that is not driven by any genetic variations. This differs from the cancer dormancy status which characterizes cancer cells genetically altered or not, that remain quiescent for long periods of time independently of the use of any pharmaceutical agent (Klein, 2011; Risson et al., 2020). In this review, we discuss the various mechanisms associated with the hallmarks of persistent cancer cell state. We also review the therapeutic approaches to targeting persistent cancer cells.

## **MECHANISMS OF CANCER CELL PERSISTENCE**

A wide variety of mechanisms that contribute to the persistence of cancer cells have been reported in recent decades. These mechanisms include epigenetic, transcriptional and translational processes that are not mutually exclusive and often co-exist (Guler et al., 2017; Sharma et al., 2010; Shen et al., 2019). As we discuss below, there are four, main non-mutually exclusive strategies that are deployed by persistent cells to survive unnoticed: 1) slowing cell proliferation; 2) adapting cell metabolism; 3) changing cell identity; and 4) hijacking the microenvironment (Figure 2).

### **Slowing down cell proliferation**

Similar to the slowly proliferating bacteria that can survive antibiotic treatment, persistent human cancer cells also display a slow proliferative rate (Chen et al., 2012; Liao et al., 2017; Roesch et al., 2013; Sharma et al., 2010). It is not always understood whether the slow proliferation rate of persistent cancer cells is pre-existing or induced by the anticancer treatment. It can result from upstream mechanisms involving epigenetic reprogramming, transcriptional regulation, or interactions with the tumor microenvironment (Figure 2).

#### *Cell-intrinsic deceleration programs*

Cell-intrinsic, epigenetic reprogramming that leads to reduced cell proliferation has been observed in various human tumor types. For example, persistent primary cancer cells derived from human glioblastoma patients enter a slow-proliferation state *in vitro* following

their treatment with the tyrosine kinase inhibitor, dasatinib. These cells upregulate the Notch1 intracellular domain, which is associated with the activation of the histone H3K27me3 demethylase, KDM6A/B (Liau et al., 2017) (Figure 3). It is known that H3K27me3-associated Polycomb repressors target different neurodevelopmental and Notch-related genes, leading to their transcriptional repression (Simon and Kingston, 2013). Widespread KDM6A/B-dependent H3K27me3 redistribution is observed in persistent glioblastoma cells exiting cell cycle upon dasatinib treatment. Knockdown of KDM6A/B significantly impairs persistent cell state, suggesting that KDM6 demethylases promote the transition and maintenance of slow-cycling, glioblastoma persistent cell state. Similarly, a slow-proliferating, persistent cell status has been observed in BRAF<sup>V600E</sup>-mutated human melanoma cells cultured *in vitro*, following their treatment with the chemotherapeutic cisplatin or with the BRAF inhibitor vemurafenib, via the up-regulation of H3K4 demethylase, KDM5B (Roesch et al., 2013). The expression of KDM5A, a paralog of KDM5B, is associated with the slow-proliferation phenotype of EGFR-mutated, human non-small cell lung cancer (NSCLC) persistent cells exposed to the EGFR tyrosine kinase inhibitor erlotinib. KDM5A reduces the level of H3K4 methylation and thus represses the expression of cell cycle-related genes in persistent cancer cells (Sharma et al., 2010). In addition to Histone 3 demethylation, the trimethylation of H3K9a is also observed in human NSCLC persistent cells, which occurs in a SET Domain Bifurcated Histone Lysine Methyltransferase 1 (SETDB1)-dependent manner. The transcriptionally repressive mark H3K9me3 is prominent on transposable elements, such as on the long interspersed repeat element 1 (LINE-1). The reduced expression of LINE-1 is required for the survival of NSCLC persistent cells (Guler et al., 2017) (Figure 3).

Direct DNA modifications can also induce a slow-proliferation phenotype, such as the oxidation of 5-Methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by Tet Methylcytosine Dioxygenase 2 (TET2) (Figure 3). This modification can induce a slow-proliferation phenotype in patient-derived xenograft of colon cancer cells treated with oxaliplatin *in vitro* or *in vivo* by repressing the expression of E2F-related cell proliferation pathways (Puig et al., 2018).

Intracellular signaling cues can coordinately define a slow-proliferation state in various tumor types. The Hedgehog pathway inhibitor, vismodegib, fosters a slow-cycling state that allows genetically engineered mouse, LGR5-positive, basal cell carcinoma (BCC) cells to persist *in vivo* in a Wnt signaling-dependent manner (Sanchez-Danes et al., 2018). Human

melanoma Nerve Growth Factor Receptor (NGFR)-positive persistent cells exposed to vemurafenib *in vitro* show an increased phosphorylation of focal adhesion kinase (FAK), leading to the downstream phosphorylation of c-Jun that in turn activates AP1 transcriptional activity and results in a slow-cycling state (Fallahi-Sichani et al., 2017). Transcriptional feedback to membrane receptor expression can also lead to cancer cell persistence. For instance, the up-regulation of the kinase receptor, AXL, is associated with a slow-cycling state in EGFR inhibitor-treated human NSCLC cells *in vitro* (Taniguchi et al., 2019), possibly through an autocrine signal provided by growth arrest-specific gene 6 (GAS6) (Shaffer et al., 2017). AXL is a target of the transcription cofactors YAP1 and TAZ (Hong and Guan, 2012). The YAP1/TAZ pairs with DNA-binding factors of the TEAD family proteins in the nucleus to regulate gene expression (Moya and Halder, 2019). The transcriptional up-regulation of AXL might therefore be mediated by YAP1/TAZ and TEAD1-4 transcription factors recruited to its promoter via TEAD-binding elements (Figure 3). This finding is reminiscent of a recent study, which showed that erlotinib inactivates MAPK pathway and enables NSCLC persistent cells to enter a non-proliferative state that is dependent on the YAP1-TEAD signaling pathway (Kurppa et al., 2020).

#### *Environment-triggered deceleration programs*

Decreased cell proliferation can also result from the effect of the tumor microenvironment, which is also known to be a critically important element of “cancer dormancy” (Sosa et al., 2014). In *BRAF*<sup>V600E</sup> mutated mouse and human melanoma, cell persistence under conditions of BRAF inhibition seems to be closely linked with the paradoxical activation of melanoma associated fibroblasts (MAFs), which trigger a tolerant “safe haven” for slow-cycling persistent melanoma cells. Indeed, the paradoxical activation of MAFs by BRAF inhibition induces the production and remodeling of the fibronectin-rich matrix, which, in turn, leads to melanoma cell persistence via the ERK re-activation dependent on the integrin  $\beta$ 1-FAK-Src signaling in tumor cells (Hirata et al., 2015). Although not directly studied in the context of persistent cancer cells, breast cancer cell-induced fibronectin organization depends on TGF $\beta$ 2-stimulated  $\alpha$ <sub>v</sub> $\beta$ <sub>3</sub> and  $\alpha$ <sub>5</sub> $\beta$ <sub>1</sub> integrin adhesion and the subsequent Rho Associated Coiled-Coil Containing Protein Kinase 1 (ROCK1)-generated matrix tension. The disruption of the fibronectin-rich matrix by Matrix Metalloproteinase 2 (MMP2) can reactivate breast cancer cells, emphasizing the essential role of the extracellular matrix in triggering the



growth arrest of tumor cells (Barney et al., 2020). Fibroblasts not only contribute to the slow-cycling, persistent cancer cell state through matrix modification but they can also be internalized and degraded by breast cancer cells through cell cannibalism. Cell cannibalism is shown to activate stress-activated jun N-terminal kinase (JNK) and is associated with upregulation of EMT (epidermal-mesenchymal transition) and stem cell-like markers (i.e. NANOG and SOX2), thus leading to growth arrest and increased tolerance to chemotherapy and radiotherapy *in vitro* (Bartosh et al., 2016; Martins et al., 2018; Overholtzer et al., 2007; Tonnessen-Murray et al., 2019). This deceleration program can also depend on mediators of inflammation secreted by senescent cells, such as the tumor necrosis factor (TNF) and interferon gamma (IFN $\gamma$ ) pathway-associated cytokines and chemokines, as it has been reported in a T antigen (Tag)-driven pancreatic carcinogenesis mouse model. Tag-specific CD4<sup>+</sup> T cells from this model selectively prevented  $\alpha_v\beta_3$  integrin expression and induced tumor growth arrest in a TNFR1- and IFN $\gamma$ - signaling-dependent manner (Muller-Hermelink et al., 2008).

It seems paradoxical that slow-cycling, persistent cells constitute a reservoir for cells to acquire drug resistance via genetic mutation (Hata et al., 2016; Ramirez et al., 2016; Shen et al., 2019), since the acquisition of mutations usually requires cells to be highly proliferative. *De novo* mutagenesis in persistent cells was originally observed in unicellular organisms (Bjedov et al., 2003; Long et al., 2016; MacLean et al., 2013), and has now been described in persistent human cancer cells. A recent study has reported that a shift in DNA repair programs occurs in microsatellite-stable (MSS), persistent human colorectal cancer cells, from mismatch repair (MMR) and homologous recombination (HR) repair, to DNA repair processes that use error-prone DNA polymerases, such as Polk and Rev1, in response to treatment with the anti-EGFR antibody, cetuximab (Figure 3) (Russo et al., 2019). Such a transient, adaptive mutagenesis leads to base mis-pairings and to the incorporation of aberrant DNA primer ends, increasing the mutation rate of these persistent colorectal cancer cells. The adaptive mutability of persistent cells might be further enhanced by the increased hydroxylation of 5mC in their DNA, as mentioned above (Puig et al., 2018). Genome-wide 5mC hypomethylation or hydroxylation can indeed lead to widespread genomic instability, and the hydrolytic deamination of genomic 5mC to thymine results in

T:G mismatches that are difficult to repair by MMR or HR (Xie et al., 2018; You and Jones, 2012).

Persistent cancer cells can thus reactivate an ancestral program in response to anti-cancer therapy, which increases their mutation rates in spite of their decreased proliferation rate. This increased mutability might lead persistent cancer cells out of a transient, slow proliferative persistent state and toward a resistant phenotype with acquired mutations. However, slowing down of cell proliferation might be insufficient for persistent cancer cells to be refractory to treatment. To survive metabolic stresses associated with cancer treatments persistent cancer cells also often have to adapt their metabolism to offending conditions by modifying their mode of energy consumption (Figure 2).

### **Adapting cell metabolism**

#### *Mitochondrial respiration*

One common characteristic of many persistent cancer cells is their ability to minimize their glucose consumption and to shift toward mitochondrial oxidative respiration. In KRAS inhibited, *KRAS*<sup>G12D</sup>-mutated mouse pancreatic ductal adenocarcinoma (PDAC) cancer, persistent cancer cells have been shown to rely on mitochondrial oxidative phosphorylation and to upregulate the mitochondrial biogenesis master regulator, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-alpha (PGC1 $\alpha$ ), and the mitochondrial marker, Voltage Dependent Anion Channel 1 (VDAC1) (Viale et al., 2014). This is consistent with an earlier study showing that BRAF<sup>V600E</sup>-mutated persistent human melanoma cells up-regulate enzymes involved in mitochondrial oxidative ATP synthesis in the presence of BRAF inhibitor *in vitro* (Roesch et al., 2013). Following cytarabine treatment, residual acute myeloid leukemia (AML) cells increase their mitochondrial mass, consistent with a high oxidative phosphorylation state (Farge et al., 2017). Targeted therapy against *BCR-ABL1*<sup>+</sup> chronic myeloid leukemia (CML) also spares a subpopulation of residual CML cells that depend on mitochondrial energetic metabolism (Kuntz et al., 2017). Thus, energy production in slowly proliferating, persistent cancer cells more closely resembles energy production in resting normal cells than in proliferating cancer cells, in which anaerobic glycolysis is diverted from mitochondrial ATP production to glycolytic lactate production and biomass synthesis (Zhu and Thompson, 2019)(Figure 4).

Glucose consumption can also vary depending on the type of cancer. Slow-cycling, mouse *Eμ-myc* lymphoma residual cells after chemotherapy are metabolically hyperactive, which is a remarkable observation in light of their non-proliferative state (Dorr et al., 2013). Although these cells are undetectable by [<sup>18</sup>F] fluorothymidine PET scan, they actively utilize glucose metabolism, and rather than producing lactate, they accumulate citrate. This indicates an increased flux into the mitochondrial tricarboxylic acid (TCA) cycle via the up-regulation of pyruvate kinase isoform 1 (PKM1). Thus, lymphoma residual cells display a hybrid form of energy metabolism, marked by a high influx of glucose metabolism into the TCA cycle and by increased mitochondrial respiration (Dorr et al., 2013). A hybrid metabolic phenotype has also been reported in human breast cancer cells that persists on their treatment with anthracycline chemotherapy. These persistent cells are made up of a subpopulation of CD44-high cells that display increased activation of both the glycolytic pentose phosphate pathway and mitochondrial oxidative respiration (Goldman et al., 2019).

Persistent cancer cells can also mobilize alternative nutrients. In a pancreatic ductal adenocarcinoma (PDAC) mouse model, mitochondrial respiration was found to be associated with autophagy and peroxisomal fatty acid  $\beta$ -oxidation (Viale et al., 2014). In a *Kras*<sup>G12D</sup>-driven murine lung cancer model, autophagy was shown to facilitate metabolic substrate recycling derived from the degradation of macromolecules, including essential amino acids and pentose phosphate intermediates, thus feeding mitochondrial TCA metabolism (Guo et al., 2016). In *BRAF*-mutant human melanoma, BRAF inhibitors induce transcriptional changes that lead to autophagy and chemoresistance (Li et al., 2019; Ma et al., 2014). By recycling metabolic substrates, persistent cancer cells can actively consume available fatty acids to fuel mitochondrial respiration and energy production. For example, human triple-negative breast cancer (TNBC) cells maintain a high level of ATP production through fatty acid  $\beta$ -oxidation (FAO) (Park et al., 2016). In return, FAO is involved in the metabolic adaptation of MYC-overexpressing TNBC, in which FAO intermediates are dramatically up-regulated (Camarda et al., 2016). In HER2-positive human breast cancers, the fatty acid transporter, CD36, is up-regulated and associated with non-genetic resistance to the EGFR inhibitor, lapatinib (Feng et al., 2019). Similarly, CD36 is involved in *BRAF*<sup>V600E</sup> mutated, persistent human melanoma cells treated with MAPK inhibitors (Aloia et al., 2019). Persistent melanoma cells display increased CD36 expression, facilitating fatty acid import and subsequent catabolism through mitochondrial carnitine palmitoyltransferase 1A (CPT1A)-

mediated fatty acid oxidation (Figure 4). A similar study showed that both mitochondrial CPT1A and peroxisomal Acyl-CoA Oxidase 1 (ACOX1), transcriptionally regulated by PPAR $\alpha$ , are required for FAO in persistent melanoma cells (unpublished data). Desaturation of intracellular fatty acid, such as the desaturation of palmitate to the unusual fatty acid sapienate, which has been reported in hepatocellular carcinomas and lung carcinomas, allows human cancer cells to become highly plastic in terms of their metabolism (Vriens et al., 2019). This plasticity highlights an exciting research path toward a better understanding of the metabolism of persistent cancer cells.

#### *Ribosome-dependent control of mRNA translation*

Since about more than half of the cell energy consumption is used for protein synthesis, it seems logical that persistent cancer cells have to minimize their protein synthesis (Li et al., 2014). Although it is sometimes unclear whether reduced protein production is a consequence or a cause of persistent cancer cell metabolism, ribosome-dependent translation seems to be directly involved in the formation of persistent cells in unicellular organisms (Culviner and Laub, 2018; Wood et al., 2019).

The dynamic adaptation of mRNA translation efficiency is determined by the supply-to-demand system in human cell lines. A genome-wide study of different types of cancers showed that this adaptation of reduced protein synthesis is largely related to the proliferative state (Hernandez-Alias et al., 2020). Thus, it is not surprising that persistent cells, which are in a slow-proliferating state, minimize their protein production. Although the underlying mechanisms of minimal translation efficiency in persistent cancer cells are still not fully understood, two recent studies have pointed to a possible role for the post-transcriptional modifications of some adenosines on mRNA to N6-methyladenosine (m6A). For example, one study showed that persistent cells in a leukemia mouse model have hypomethylated m6A in their mRNA and reported that the up-regulation of the m6A demethylase, FTO (Fat mass and obesity-associated protein) was highly tolerant to tyrosine kinase inhibitors. FTO-dependent m6A demethylation enhances selective mRNA substrate stability, thus maintaining the cells' minimal translation level (Yan et al., 2018). Another study showed that a subset of mRNAs that are m6A-modified in their 5' untranslated regions is up-regulated in persistent melanoma cells upon their treatment with BRAF and MEK inhibitors (Figure 4). Similar to the observations made in leukemia, these mRNAs are actively

translated despite a global reduction of translation efficiency in persistent cells (Shen et al., 2019).

### *Oxidative energy trade-off*

Persistent cancer cells become oxidatively stressed as a result of increased mitochondrial oxidative respiration due to the production of periplasmic superoxides from the mitochondrial respiratory electron carriers (Anand et al., 2019). Thus, persistent cells require a robust anti-oxidant process to counteract the overproduction of superoxides. Such a process might include the glutathione-dependent reduction of lipid peroxides, which is commonly observed in various persistent cancer cells (Hangauer et al., 2017). In particular, phospholipid glutathione peroxidase 4 (GPX4) is up-regulated in persistent human breast cancer, lung cancer, and melanoma cells (Hangauer et al., 2017) (Figure 4). Increased GPX4 catalyzes the glutathione-dependent reduction of intracellular lipid peroxides to avoid oxidative stress. In contrast, GPX4 inhibition in persistent cells induces ferroptosis, a non-apoptotic oxidative cell death (Dixon et al., 2012). Consistent with this finding, an earlier work in NSCLC persistent cells demonstrated that aldehyde dehydrogenase (ALDH) is required for the maintenance of a persistent cell population by protecting them from the toxic effects of increased reactive oxygen species derived from mitochondrial respiration carriers (Raha et al., 2014). The regulation of anti-oxidant enzyme expression might also depend on NF-E2-Related Factor 2 (NRF2), a master regulator of redox homeostasis. In a transgenic mouse model of HER2-driven breast cancer, NRF2 is activated in residual dormant cells upon HER2 inhibition, re-establishing a higher glutathione/oxidized glutathione (GSH/GSSG) ratio in a glutathione metabolism-dependent manner (Fox et al., 2020). In another study in human breast and lung cancer cells, NRF2 was found to directly control the expression of the neuronal redox-sensing channel TRPA1 (Transient Receptor Potential Cation Channel subfamily A) that up-regulates Ca<sup>2+</sup>-dependent anti-apoptotic pathway (Takahashi et al., 2018). In summary, persistent cancer cells can use various metabolic pathways to survive cancer treatment attacks. However, their plasticity goes beyond their energy consumption modalities, since they also can change their identity in order to conceal themselves from immune surveillance and from being targeted by therapeutics.

## **Changing cell identity**

In this section, we discuss some of the common cell identity alterations that have been recently reported in persistent cancer cells, beginning with the epithelial to mesenchymal transition (EMT) (Figure 5).

### *Epithelial-to-mesenchymal transition (EMT)*

EMT is the process by which epithelial cells convert to mesenchymal cells via the loss of polarity and intercellular adhesion and the acquisition of migration and proliferation capabilities. EMT is implicated in several biological processes such as embryo development, wound healing, tissue fibrosis and tumor invasiveness and metastasis (Nieto, 2011). The acquisition of mesenchymal features by epithelial carcinoma cells renders them more resistant to cell death (Lamouille et al., 2014). Raoof et al identified an EMT program in mesenchymal cell lines derived from a patient's EGFR-mutated lung tumor samples, which were used as surrogates for persistent populations. This program depended on FGFR3 signaling and was activated following the cell lines' treatment with the third-generation EGFR-inhibitor, osimertinib. Sustained activation of FGFR3 leads to ERK signaling re-activation, despite the presence of osimertinib (Raoof et al., 2019). The up-regulation of EMT markers, including N-cadherin, vimentin and Slug, is also observed in persistent human epithelial lung cancer cells following the cells' treatment with paclitaxel chemotherapy, allowing collateral persistence to EGFR tyrosine kinase inhibition (Aldonza et al., 2020). A mesenchymal identity is also adopted by other types of persistent cancer cells (Hangauer et al., 2017). For example, MEK and PI3K/mTOR inhibitor-driven basal-like breast cancer persistence in human is shown to arise through EMT-related cell state transitions, which depend on the Bromodomain Containing 4 (BRD4)-mediated dynamic remodeling of open chromatin architecture (Risom et al., 2018). EMT might also conceal persistent cells from immune surveillance. Indeed, in a large dataset analysis of lung adenocarcinomas, EMT promotes the expression of diverse immune inhibitory ligands, including PD-L1, TIM3, LAG3, accompanied by an enrichment of CD4<sup>+</sup>FoxP3<sup>+</sup> immune-suppressive regulatory T cells (Lou et al., 2016).

### *Transdifferentiation*

Transdifferentiation refers to the conversion of cells from one mature lineage into another without returning to a pluripotent state (Graf and Enver, 2009). Persistent cancer cells can also change their cell identity, depending on the lineage history of the cancer cell type. For instance, the skin epithelium is sustained by various pools of stem cells, which reside in the interfollicular epidermis (IFE), bulge, isthmus and sebaceous gland. Basal cells in the IFE are known to be reprogrammed by the oncogenic Hedgehog (Hh) signalling pathway toward a bulge-like cell state and to consequently initiate BCC (Bakshi et al., 2017). When the Hh pathway is inhibited by vismodegib (Migden et al., 2015; Sekulic et al., 2012), residual BCC cells transdifferentiate into a mixed isthmus and IFE cell phenotype, which is independent of Hh pathway activity. This mixed cell state is not observed in untreated BCC populations, hinting at a process of drug-induced transdifferentiation (Biehs et al., 2018). In melanoma, cancer cells can survive targeted therapy by exploiting their developmental plasticity. By using a panoply of patient-derived xenografts, Rambow et al found that residual persistent melanoma cells transdifferentiate into a neural crest stem cell-like state that can co-exist with three other distinct cell phenotypes during tumor relapse (Rambow et al., 2018). Such Larmakian-like cell identity changes have also been observed in prostate cancer and lung cancer cells that undergo a drug-induced, neuroendocrine transdifferentiation. For example, human castration-resistant prostate cancer (CRPC) cells treated with an androgen-deprivation therapy undergo an androgen receptor-independent lineage conversion; this conversion produces cells that histologically resemble neuroendocrine prostate cancer (NEPC) cells and that express the neuroendocrine markers, chromogranin A and synaptophysin (Davies et al., 2018; Zou et al., 2017). Interestingly, these transdifferentiated NEPC-like cells retain the genetic alterations that were initially present in the prostate adenocarcinomas, including *TMPRSS2-ERG* fusions, indicating that these persistent cells have indeed transdifferentiated from the initial cancer cell clone (Beltran et al., 2016). This transdifferentiation process is also reversible; when the cells are re-exposed to androgen, they revert to an androgen receptor-dependent cell state (Nouri et al., 2017). Similarly, EGFR-driven human NSCLC cells have been reported to convert into a small cell lung cancer (SCLC) phenotype upon EGFR inhibition (Lee et al., 2017). In one rare case, NSCLC cells were reported to transdifferentiate into neuroendocrine-like cells histologically, including large cell neuroendocrine carcinoma and small/large cell carcinoma, in response to EGFR inhibition (Marcoux et al., 2019).

Thus, persistent cancer cells can evade therapeutic insults by changing their cell identity via EMT or transdifferentiation in response or in association with various cancer treatments. However, there are situations where cancer persistent cells can exist through a collaboration with other cells, which can be tumor cells or hijacked microenvironmental cells (Figure 2).

### **Hijacking the microenvironment**

#### *Tumor cell symbiosis*

Symbiosis describes a commensal interaction between two different biological organisms. In the cancer ecosystem, cancer cells in different states can form an economic community to better manage energy consumption. This has been well documented in multiple cancer types, including in breast cancer, pancreatic cancer and in renal cell carcinoma, treated with anti-angiogenesis therapeutics (Allen et al., 2016; Jimenez-Valerio et al., 2016; Pisarsky et al., 2016). For example, the treatment of murine pancreatic neuroendocrine tumors with the VEGFR inhibitors, sunitinib and nintedanib, has been reported to generate two groups of cancer cells, according to the relative distance of these cancer cells from the remaining blood vessels. Cancer cells located distal to the remaining blood vessels increase their anaerobic glycolysis to secrete lactate by expressing the MCT4 lactate transporter in a HIF1 $\alpha$ -dependent manner, while those located proximal to the blood supply uptake the secreted lactate by expressing the MCT1 lactate transporter (Allen et al., 2016). Lactate in turn activates mTOR signaling, leading to the survival of cancer cells upon anti-angiogenesis inhibition (Jimenez-Valerio et al., 2016). Cancer cells thus can adopt two different metabolic states, which together enable both cell populations to survive their exposure to anti-cancer therapeutics, in a form of symbiosis. One of the cell populations has a metabolic status similar to the one described in persistent cancer cells, suggesting that tumor cell symbiosis may facilitate the cancer persistence status (Figure 6).

#### *Cancer-associated fibroblasts*

Cancer-associated fibroblasts (CAFs) are a well-documented, stromal cell population that is involved in tumor response to drug treatment. For example, stromal cells secrete hepatocyte growth factor (HGF), which activates the HGF receptor, MET, activating the downstream PI3K-AKT signaling pathway (Figure 6). This pathway confers immediate tolerance to BRAF inhibition in BRAF-mutant melanoma, colorectal and glioblastoma human



and mouse cell models (Straussman et al., 2012). CAF-secreted HGF performs a similar function in breast cancer cells and attenuates HER2 inhibitor sensitivity in basal-like HER2<sup>+</sup> human breast cancer (Watson et al., 2018). Lactate, produced by cancer cells, might be the key molecule that stimulates CAFs to produce HGF in a NF- $\kappa$ B-dependent manner (Apicella et al., 2018). HGF might also be important in adaptive tumor tolerance to immunotherapy. Indeed, it has been reported in several murine cancer models (melanoma, lung cancer, colon adenocarcinoma), that HGF recruits immunosuppressive neutrophils to the T cell-inflamed tumor microenvironment, leading to restrained T cell expansion and effector functions (Glodde et al., 2017). CAFs can also transfer microRNA-containing exosomes to tumor cells. For example, CAF-derived microRNA-21 binds directly to its target apoptotic protease activating factor 1 (APAF1) mRNA in human ovarian cancer cells and suppresses paclitaxel-induced apoptosis (Au Yeung et al., 2016). With the increasing evidence that CAFs play a role in the tumor drug response, it is important to dissect their heterogeneity in different tumor types. Two major types of CAF exist in the desmoplastic pancreatic cancer: peritumoral CAFs, which are in direct contact with tumor cells; and tumor-educated distal CAFs, which communicate with tumor cells by secreting cytokines (Ohlund et al., 2017). In addition to this heterogeneity, a recent study in human samples and xenograft models of breast and lung cancer has identified a new subset of CAFs that expresses CD10 and GPR77 and that promote cancer chemoresistance by providing a survival niche for tumor cells via IL-6 and IL-8 secretion (Su et al., 2018).

#### *Tumor-associated macrophages*

Tumor-associated macrophages (TAMs) represent another critically important stromal population that co-evolves with tumor cells (Figure 6). During tumor progression, cancer cells reprogram macrophages in the tumor toward an anti-inflammatory M2-like phenotype that is associated with the secretion of growth factors, pro-angiogenic molecules, and immunosuppressive factors (Cassetta and Pollard, 2018). In melanomas, the polarization of macrophages to an M2-like TAMs is likely mediated by a mechanism that involves a G-protein-coupled receptor (GPCR) that senses acidosis induced by melanoma cells (Bohn et al., 2018). M2-like TAMs secrete multiple cytokines, including IL-6, TNF, C-C motif chemokine ligand 5 (CCL5) and CCL18, into the tumor microenvironment. TAM-derived IL-6 facilitates tumor cell glycolysis by increasing the activity of 3-phosphoinositide-dependent protein

kinase 1 (PDPK1) (Zhang et al., 2018). CCL5 increases upon HER2 inhibition and promotes the infiltration of CCR5-expressing TAMs into the residual tumors, leading to the activation of TNF $\alpha$ -NF- $\kappa$ B signaling in a murine breast cancer model (Walens et al., 2019). TAMs also secrete pyrimidine species, such as deoxycytidine, that compete with gemcitabine for deoxycytidine kinase (DCK), thereby promoting tolerance to chemotherapy (Halbrook et al., 2019).

## **THERAPEUTIC PERSPECTIVES**

Given the role of persistent cancer cells in tumor relapse, targeting this residual cell population represents an attractive strategy by which to turn an invincible disease into a curable one. One seemingly straightforward strategy would be to wake up the sleeping residual cells and then kill them with anti-proliferative chemotherapies. This is the basis of some intermittent treatment protocols that aim to re-sensitize the persistent cells and that rely on the reversible nature of their persistence (Kuczynski et al., 2013). In a murine BRAF<sup>V600E</sup> mutant melanoma mouse model, an intermittent dosing schedule of vemurafenib has been shown to forestall the emergence of genetic resistance (Das Thakur et al., 2013). However, this strategy does not completely prevent the development of drug resistance and it faces complications due to the unpredictable dynamics of cancer evolution and the potential for unmanageable explosive tumor growth to occur upon drug removal.

Targeting specific persistent cancer cell hallmarks (Table 1) might offer a more appropriate approach to killing persistent cancer cells. For example, targeting the slow cell proliferation of persistent cells in combination with primary treatment can potentially eradicate this residual population. In one such approach using a human lung cancer cell line, PC9, slowly proliferating, IGF1R-dependent, persistent PC9 cells were eliminated by using a combination of erlotinib and AEW541, an IGF1R inhibitor (Sharma et al., 2010). IGF1R signaling promotes histone H3K4 demethylation by KDM5, the inhibition of which by the histone demethylase CPI-455 increases H3K4 trimethylation and decreases the number of persistent cells in several cancer cell line models (Vinogradova et al., 2016). Similarly, HDAC inhibitors, such as trichostatin A and entinostat, de-repress the expression of the repetitive element *LINE-1*, possibly by increasing histone acetylation and heterochromatin formation, leading to the ablation of persistent cancer cells in a human xenograft NSCLC (Guler et al., 2017). Deregulated H3K27 acetylation and H3K4 trimethylation are often linked to transcriptional

repression in persistent cancer cells. In combination with erlotinib in NSCLC or with BGJ398 in FGFR-mutant bladder carcinoma, THZ1, a CDK7/12 inhibitor, shows the potential ability to impede the emergence of drug resistant cell populations (Rusan et al., 2018). The environment-mediated deceleration of persistent cell proliferation can also offer a druggable target. In human glioblastoma, Notch-mediated persistence is ablated by Compound E, a Notch inhibitor (Liau et al., 2017). Similarly, extracellular fibronectin-mediated integrin-FAK activation in persistent melanoma cells is inhibited by FAK inhibitors, such as PF573228 and PF562271. Thus, the joint inhibition of BRAF and FAK in melanoma abolishes ERK reactivation and leads to the effective control of BRAF<sup>V600E</sup>-mutant melanoma (Hirata et al., 2015).

Mitochondrial respiration is accompanied by an anti-oxidant program to avoid oxidative stress. Therefore, increasing oxidative stress in persistent cancer cells might overwhelm the cellular anti-oxidant program and thus lead to cell death. GPX4 inhibitors RSL3 and ML210 effectively eradicate persistent cells by inducing ferroptosis in various cancer types (Hangauer et al., 2017). Targeting an anti-oxidant process through the TRPA1 with AM0920 downregulates Ca<sup>2+</sup>-dependent anti-apoptotic pathways, thus increasing chemosensitivity in breast and lung human xenograft preclinical animal models (Takahashi et al., 2018).

Taking advantage of the translation reprogramming observed in the persistent human BRAF<sup>V600E</sup>-mutant melanoma cells, inhibiting the translation initiation factor eIF4A with silvestrol specifically eradicates persistent cells, and combining silvestrol with vemurafenib and MEK inhibitors prevents the emergence of drug-resistant populations *in vitro* (Shen et al., 2019).

Another strategy is to directly target the new cell identity adopted by persistent cancer cells. In human melanoma cell lines, the inhibition of RXR transcriptional activity with HX531 produces a synergistic effect with vemurafenib to ablate the neural crest stem-cell-like persistent state and forestalls the emergence of resistant populations (Rambow et al., 2018). The inhibition of WNT signaling by LGK-974 abrogates BCC persistence, possibly by inhibiting the transdifferentiation process induced by vismodegib (Sanchez-Danes et al., 2018). In a similar way, transdifferentiation of human squamous cell carcinoma cells under chemotherapy selection can be targeted using JQ1, an inhibitor of the epigenetic reader BRD4. In combination with cisplatin, BRD4 inhibition thus impedes persistent cell adaptation and delays tumor relapse (Sharma et al., 2018; Sharma et al., 2010). Another example is

seen in human prostate tumor cells that classically lose their androgen receptor-dependence and can acquire a neuroendocrine transdifferentiation. IL-6 blockade with neutralizing antibody selectively abrogates neuroendocrine differentiation of prostate cancer cells and re-sensitizes them to androgen receptor inhibitors (Wallner et al., 2006).

Finally, targeting environmental factors that are hijacked by persistent cells in a tumor-educated ecosystem also shows therapeutic benefits in preclinical models. For example, in a human xenograft NSCLC model, taking advantage of the direct relationship between inflammation-induced IL-6 and the decreased sensitivity to erlotinib, selectively blocking IL-6 signalling re-sensitizes the tumors to erlotinib (Yao et al., 2010). In addition, stromal cell-secreted HGF can decrease vemurafenib sensitivity by activating the HGF/MET axis in BRAF mutated cancer cell lines. The MET inhibitor, crizotinib, in combination with vemurafenib can thus have a therapeutic effect on BRAF mutated cell lines refractory to BRAF inhibition (Straussman et al., 2012).

## **CHALLENGES AND PERSPECTIVES**

As discussed above, persistence has been extensively studied in the context of chemotherapy or targeted therapy. These studies often disregarded the fact that persistent cancer cells must also evade from or be tolerated by immune surveillance, in order to survive in the immunocompetent organisms. Indeed, few information is available concerning the mechanisms of immune ignorance or immune evasion developed by persistent cancer cells. Along the same line, it is not clear whether similar persistent cell state can also exist following immunotherapy, for example in the form of an immune checkpoint blockade or adoptive transfer T cells (ACT). It is known that tumor cells might exploit different mechanisms to escape immune surveillance, such as reduced expression of Major histocompatibility complex (MHC) or increased expression of negative immune checkpoint factors, such as TIM3 or LAG3 (Sharma et al., 2017). Stem cell-like mouse squamous cell carcinoma cells that experience ACT immunotherapy selectively express CD80, which enables them to upregulate cytotoxic T lymphocyte antigen-4 (CTLA4) to directly dampen cytotoxic T cell activity (Miao et al., 2019). Tumor-derived CCL21 creates an immune-tolerant microenvironment by inducing lymphoid-like reticular stromal networks that depend on host CCR7-expressing cells in human melanoma (Shields et al., 2010). Indeed, upon stimulation by CCL21, as well as by IL-7, the host CCR7-expressing cells, in particular fibroblastic reticular

cells, transform the tumor-draining lymph node into an immunosuppressive privileged site and promote tumor immune evasion (Riedel et al., 2016) (Figure 6). Therefore, in the context of immunotherapy persistence, residual tumor cells tend to use cell-to-cell communication processes to mitigate the cytotoxicity of the immune system. Although these pioneer studies open an intriguing area for future research directions of persistent cancer cells, they did not rigorously evaluate the persistent cell state with regards to the proliferation rate or the reversibility of the cell status.

In general, regardless of the anticancer therapy used, there is no consensus on how to define the cancer persistent cell state. We propose that all four criteria described below should be experimentally evaluated: 1) verifying the low proliferative rate of the surviving cells upon treatment (Sharma et al., 2010)(Risom et al., 2018); 2) confirming the decreased sensitivity of the persistent cancer cells toward the anti-cancer agent in comparison with the treatment-naïve parental cells (Hangauer et al., 2017; Shen et al., 2019); 3) testing the reversibility of the process in terms of proliferation rate and sensitivity of the cells to the same treatment (Shen et al., 2019); 4) showing that the surviving cells can give rise to genetic resistance upon continuous anti-cancer treatment (Hata et al., 2016; Ramirez et al., 2016; Shen et al., 2019). These evaluations would be helpful to avoid confusion with other cell states, including disseminated cancer cell dormancy and cancer stem-cell like phenotype.

In addition to these *in vitro* experiments, it is still an enormous challenge to study the complex ecosystem of persistent cancer cells *in vivo*. Genetically engineered mouse melanoma model (for instance the Yale University Mouse Melanoma, YUMM) (Meeth et al., 2016) is a suitable syngeneic mouse model to study the persistence *in vivo* (Erkes et al., 2020), but it is restricted to targeted anti-BRAF therapy. Therefore, future efforts should be directed toward the development of clinically relevant models. Another challenge is related to the difficulty to isolate live persistent cancer cells from *in vivo* models due to their very low numbers. However, a recent lineage tracing method (CRISPRa tracing of clones in heterogeneous cell populations, CaTCH) may help spotting and following very rare cell populations (~0.001%) (Umkehrer et al., 2020). Finally, the main challenge is related to the highly dynamic property of the persistence status and to the heterogeneity of the population of persistent cancer cells. In this regard, innovative single-cell multi-omics

methods should be developed to track persistent cancer cells in the tumors (Rambow et al., 2018) as well as in the blood (Pantel and Alix-Panabieres, 2019), and together with multidisciplinary modeling of the dynamics of persistence (Grassberger et al., 2019), these methods will hopefully provide us with useful tools with which to unravel the complex roadmap to better targeting persistent cancer cells in the future.

## **CONCLUSION**

Overall, cancer persistence allows a population of cancer cells to survive chemotherapeutic assault and to remain hidden in the patient's body until they eventually give rise to clinical progression. As such, cancer cell persistence appears to be the most challenging obstacle to effective and durable anticancer treatment. Diverse mechanisms have been reported to explain this persistence phenomenon. Although a multitude of potential therapeutic approaches to target cancer persistence have been postulated, as yet none has been successfully translated into clinical use. This might be due to the fact that current therapeutic methods have not integrated the many mechanisms of persistence to simultaneously combat their metabolic and proliferation characteristics, their dynamic plasticity, and their complex interactions with the tumor microenvironment. New therapeutic strategies targeting persistent cancer cells are thus urgently needed to break the ceiling glass of drug resistance.

**Figure 1. Presence of persistent cancer cells across various types of therapeutic responses.** Metastatic cancers can have various types of response (clinical and radiological) to a given anti-cancer therapy. At the macroscopic level, in a “complete response” (light green), the metastases disappear upon treatment (100% decrease in tumor size). In a “partial response” (dark green), there is >30% decrease of the size of the metastases. If the sum of the size of the metastases varies between -30% and +20%, the patient is considered in a “stable disease”. In a “progressive disease” (red), the tumor size increase is more than 20%. Tumor responses are usually represented under the form of a corresponding waterfall plot, showing the magnitude of tumor size changes upon treatment, relative to tumor size before treatment. Each bar on the plot represents one patient. Grey arrows represent the clinical evolutions of different tumor responses to treatment. At the microscopic level, representative magnified fields show that residual persistent cancer cells can exist even with a complete response at the macroscopic level. Residual persistent cells adapt to the environment where they remain invisible for long periods of time and can eventually be a reservoir for the development of genetic resistance.

**Figure 2. The different ways in which persistent cancer cells can evade treatment.** Four main strategies employed by persistent cancer cells, including slowing down proliferation, adapting cell metabolism, changing cell identity and hijacking microenvironment, are associated with anti-cancer treatment. Different and non mutually exclusive molecular mechanisms are involved in the four major strategies. Ten different mechanisms are described: epigenetic alteration, adaptive mutability, ribosome dependency, mitochondria respiration, oxidative trade-off, epithelial-mesenchymal transition, transdifferentiation, tumor cell symbiosis, immune suppression, and microenvironment alteration.



**Figure 3. Biological mechanisms implicated in slowing down proliferation.** Intracellular signaling and epigenetic alterations are involved in promoting slow-cycling persistent cell state. Various receptor-mediated intracellular signaling pathways can induce epigenetic changes via histone (de)methylation, DNA methylation or transcription factor modifications. The slow-cycling status of persistent cells can be related to an adaptive mutability resulting from MMR and HR downregulating (green arrow) and upregulation of error-prone DNA polymerases (red arrow). These mechanisms also interact with other regulatory pathways, for instance, cell metabolism described in Figure 4 and extracellular communication described in Figure 5. *Abbreviations:* KDM: Histone lysine demethylase; TET2: Tet methylcytosine dioxygenase 2; SETDB1: SET Domain Bifurcated Histone Lysine Methyltransferase 1; N1ICD: C-terminal deletion of NOTCH1 intracellular domain; AP1: Activator protein 1; p-FAK: phosphorylation of Focal adhesion kinase; NGFR: Nerve Growth Factor Receptor; PPAR $\alpha$ : Peroxisome Proliferator Activated Receptor alpha; PGC1 $\alpha$ : Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; MMR: DNA mismatch repair; HR: Homologous recombination; Pol: polymerase.

**Figure 4. Biological mechanisms implicated in adapting cell metabolism.** Increased mitochondrial respiration is observed in various types of persistent cancer cells. This can be fueled by peroxisomal fatty acid oxidation or autophagy-dependent recycling via the production of Acetyl-CoA. Persistent cells can upregulate the membrane expression of fatty acid transport proteins, such as CD36, to facilitate the uptake of extracellular lipids. The fatty acids can be further used by CPT1A-mediated mitochondrial fatty acid oxidation or ACOX1-mediated peroxisomal oxidation. Increased mitochondrial respiration promotes intracellular reactive oxidant production and lipid peroxidation, which can be neutralized by autophagy or phospholipid hydroperoxidases, such as GPX4. Alternative metabolism in persistent cells is associated with mRNA translation reprogramming, which may be dependent on the status of the mRNA methylation regulated by both RNA methylase (i.e. METTL3) and demethylase (i.e. FTO). This leads to the translation of a subset of mRNAs, which promotes the persistent cell state. Adaptive metabolism is not mutually exclusive with other mechanisms, in particular, key metabolic enzymes are transcriptionally regulated by intracellular signaling described in Figure 3. *Abbreviations:* CD36: fatty acid translocase; ACOX1: Acyl-CoA Oxidase 1; GSH: Glutathione; GPX4: Glutathione Peroxidase 4; CPT1A: Carnitine Palmitoyltransferase 1A; METTL3: Methyltransferase Like 3; FTO: Fat Mass and Obesity-Associated Protein.

**Figure 5. Changing cell identity via EMT or transdifferentiation.** (A) Persistent cancer cells can transit from an epithelial cell state toward a mesenchymal cell state (i.e. upregulated expression of N-cadherin or FGFR) upon anti-cancer treatment, which is associated with increased expressions of immunosuppressive membrane proteins (i.e. PD-L1 and TIM3). Secreted factors from persistent cells which undergo EMT induce an immune suppressive microenvironment, such as augmenting the presence of FoxP3+ regulatory T cells. Persistent cell-associated microenvironmental fibronectin matrix can thus interact with signaling receptors expressed on the membrane of persistent cancer cells, which promote the communications with intracellular pathways described in Figure 3. (B) Different types of cancer cells can also transdifferentiate into different lineage cell types depending on the origin of the tissue. *Abbreviations:* FGFR: Fibroblast Growth Factor Receptor; IFN $\gamma$ : Interferon gamma; TIM3: T Cell Immunoglobulin Mucin 3; BRD4: Bromodomain Containing 4; PD-L1: programmed death-ligand 1. BCC: Basal cell carcinoma; IFE: interfollicular epidermis; NSCLC: Non small cell lung cancer; SCLC: small cell lung cancer, CRPC: castration-resistant prostate cancer; NEPC: neuroendocrine prostate cancer.

**Figure 6. Persistent cancer cells hijack microenvironmental cells to survive anti-cancer treatment.** Tumor cells can cooperate to create a metabolic symbiosis involving lactate utilization. Residual cancer cells can also educate other microenvironmental non-tumor cells, for instance, cancer-associated fibroblast (CAF), neutrophil and tumor-associated macrophage (TAM). These non-tumor cells can secrete cytokines, chemokines, small RNAs and metabolites to support the survival of persistent cancer cells upon anti-cancer treatment. *Abbreviations:* CCL21: C-C Motif Chemokine Ligand 21; IL-17: interleukin 17; HGF: Hepatic growth factor; MCT: Monocarboxylate Transporter; MHC-I: Major histocompatibility complex I; TCR: T cell receptor; CTLA4: Cytotoxic T-Lymphocyte Associated Protein 4; CCR5: C-C motif chemokine receptor 5.

Table 1. Therapeutic strategies that directly target persistent cancer cells.

Cancer Type	First-line treatment	Persistence Strategy	Persistence Target	Drug	References
Breast and lung cancer	Carboplatin	Alternate metabolism	TRPA1	AM0902	Takahashi, N. et al. <i>Cancer Cell</i> <b>33</b> , 985-1003 e7 (2018).
Pan-cancer	TKI	Alternate metabolism	GPX4	RSL3, ML210	Hangauer, M.J. et al. <i>Nature</i> <b>551</b> , 247-250 (2017).
Pan-cancer	TRAIL	Alternate metabolism	Ribosome	Cycloheximide	Baskar et al. <i>Life Sci Alliance</i> , 2 (6) 2019
Liver cancer	Sorafenib	Alternate metabolism	HK2	2-DG	Wang et al. <i>Onco Targets Ther</i> , 12, 5359-5373, 2019
Melanoma	Vemurafenib	Alternate metabolism	Autophagy	Chloroquine	Sanduja et al. <i>Oncogene</i> , 35 (40), 5295-5303, 2016
Melanoma	Vemurafenib+MEKi	Alternate metabolism	EIF4A	Silvestrol	Shen et al. <i>Nature Communications</i> , 2019
Squamous cell carcinoma	Cisplatin	Change identity	BRD4	JQ1	Sharma et al. <i>Nat Commun</i> , 9 (1), 4931, 2018
Prostate cancer	AR inhibitors	Change identity	IL-6	Siltuximab	Wallner et al. <i>Cancer Res</i> , 66, 3087, 2006
Basal cell carcinoma	Vismodegib	Change identity	WNT	LGK-974	Sanchez-Danes et al, <i>Nature</i> 2018
Melanoma	Vemurafenib+MEKi	Change identity	RXR	HX531	Rambow et al, <i>Cell</i> 2018
NSCLC	Erlotinib	Change identity	AXL	SGI-7079	Zhang et al <i>Nature Genetics</i> , 2012 Jul 1;44(8):852-60.
Pancreatic cancer	Sunitinib/Axitinib	Hijack TME	mTOR	Rapamycin	Allen, E. et al. <i>Cell Rep</i> <b>15</b> , 1144-60 (2016).
Skin squamous cell carcinoma	Adoptive T transfer	Hijack TME	TGFB/CTLA4	Blocking antibody	Miao Y et al. <i>Cell</i> . 2019 May 16;177(5):1172-1186
Melanoma	Vemurafenib	Hijack TME	MET	Crizotinib	Straussman R et al, <i>Nature</i> , 2012
Melanoma	Vemurafenib	Hijack TME	FAK	PF573228, PF562271	Hirata E et al, <i>Cancer Cell</i> , 2015
NSCLC	Erlotinib	Hijack TME	IL6	Blocking antibody	Yao et al, <i>PNAS</i> , 2010 Aug 31;107(35):15535-40.
Colon cancer	Cisplatin	Slow proliferation	IAP	LCL-161	<i>Cell</i> , 165 (3), 631-42 2016 Apr 21
Lung cancer	TKI	Slow proliferation	CDK7/12	THZ1	Rusan, M. et al. <i>Cancer Discov</i> <b>8</b> , 59-73 (2018).
Pan-cancer	Doxorubicin, cisplatin	Slow proliferation	BCL6	FX1	<i>Cancer Discov</i> , 9 (5), 662-679 May 2019
Glioblastoma	Dasatinib	Slow proliferation	Notch	Compound E	Liau, B.B. et al. <i>Cell Stem Cell</i> <b>20</b> , 233-246 e7 (2017).
NSCLC	Erlotinib	Slow proliferation	IGF1R	AEW541	Sharma et al, <i>Cell</i> , 2010
NSCLC	Erlotinib	Slow proliferation	HDACs	Trichostatin A, Entinostat	Guler et al. <i>Cancer Cell</i> , 2017 Aug 14;32(2):221-237.e13.
Melanoma	Vemurafenib	Slow proliferation	MITF	Nelfinavir	Smith et al, 2016, <i>Cancer cell</i>
NSCLC	Erlotinib	Slow proliferation	KDM5	CPI-455	Vinogradova et al. <i>Nat Chem Biol</i> . 12, 531–538(2016)

**Abbreviations:**

NSCLC: Non-small cell lung cancer; TKI: Tyrosine kinase inhibitor; TRAIL: Tumor Necrosis Factor Ligand Superfamily Member 10; TRPA1: Transient Receptor Potential Cation Channel Subfamily A; GPX4: Glutathione Peroxidase 4; HK2: Hexokinase 2; EIF4A: Eukaryotic Translation Initiation Factor 4A; BRD4: Bromodomain Containing 4; IL-6: Interleukin 6; WNT: Wingless-Type MMTV Integration Site Family; RXR: Retinoid X Receptor; AXL: AXL Receptor Tyrosine Kinase; mTOR: Mechanistic Target Of Rapamycin Kinase; TGFB: Transforming Growth Factor Beta; CTLA4: Cytotoxic T-Lymphocyte Associated Protein 4; MET: Hepatocyte Growth Factor Receptor; FAK: Focal adhesion kinase; IAP: Inhibitors of apoptosis protein; CDK7/12: Cyclin Dependent Kinase 7/12; BCL6: B-Cell Lymphoma 6 Protein; Notch: Translocation-Associated Notch Protein; IGF1R: Insulin Like Growth Factor 1 Receptor; HDAC: Histone Deacetylase; MITF: Melanocyte Inducing Transcription Factor; KDM5: Lysine Demethylase 5B;

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**Declaration of interests**

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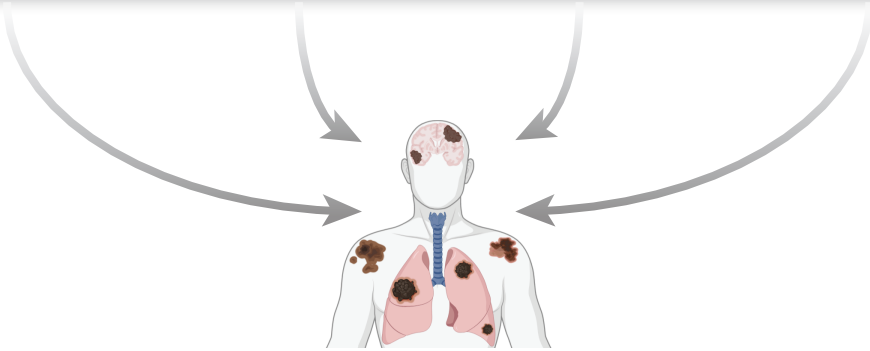
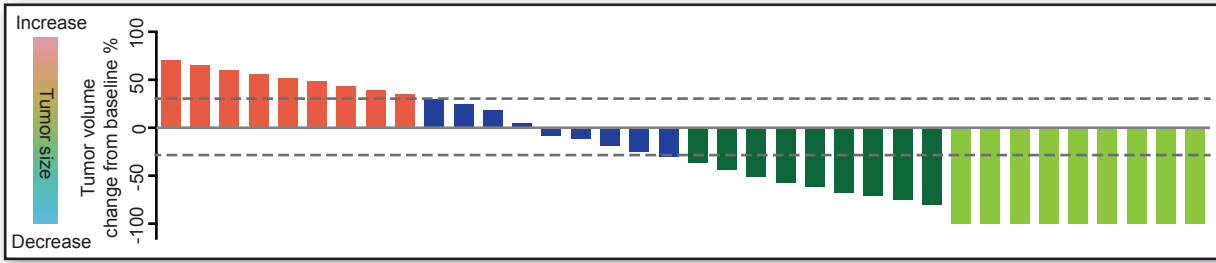
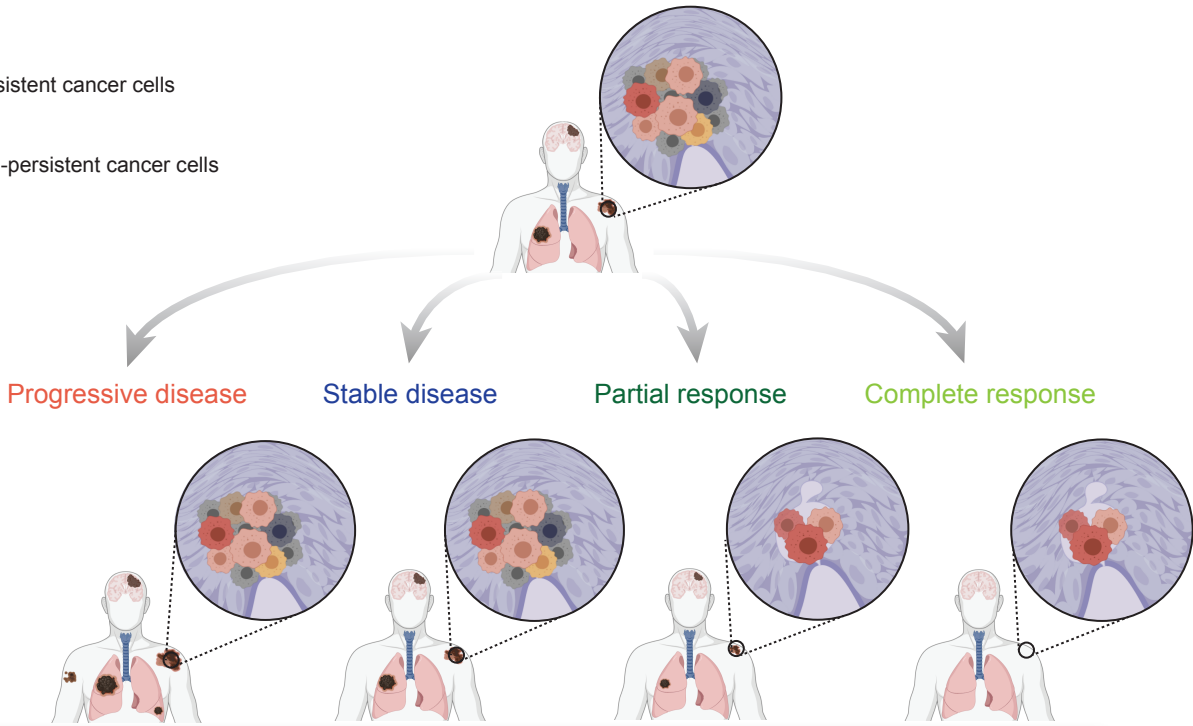
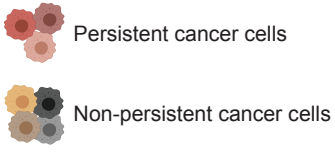
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Treatment initiation

Response evaluation

Relapse

