Dormant, quiescent, tolerant and persister cells:

four synonyms for the same target in cancer

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Abstract

Although many drugs/treatments are now available for most diseases, too often, resistance to these treatments impedes complete therapeutic success. Acquired resistance is a major problem in many pathologies but it is an acute one in cancers and infections. This is probably because these diseases often require long durations of treatment, which ascribe to the selection of resistant cells. However, the actual mechanisms implicated in the selection process are still under debate. It is becoming increasingly clear that resistance is associated with the heterogeneity of cancer cells or micro-organisms and that multiple mechanisms underlie the emergence of drug-resistant subpopulations. Recently, it has been suggested that a subpopulation of drug tolerant cells present in cancer populations and called "persisters" play a major role in this resistance. Recent studies have shown that microorganisms share similar properties. Still, how persister/tolerant cells intervene in the development of resistance is not completely elucidated but seems to be related to epigenetic changes in treated cells and the capacity of persisters to modulate and/or highjack their microenvironment. Due to the complexity of this process, the input from mathematicians, as well as new methods of bioinformatics and statistics, is necessary to fully comprehend the acquisition of resistance/tolerance deriving from and leading to the heterogeneous cell populations. The present review will give a brief overview of the most recent data available on drug tolerant cells in cancers and their similarities with microorganisms.

Key words: persisters, drug resistance, drug tolerance, recurrent disease, cell dormancy, quiescence

1. Introduction

Tumor development has originally been described as a linear process initiated by the transformation of normal cells following overexpression or mutations of oncogenes. Additional mutations and/or epigenetic alterations would markedly contribute to increase the tumor heterogeneity and progression (Figure 1) [1-5]. However, genomic studies of cancer cells and clinical observations have strongly altered the vision of linear and hierarchical events leading to homogenous tumors. Asymmetric division is a fundamental biological process resulting in the generation of distinct cell types within multicellular organisms and consequently plays a key role in tissue homeostasis [6]. During asymmetric division, components of cell machinery (e.g. organelles, RNA, proteins) are differentially segregated into the two daughter cells leading to the generation of two different cells such as one stem cell (or undifferentiated cell) that guarantee the self-tissue renewal and one differentiated cell dedicated to functional tasks. A similar process has been observed in tumors allowing tumors to generate a pool of cancer "stem-like" cells contributing to a continuous enrichment of the tumor heterogeneity [7]. This asymmetrical mitosis would contribute to the disruption the linear concept of tumor progression (Figure 1). Long-term relapse in patients considered clinically disease-free is a conventional clinical feature of numerous cancers including breast, prostate, thyroid or renal carcinomas and reinforce the notion of non-linear tumor growth. Breast cancer perfectly illustrates this; late relapse in about 20% of disease-free patients show recurrence of the disease locally or in distant organs 7-25 years after resection of the primary tumor [8,9]. This process called clinical cancer dormancy in patients without any apparent clinical symptoms or detectable disease; is characterized by persistent cancer cells. Based on recent observations, the notion of dormant, quiescent or persistent cells has progressively emerged. The present review will give a brief overview on their origin, properties, control and main clinical impacts and will discuss the stop or grow processes implicated. Thus,

characterization of this particular population of cancer cells is a major challenge in cancer biology.

2. Dormant, quiescent, tolerant and persister cells in cancer: are these four terms designating the same population?

Even if there is no clear consensus on the latency period, the risk of recurrence in cancer is directly related to the persistence of undetectable cancer cells after the resection of the primary tumor [10]. The latency can occur after long periods of time and this state is called "dormancy" in which cancer cells exhibit slow cycling, low metabolism and fitness, and long-term survival mechanisms. Probably due to environmental changes, these cells become active and this occurs usually at a distance from the primary tumor site. Several teams were able to isolate tumor cells in the blood and bone marrow following the resection of the primary tumor demonstrating the early dissemination of solitary cancer cells from the primary site [11-14]. On the other hand, Meng *et al.* isolated circulating tumor cells in breast cancer patients 7-22 years after removal of the initial disease years and without any sign of metastatic dissemination [15]. More recently, Vishnoi *et al.* have isolated circulating tumor cells with stemness properties in patients diagnosed with and without breast cancer brain metastasis [16]. The half-life of these rare cell events was estimated to be a few hours and revealed that dormancy is a dynamic persistence in peripheral organs by cancer cells with distinct properties [17]. Dormancy has been considered as a steady state characterized by a balance between low cell proliferation and active cell death programs. However, the cell death status of these cells is still a matter of debate (autophagy, apoptosis or…?) and its implication in dormancy not firmly established. Exiting dormancy means the cells re-enter the cell cycle, have increase fitness and adapt their metabolism for faster growth. The implication of immune system and/or inflammation in this process remains to be investigated in depth both for its role in induction and reactivation of these cells [18]. Similarly, micro-environmental

constraints (O_2 pressure, acidic environment, non cancer cell contacts and stiffness) have been implicated in the induction and maintenance of dormancy [19] and the implication of treatments on the induction of quiescence/dormancy through tumor editing remains also a matter of debate.

Dormant/quiescent/persister cells exhibit the molecular profile of stem cells. The concept of cancer initiating cells or cancer stem-like cells refers to a subset of cells within the tumor that uniquely sustains malignant growth. “Cancer initiating cells” or “cancer stem-like cells” are used interchangeably although the first denomination is more associated with the cell of origin and the second to the propagation of cancer [20]. Although numerous publications have described the self-renewing and stemness nature of these cells in different types of cancer, the concept of “stem-like cells” in cancer is subject to controversy for certain authors. Cancer stem-like cells define a subpopulation of self-renewing cells that are able to reproduce all the features of tumor cells, regardless of their tumorigeneic status. In addition, they should express likeness markers, be able to remain viable in a quiescent state, to divide in response to appropriate stimuli and to form spheroids (“oncospheres”) under three-dimensional (3D) conditions [20]. Consequently, stemness in cancer can be defined as cells with an enhanced plasticity, a loss of cell identity, an altered self-renewed/differentiation balance and an acquisition of *de novo* self-renewal with daughter cells committed to specific differentiation [21]. These stem cells express markers such as high aldehyde dehydrogenase (ALDH) activity, CD44, CD133 and can be identified by flow cytometry as a side population (Hoechst exclusion) and be potentially responsible for tumor relapse like dormant/quiescent/persister cells [22-26]. Furthermore, the link between stemness properties and dormancy has been already established in various cancer types including for instance glioblastoma [27], sarcoma [25], colon [28], breast [29,30], prostate [31], lung [32,33], and ovarian cancers [34].

In this context, after long-term treatment without detectable tumor regrowth, a fraction of these cells can persist and gain the ability to expand in the presence of drugs and become progressively drug tolerant and/or drug resistant (Figure 2). The later stage is directly linked to patients with high numbers of recurrence [8,9]. It is confusing to use so many different names (persistant/tolerant/dormant/quiescent) for a population, which stands in between sensitive and resistant cells. Indeed, Ramirez *et al.* has demonstrated recently that drug tolerance is a stage between sensitivity and resistance from which can emerge resistant clones with diverse drug-resistance properties [35]. A reduction in the proliferation rate gives a selective advantage to cancer cells to resist to drug pressure and this in turn would cause the enrichment of dormant cells with a stem-like phenotype of dormant cells as shown by Zhou *et al.* in ovarian cancer [34]. Similarly, Touil *et al.* demonstrated that colon cancer cells escape 5-FU-induced cell death by expressing a cancer stem-like cell profile and enter into a reversible dormant/quiescent G0 state [36]. This process required the activation of the tyrosine kinase c-Yes leading to the dissociation of Yes/YAP (Yes-associated protein) and a depletion of nuclear YAP. Silencing of YES1 mimicked the effect of 5-FU by inducing cell dormancy [36]. In addition, dormant/quiescent/tolerant/persister cells may hijack their environment, which could become an immune tolerant environment and consequently a sanctuary for drug resistance and tumor development [37]. For instance, it has been shown that cancer stem-like cells are able to facilitate the selection of TH2 type T cells in an *in vitro* co-culture of peripheral blood lymphocytes and autologous cancer stem-like cells. Similarly, cancer stem-like cells have been characterized by a defective expression and/or function of HLA class I antigen-processing machinery [37]. These cells then regulate the expression of tumor-associated antigens and molecules involved in antigen processing and presentation on their membrane that facilitate the immune evasion and yield to more aggressive tumors

[38,39]. Previous studies have revealed the low immunogenicity of dormant/quiescent/tolerant/persisters by the regulation of tumor-associated antigens and molecules involved in antigen processing and also by the release of a large variety of immunosuppressive cytokines (e.g. IL-4, IL-10, TGF-β) [40]. Thus, defective expression of tumor-associated antigens and molecules involved in antigen processing could result in a lower sensitivity of persister cells to interferon stimulation and a failure to elicit a T cell-mediated response [41,42]. The intrinsic and acquired properties (e.g. low cycling level, low immunogenicity, drug resistance) of dormant/quiescent/tolerant/persister cells result in a key selective advantage (Figure 3).

3. Involvement of the tumor microenvironment in the control of dormancy

Using *in silico* modeling, Poleszczuk *et al.* hypothesized the competition between the different cell clones which compose the tumor mass and more particularly between cancer cells with stem-like phenotype and the others. In their models, within a proliferative tumor, non-stem cancer cells tend to inhibit cancer stem-like cell division (dormant/quiescent/tolerant/persisters) while in a low proliferative tumor these cells advantages an increase in persister cells by facilitating their division [43]. Furthermore, in addition to their internal fight for cell survival, the local tumor microenvironment (TME) acts as a referee regulating this fragile balance. Sir James Paget presented the first evidence for the role of the TME in tumorigenesis at the end of the 19th Century with the "seed and soil" theory [44]. Indeed, he postulated that a combination of genetic events and a favorable microenvironment drive tumor initiation and growth and allows for the maintenance of dormant cells. This theory is now recognized by the scientific community and observes most of the cancer entities. Consequently, Cahu *et al.* recently compared the characteristics of T-cell acute lymphoblastic leukemia cells located in various bone marrow sites of the body and

demonstrated that cancer cells exhibited a high proliferation profile in the bone marrow of tail vertebrae with a phenotype of dormancy (e.g. decreased metabolism and cell cycle progression) compared to the bone marrow of thorax vertebrae [45]. Interestingly, cancer cells isolated from bone marrow of tail vertebrae displayed higher drug resistance illustrating the role of the local TME in the orchestration of T-cell acute lymphoblastic leukemia propagation, dormancy and drug resistance.

Bone is also a sanctuary for breast and prostate cancer cells. Bone is the physiological niche for hematopoietic stem cells, and one of the roles of osteoblast as reticular cells is the control of the self-renewal and differentiation of hematopoietic stem cells through cytokine release and specific inter-cellular contacts [46]. In oncologic context, the compartment of hematopoietic stem cell competes with cancer cells. This territorial fight leads to the replacement of hematopoietic stem cells by cancer cells [47]. This mechanism has been recently confirmed by Jeong *et al.* who demonstrated the dynamic adaptation of the bone niche to changes in stimulation like chemotherapy [48]. 5-FU inhibited the proliferation of leukemic cells and concomitantly induced the remodeling of the bone marrow niche, reorienting towards a regeneration of normal hematopoietic stem cells. This observation clearly demonstrated the influence of adaptive changes of the bone marrow niche to the host cell. Such a switch in bone niche activity may be a potential therapeutic strategy for bone-associated cancers [49]. In the bone marrow niche, osteoblasts play a crucial role in the physiological control of hematopoiesis and in the pathological context by regulating the phenotype of disseminated cancer cells [50]. In this context, Shiozawa *et al.* studied the behavior of prostate cancer cells disseminated in bone marrow. These authors demonstrated the contribution of Gas-6 released by osteoblasts in the conversion of prostate cancer to the cancer stem-like cells within the marrow microenvironment [50]. Furthermore, osteoblasts release soluble factors mediating the dormancy of prostate cancer in the bone environment

[51]. Growth Differentiation Factor (GDF)-10 and Transforming Growth Factor (TGF)-β2 are two protagonists involved in this process and regulate dormancy through activation of the TGFβRIII-p38MAPK-pS249/T252RB pathway. Leukemia Inhibitory Factor (LIF), a member of the IL-6 cytokine, is produced by osteoblasts [52] and provides a pro-dormancy signal to breast cancer cells [53]. Secreted Protein Acidic and Rich in Cysteine (SPARC) induces similar activity on prostate cancer cells and increases Bone Morphogenetic Protein (BMP-7) expression by bone marrow stromal cells [54]. By using osteoblast-specific Jagged1 transgenic mouse, Zheng *et al.* demonstrated Jagged1 mediated tumor-stromal interactions and provided a survival niche for cancer cells thereby validating the osteoblast niche as a drugable target [55].

The notion of tumor niche should not be restricted to bone but to all vascularized tumor sites [56]. Indeed, cancer cell dormancy appears tightly regulated by the microvasculature. Ghajar *et al.* used engineered organotypic vasculature niches to determine the involvement of endothelia cells in cancer cell growth and demonstrated that endothelial-derived thrombospondin-1 supported breast cancer cell dormancy and that the stable microvasculature formed a perfect niche for promoting dormancy [57]. Angiogenesis is a critical process of tumor growth and cancer cell spreading. The hypoxic environment within the tumor tissue activates of specific signaling pathways, which initiate cancer cell invasion and neo-angiogenesis. Among the key molecular pathways involved in hypoxia, hypoxia-inducible factor (HIF)-1 and lysyl oxidase (LOX) appear as two central mediators of the metastatic niche and regulate cell dormancy and stem-like phenotype [58-62]. In addition to polypeptidic mediators, lipids fuel metastatic cells. Recently, Pascual *et al.* identified a subpopulation of $CD44^{bright}$ cells in human oral carcinomas characterized as slow-cycling, no overexpression of mesenchymal markers, expressing high levels of the fatty acid receptor CD36 and lipid metabolism genes, and capable of initiating metastasis. They demonstrated that suppression

of CD39 impaired the metastatic process [63]. Overall, the literature demonstrates the central role of all components (cell, extracellular matrix, physicochemical parameters) of tumor niche in the induction, maintenance and regulation as well as drug resistance of dormant/quiescent/tolerant/persister cells [64].

4. Persisters and drug resistances: a common property shared between cancers and infectious diseases

In bacteria, a link between dormant/quiescent cells and cell with properties of long-term persistence (persister cells) as a response to antibiotics has been established [65]. Persisters in microorganisms have been defined as dormant variants of regular cells formed stochastically or in response to stress (including antibiotics to bacteria). Most importantly, the formation of persister cells can induce a non-genetic heterogeneity within a bacterial population (especially as “biofilms”), which in turn may contribute to the functional adaption to environmental changes [66]. It is well-known that pathogens can easily acquire resistance mechanisms to survive the effects of antimicrobial agents. Similarly to cancer cells, pathogens can reduce their metabolism to move progressively to a dormancy/quiescence/tolerance/persistence state without any genetic modifications [67]. Present at low frequency in the normal population, persisters increase under drug pressure and could explain chronic bacterial and fungal infections [68]. In cancers, the notion of persisters has been introduced in lung cancer treated with EGFR inhibitors [69]. These data suggested that tumor resistance could be mediated by a dynamic process that accumulated temporarily into distinct subpopulations [35,70]. Similar observations in bacteria and yeast also suggested that the dormancy/quiescence/tolerance/persistence phenomenon is a dynamic process with different genes playing roles of variable significance at different times [71,72]. Thus cancer cells and pathogens seem to follow similar patterns.

Mycobacterium tuberculosis is one of the best examples of dormancy in bacteria. Epidemiological studies revealed the resurgence of the infection despite multiple antibiotic therapies and showed the high risk of relapse associated with the emergence of antibiotic resistance and the ability of Mycobacterium tuberculosis to become dormant in infected organs. Similarly to dormant cancer cells, Mycobacterium tuberculosis dormancy is also characterized by its capacity to modulate the local immune cells and as such escape from immune system control [73,74]. Bacteria infect pneumocytes and alveolar phagocytic cells such as dendritic cells in which they replicate before spreading to pulmonary lymph nodes and to several distant foci. Mycobacterium tuberculosis hijacks the environment to facilitate its dormancy and drug resistance by masking the pathogen-associated molecular patterns of macrophages or by regulating TLR4 expression on the cell surface of mononuclear phagocytes causing a down-regulation of TH1 cytokine release [75]. In addition, immunosuppression can also result from a bystander effect in phagocyte population with an inhibition of dendritic cell differentiation and apoptosis of T cells leading to a survival advantage of the pathogen [76,77]. A similar bystander effect has been observed in the control of dormancy in thyroid cancer [78]. Using a fibrosarcoma model, Liu *et al.* demonstrated the protection of proton-irradiated cancer stem-like cells by bystander cancer stem-like cells [79].

Finally, adaptive resistance emerges in populations of bacteria in the presence of antibiotic. Antibiotic resistance can be driven by epigenetic inheritance of variant gene expression patterns similarly to drug resistance observed in cancer [80]. Knoechel et *al.* provides evidence of a mechanism of drug resistance that appears to reflect an alternative epigenetic cell state and establish a role for epigenetic heterogeneity in leukemia resistance that may be addressed by incorporating epigenetic modulators in combination therapy [81]. Several other studies have concurred with these results and recently persisters have been

shown to be the target for epigenetics drugs in lung cancer [82]. As such dormancy in cancer could have a noncoding origin through epigenetic control of cell division [83]. Together, these data suggest that the acquisition of therapeutic resistance may occur in multiple stages, through rare cells, which later develop into stable resistant cells through epigenetic reprogramming.

Thus, similar to cancer, the host-pathogen arms race implicate deception plus hide and seek strategies within heterogeneous populations, which can include non-pathogenic cells if necessary.

5. Dormancy/Quiescence/Tolerance/Persistance: a specific process in response to environmental stress for cell survival

Mechanisms of resistance of biofilms formed by microorganisms possess many common points [84]. The mechanisms proposed to account for the dormant/quiescent/tolerant/persistent cell subpopulation in bacteria are nonetheless multiple. However, recent studies have indicated that conserved genes can be identified and are implicated in basic cellular functions such as the anti-oxidative defense pathway, heat shock proteins, energy production and specific bacterial mechanisms (e.g. toxin-antitoxin, module and SOS systems) [72]. The latter study also suggests that the persistence phenomenon is a dynamic process with different persister genes playing roles of variable significance at different times. Many other publications emphasize the role of the stress response to the generation of the persister population [85]. Interestingly, many known mechanisms of resistance in cancer appear to be related to similar pathways [86] (Figure 4). Dormancy and the related functional consequences (e.g. drug resistance) is a conserved mechanism of survival that allows an evolutionary adaptation to various hostile environments. This parameter of evolution has been observed in *C.elegans,* which is able to sense stress and to induce cellular dormancy in order to resist to nutrient deprivation [87]. In cancer cells, stress-

induced pathways combine the endoplasmic unfolded protein response and autophagy [88,89]. The endoplasmic reticulum plays a central role in this process. Eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) also known as protein kinase R-like endoplasmic reticulum kinase (PERK) inactivates EIF2 by phosphorylation that results in a marked blockade of global protein synthesis triggering dormancy by initiating a cell cycle arrest in G0/G1 phase thereby fostering the survival of drug-tolerant persister cells [88,90]. Al Emran *et al.* identified early stress-induced multi-drug tolerant cancer cells in different cancers [91]. In response to drug exposure or nutrient starvation, epigenetic changes were observed (e.g. loss of the H3K4me3 and H3K27me3 and gain of H3K9me3) leading to cell survival. Autophagy is another mechanism for dormant cells to improve their survival and to initiate recurrent disease [89]. Autophagy gene autophagy-related 7 (ATG7) appears to be essential for dormancy-associated autophagy and the inhibition of the autophagy in dormant breast cancer cells leads to the accumulation of altered mitochondria and reactive oxygen species (ROS) and finally to apoptotic cell death [89]. In addition to the conventional mechanisms (e.g. reticulum endoplasmic response, autophagy) of cell survival to stress environment, cancer cells practice cannibalism of their neighbors to survive [92]. Indeed, Bartsoch *et al.* observed the entrance to dormancy of breast cancer cells deprived in nutrients after cannibalizing mesenchymal stem cells. Tumor dormancy is thus a selective, adaptive and evolutionary mechanism in response to stress.

6. An ecological/sociological/Darwinian view of drug resistance: interest of mathematic models

The current “Darwinian” description of cancer implies that multiple clones with different levels of fitness interfere with each other in different ways (positive or negative) and are dependent on the environment [93]. From these interactions, the average “fitness” of the

tumor can be increased when equilibrium between clones is achieved and if cancer phenotypes are plastic enough to sustain small variations in the environment. The selective pressure induced by the treatment allows the Darwinian selection of the fittest cells (or group of cells) not necessarily of the "strongest" or more aggressive at first. This is similar in essence to what is observed in an ecological habitat shared by diverse species, heterogeneous populations of cancer cells that reside in close proximity are thought to engage in a variety of interactions that may influence their fitness and survival. Thus targeting one dominant clone would affect the equilibrium but not necessarily eradicate the tumor.

The cellular interactions can be direct or be mediated via the TME. Similar interactions can be found in microorganism biofilms. Very broadly, the interactions, similar to that observed in ecological systems, can be classified into negative (competition or amensalism for example) or positive interactions (commensalism, mutualism or synergism) [94]. Of note, there are now plenty of examples of the predatory mechanisms used by cancer cells on the non-cancer components of the TME. Drug treatment is likely to significantly disrupt the equilibrium attained by cancer cells at the time of the treatment. This implies that minor sub-clones can determine the clinical course and response of disease, and that temporal and spatial heterogeneity needs to be considered not only before but also post-treatment [95]. There are numerous examples that suggest the possibility to exploit the ecology of tumors for treatment [96].

Predictive models demonstrate the potential of applied evolutionary biology to improve public health and disease control. The mathematical modeling of cancer has increasingly been sought in the past years [97], not only for improving treatments and drug discovery [98], but also for modeling cancer interaction with its microenvironment [99]. The impact of tolerant cells has also been studied both in bacteria and in cancer [100]. However, modeling resistance to treatment needs to take in consideration many aspects of population

biology (cell types and clones differential dynamics, metabolism adaptation, positive and negative influences of micro-environmental factors, drug concentrations…) in order to elucidate the roles of phenotypic plasticity and selection pressures in tumor relapse [101]. So next-generation models capable of simulating highly detailed somatic genetic and epigenetic events are probably still to be created.

7. Perspectives and conclusions

It is becoming increasingly clear that there is significant heterogeneity to drug response within a bacterial and cancer populations and that multiple mechanisms underlie the emergence of drug-tolerant and drug-resistant subpopulations. The emergence of dormant/quiescent/tolerant/persistent cells is an ancestral and conserved mechanism directly related to cell survival and involves multiple parameters: genetic, epigenetic, selectivity, relationship with the local microenvironment (e.g. cell cannibalism, immune repression), diversity/heterogeneity. Together, these data suggest that the acquisition of therapeutic resistance may occur in multiple stages, through rare cells, which later develop into stable resistant cells through epigenetic reprogramming. Strikingly, the comparison of the two seemingly different types of diseases: cancer and infectious, show common traits and strategies to evade drug effects. Further analyses of the resemblance and the differences between the different pathologies could provide new therapeutic tactics for treating resistant cells. This study would require multidisciplinary approaches from the clinic to cell biology and molecular biology as illustrated by the recent works of Hangauer *et al.* who demonstrated the dependency of persister cells on the lipid hydroperoxydase GPX4 and showed that loss of GPX4 function led to selective ferroptotic death of persister cells [102]. This loss of function was directly associated with a prevention of tumor relapse and supports the potential targeting GPX4 as therapeutic option to prevent or cure acquired drug resistance. New mechanisms of drug resistance enrich the panel of existing process and illustrate the unlimited “imagination”

of cancer cells to survive. Thus, loss of cilia was able to confer resistance to Smoothened inhibitor sonidegib in medulloblastoma cells illustrating a novel mechanism driven drug resistance [103].

Because of the complexity of the problem and the connections with Darwinian mechanisms, the input of mathematicians and ecologists is a necessity to fully comprehend the acquisition of resistance in a heterogeneous population. In addition, a better understanding of coding and noncoding (epigenetic) mechanisms controlling cell dormancy would lead to the development of new drug resistant therapies to eradicate residual diseases and to prevent tumor recurrence.

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Figure Legends

Figure 1: High heterogeneity of cancer cells in a tumor mass. The appearance of a first mutation in cancer driver gene, which can occur in the bulk population or in a cancer stem-like cell, **(1**) leads to a clonal expansion of the damaged cell (**2**). The abnormal clones exhibit consequently chromosome instabilities resulting in polyclonal proliferation of cancer cells harboring diverse mutation profiles following secondary oncogenic events **(3**). From this highly heterogeneous population, cancer cells spread to distant organs and establish metastatic foci (**4**). Asymmetric division is a basic biological process leading to the formation of distinct cell types within multicellular organisms and which markedly contribute to maintain a pool of cancer stem-like cells in a tumor mass. Migrating and/or disseminating cancer cells can be in a dormant state and be reactivated by unknown mechanism sometime many years after their implantation. In addition to genetic alterations, epigenetic modifications markedly contribute to increase the tumor heterogeneity and cell dormancy.

Figure 2: Origin of dormant/quiescent/tolerant/persistent cells. (**A)** Conventional model of acquisition of resistance through mutations present before the treatment (heterogeneous population) or induced by the drug followed by Darwinian selection. Mutations occurring in blue cancerous cell population lead to the development of red and green mutated cancer cells and then to a polyclonal population. Drug induces the selection of specific resistant red cells and to a clonal expansion. **(B**) Persisters (red cells) are present in the initial population of

cancer or normal cell cells (blue cells) and these cells have low metabolic activities or slow proliferation. As such they are not targeted by the drugs and hence escape the treatment. Population regrowth occurs identical (similar) to the original population or mutations lead to a new population with similar persister properties (other colored cells). (**C)** Persisters (red cells) compose a side population, which is genetically different (heterogeneity) or in equilibrium with "normal" or proliferating cancer cell population due to stress signaling (blue cells). Obviously, the two models are not exclusive.

Figure 3: A model for the relationship between dormant/persisters and cancer stem-like cells. Similar to what has been described for hematopoietic stem cells (HSC), a small population of cancer cells with self-renewal and asymmetric division properties exist under 2 classes: long-term (LT) cells with slow cycling and long-lived properties or short-term (ST), cells which are derived from LT cells and give rise to cancer cells. Similar to HSC these cells could be in contact/dependent on their interactions with normal cells such as mesenchymal stem cells, while cancer cells would interact with other cell types (**A**). Treatment would eradicate cancer cells but have little affect LT cells (**B**). At this stage LT cells would migrate away from the original site and enter latency (**B**), possibly under the control of the immune system (**C**). ST cells would undergo apoptosis and as such would not give rise to cancer cells (**C**). Stress or other changes in the environment would rescind the cell death induction in ST cells, paving the way for tumor formation. LT cells could be considered as dormant/persister cells while ST cells may be considered as cancer stem-like cells.

Figure 4: Dormancy is a conserved mechanism dedicated entirely to cell survival in response to stress signals. Summary of the main characteristics of cancer cell dormancy (in blue) and their key regulators (in red).

Figure 1

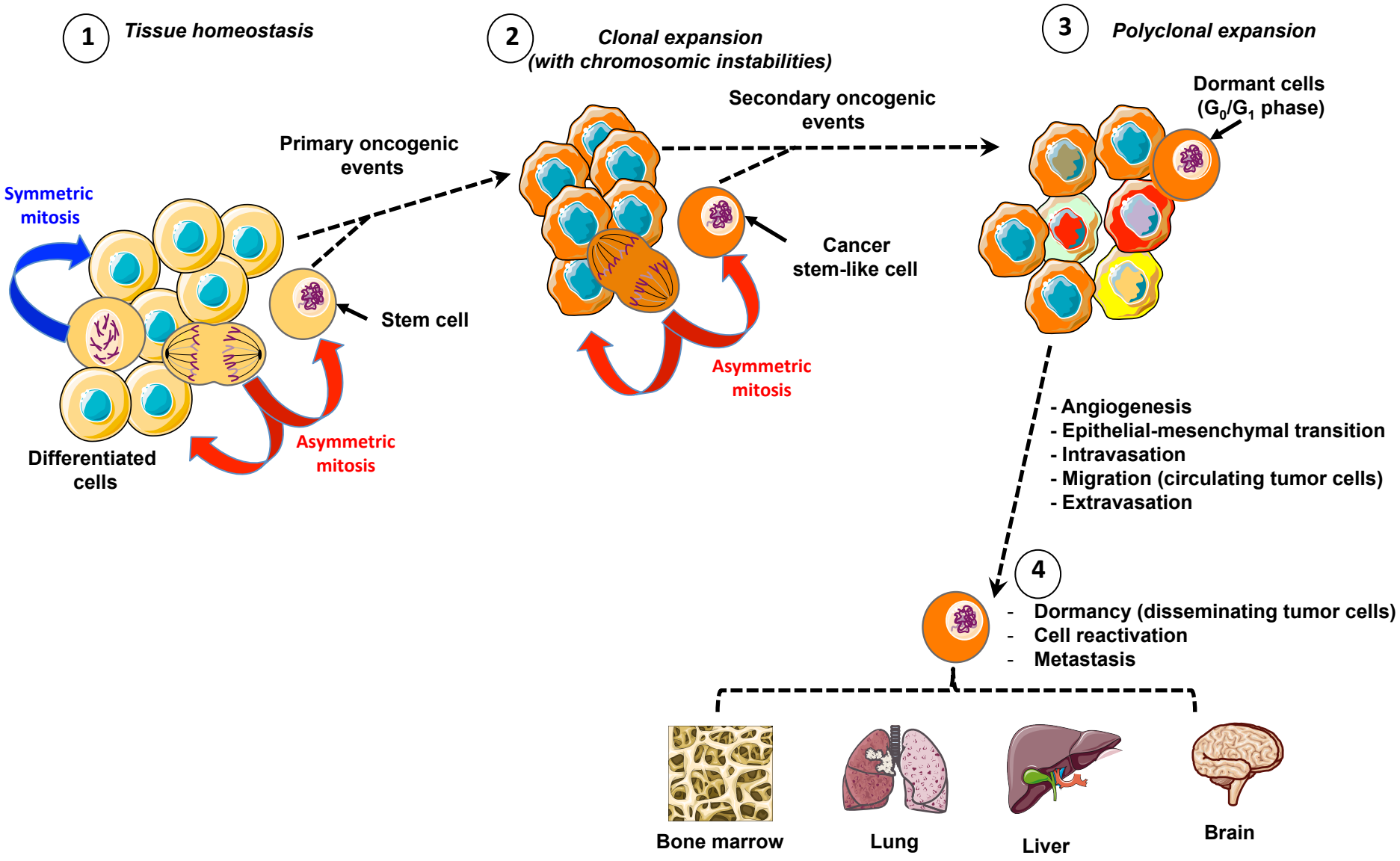

1
Tissue homeostasis
2
Clonal expansion
(with chromosomic instabilities)
3
Polyclonal expansion
Symmetric
mitosis
Primary oncogenic
events
Stem cell
Differentiated
cells
Asymmetric
mitosis
Secondary oncogenic
events
Cancer
stem-like cell
Asymmetric
mitosis
Dormant cells
(G_0/G_1 phase)
- Angiogenesis
- Epithelial-mesenchymal transition
- Intravasation
- Migration (circulating tumor cells)
- Extravasation
4
- Dormancy (disseminating tumor cells)
- Cell reactivation
- Metastasis
Bone marrow
Lung
Liver
Brain

Figure 2

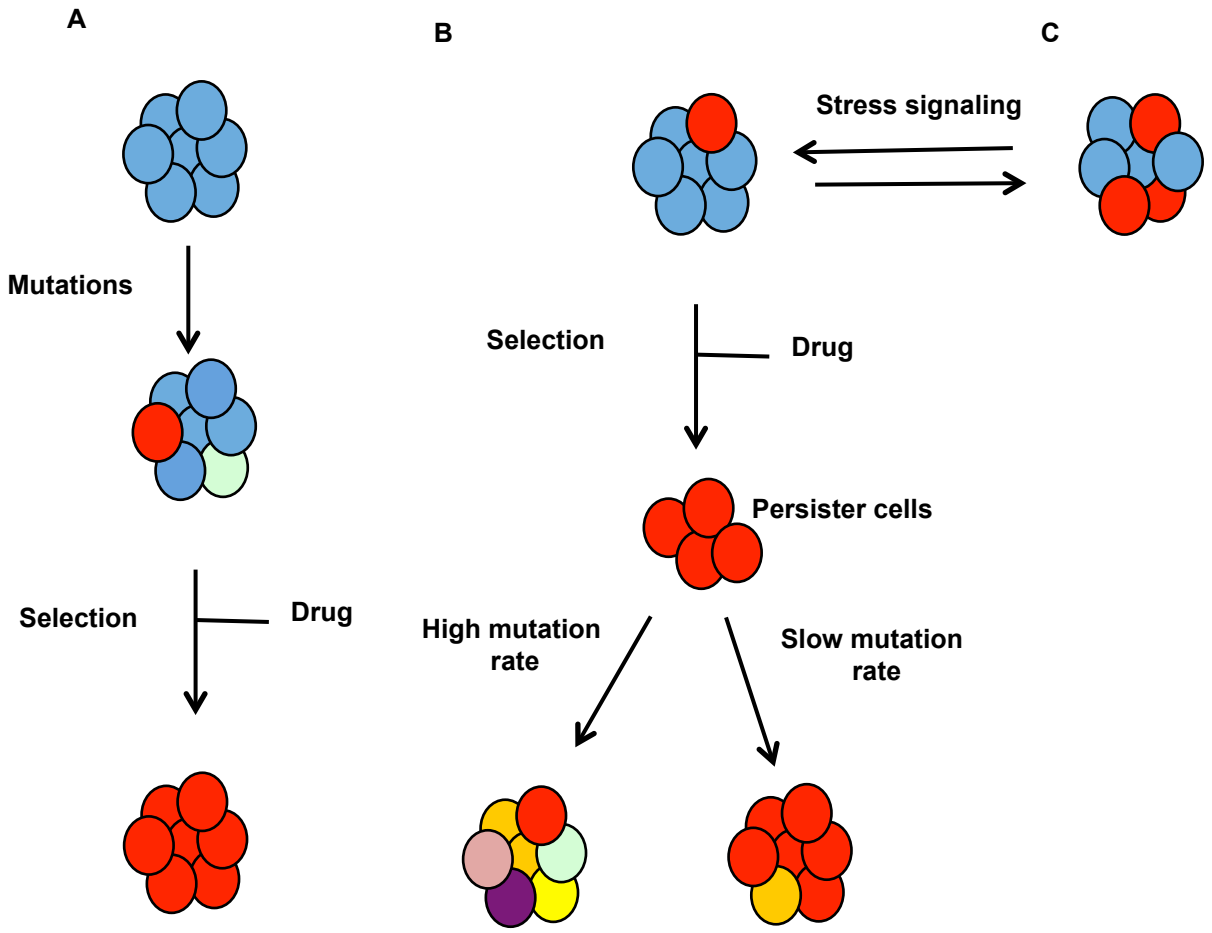

A
Mutations
Selection
Drug
B
C
Stress signaling
Selection
Drug
Persister cells
High mutation rate
Slow mutation rate

Figure 3

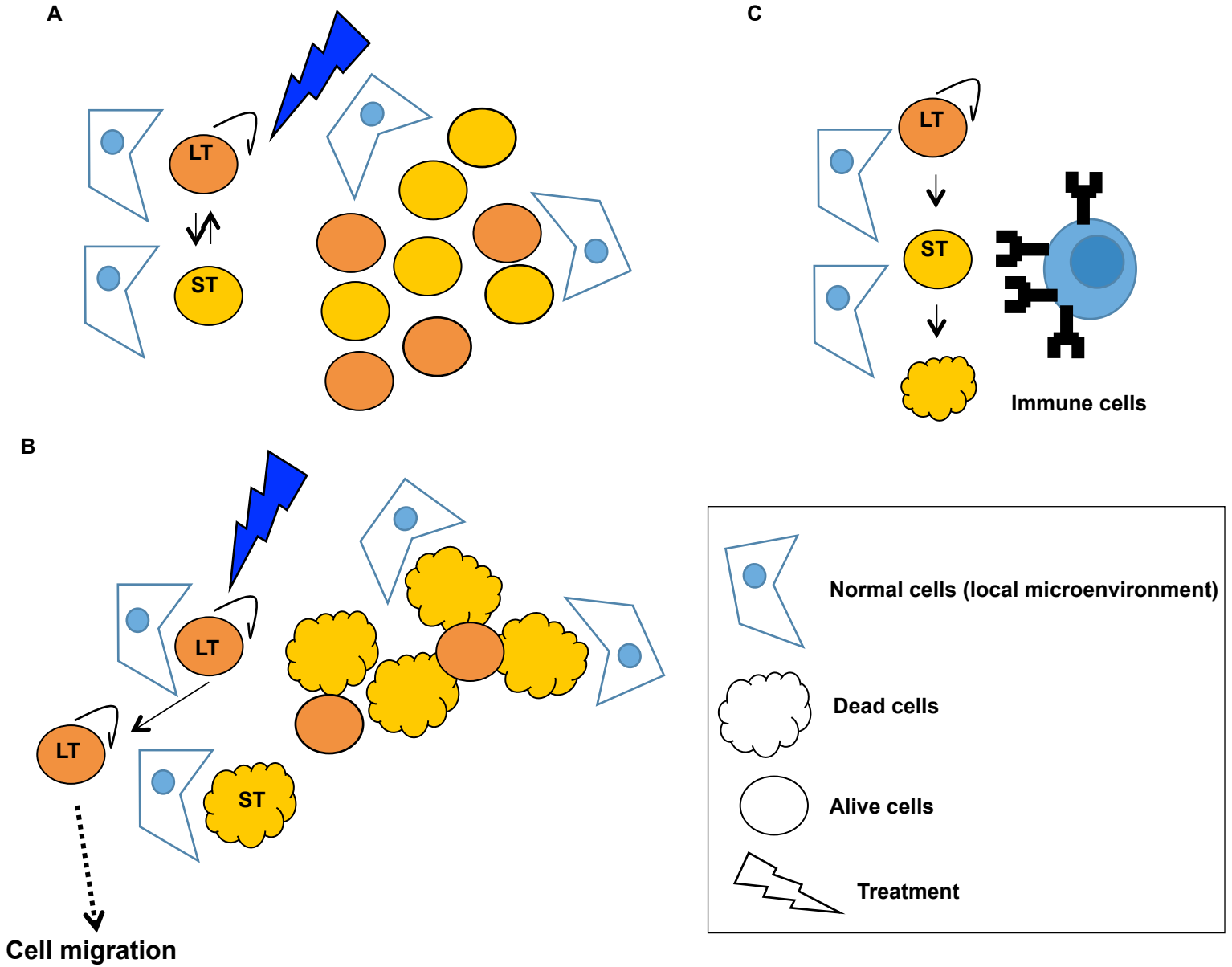
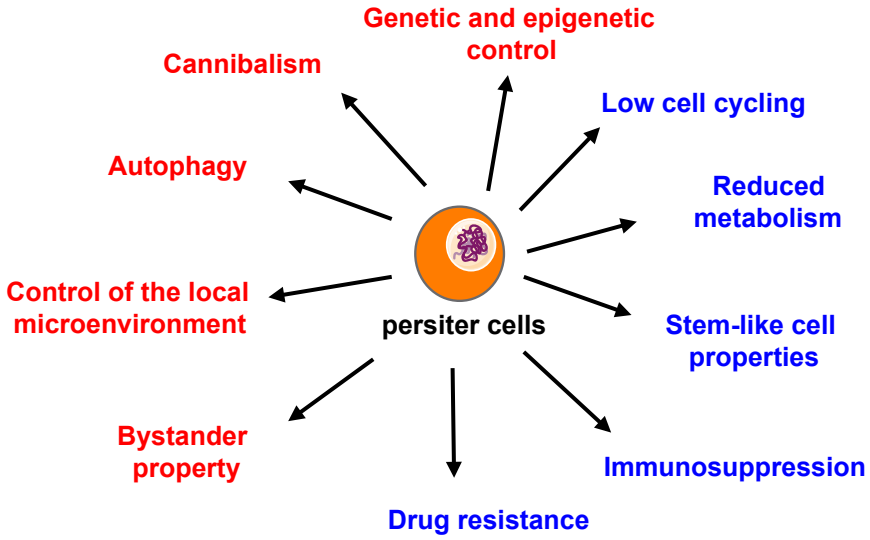

A
LT
ST
C
LT
ST
Immune cells
B
LT
LT
ST
Cell migration
Normal cells (local microenvironment)
Dead cells
Alive cells
Treatment

Figure 4


Genetic and epigenetic control
Cannibalism
Low cell cycling
Autophagy
Reduced metabolism
Control of the local microenvironment
persiter cells
Stem-like cell properties
Bystander property
Immunosuppression
Drug resistance