*Review*

# Evolutionary Oncology: Non Genetic Mechanisms of Ancestral Origin Involved in Cancer Drug Resistance

**Vladimir F. Niculescu**

Kirschenweg 1, Diedorf 86420, Germany; vladimir.niculescu@yahoo.com

**Abstract**

From the perspective of evolutionary oncology, cancer represents a unicellularized cell system consisting of a self-renewing, oxygen-sensitive stemgermline and an oxygen-resistant somatic tumor bulk cell population generated through germ-to-soma transition processes and somatic proliferation. In this configuration, cancer shows limited analogy to the multicellular organization of its host but displays deep evolutionary homology with the unicellular systems of parasitic amoebae, reflecting its origin in the common ancestor of Amoebozoans and Metazoans. The hypoxic stemgermline is the central driver of cancer's cell system. Because its genome is highly vulnerable to hyperoxia, it requires continuous protection and, when necessary, replacement through reconstituted sublines. The non-genetic mechanisms underlying cancer drug resistance are interpreted here as ancestral survival programs that originated in the common ancestor approximately one billion years ago during two major evolutionary oxygen transitions. These programs are reactivated in the unicellularized cancer system to protect genome integrity under environmental and therapeutic stress. Over evolutionary time, these survival mechanisms evolved into highly robust and resilient networks, which may explain the remarkable persistence of the parasitic cancer cell system despite therapeutic intervention. Survival is maintained through coordinated programs of genome reconstruction and cycles of cellular plasticity that are activated when either the stemgermline or the somatic tumor population is exposed to intra-tumoral stressors or anticancer therapies. By placing cancer drug resistance within this deep evolutionary framework, evolutionary oncology offers a new conceptual basis for understanding cancer resilience and may guide the development of future therapeutic strategies aimed at targeting these ancient survival programs.

**Keywords:** cancer; evolution; entamoeba; epigenetic protection; epigenetic wraps; desensitization

## 1. Introduction

The present work analyzes the current understanding of cancer drug resistance from the perspective of evolutionary cancer cell biology (ECCB) and argues that the non-genetic mechanisms involved in cancer drug resistance can be traced back to ancestral genome-protection programs evolved by the common ancestor of amoebozoans, metazoans, and fungi (AMF) many millions of years ago (Mya), during the early oxygen enrichment of the world's ocean.

In recent years, it has become increasingly clear that the cancer cell system is not a diseased or dysfunctional multicellular program, but rather a reactivated, unicellularized biological system derived from ancestral genome compartments, whose organization, regulation, and evolutionary logic are fundamentally distinct from those of the multicellular host organism. (Nic 1–4). This conceptual shift has made it possible to resolve many previously unanswered questions in cancer biology and to explain the profound differences between the biology of cancer cells and the biology of the multicellular host cell system, including the stem cell complex. Unicellular and unicellularized cancer cell systems follow their own rules and rely on ancestral mechanisms inherited from the common AMF ancestor, many of which are no longer present or active in multicellular organisms.

### *1.1. The Ancestral Germ and Soma Concept*

Evolutionary cancer cell biology ECCB does not support a close relationship between the highly evolved, multipotent stem cells of multicellular organisms and humans and the primitive cancer stem cells (CSCs). Instead, CSCs are evolutionarily much closer to the Ur-germline (stemgermline) of the common AMF ancestor than to multipotent human stem (HSCs). HSCs are characterized by extensive differentiation capacity and function as central regulators of tissue and organ development. In contrast, CSCs represent primitive, largely unipotent stem cells with little differentiation capacity.

In the evolutionarily related cell system of parasitic amoebae, committed stem cells differentiate into cysts capable of polyploidization, genomic amplification, and the generation of stemgermline precursor cells. In cancer, committed cancer stem cells (com-CSCs) give rise to a homologous cellular structure with comparable functional capacity, but without the formation of a cyst wall.

Because of its evolutionary origin and its system-controlling role, the stemgermline constitutes a fundamental biological unit that is critically involved in cancer drug resistance. Similar to the Urgermline of the AMF ancestor, the cancer stemgermline consists of a strongly hypoxic self-renewing germ line with high sensitivity to oxygen excess and tumor-associated stressors. Hyperoxic conditions about 2-3% $O^2$ irreversibly damage the oxygen sensitive parts of the stemgermline genome.

Historically, this problem was resolved at the onset of the era of increasing environmental oxygenation, when the AMF cell system evolved a second, oxygen-resistant cell lineage (the soma). This lineage functionally inactivates the sensitive stemgermline genome and protectes it through an epigenetic shielding mechanism. From this evolutionary innovation emerged an autonomously *developmental program* that remains active in dual germ and soma cell systems such as cancer and protists.

### *1.2. Tumors and Oxygen Gradients*

Tumors consist of a dual-structured ancestral cellular system comprising a central, oxygen-sensitive stemgermline that accounts for approximately 1–2% of the total tumor cell population and is localized within the tumor core, and an oxygen-resistant somatic cell population representing roughly 98% of the tumor mass (bulk tumor cells). This somatic compartment surrounds and functionally supports the stem–germline population. Importantly, bulk tumor cells *are not differentiation products* of the stemgermline; instead, they represent the somatic lineage of the cancer cell system that arises naturally through a germ-to-soma transition process (GST) The bulk tumor mass develops due to its high proliferative capacity and not through differentiation from self-renewing stemgermline cells.

The somatic compartment protects the oxygen-sensitive stemgermline genome in two complementary ways. First, somatic tumor cells actively consume oxygen within the tumor microenvironment, thereby maintaining the hypoxic conditions required for full functionality of the core stemgermline system. Second, the somatic cells harbor an epigenetically inactivated stemgermline genome and serves thereby as a protected stemgermline genome shielded from extrinsic oxygen and intrinsic oxygen-derived stressors.

Schematically, the tumor can be represented as a structure composed of two concentric compartments: a hypoxic core and a multilayered hyperoxic periphery with varying oxygen content (Figure 1). Angiogenic processes establish a dynamic oxygen gradient and promote extensive cellular trouble within the tumor. As somatic bulk tumor cells are unable to consume all available oxygen immediately, excess oxygen diffuses inward from the periphery toward the hypoxic core. This oxygen excess can irreversibly damage the genome of stemgermline cells, leading to dysfunction. In response, somatic bulk tumor cells initiate reverse soma-to-germ transition (SGT) processes, generating intact replacement stemgermline clones and sublines that restore functionality within the hypoxic core compartment.

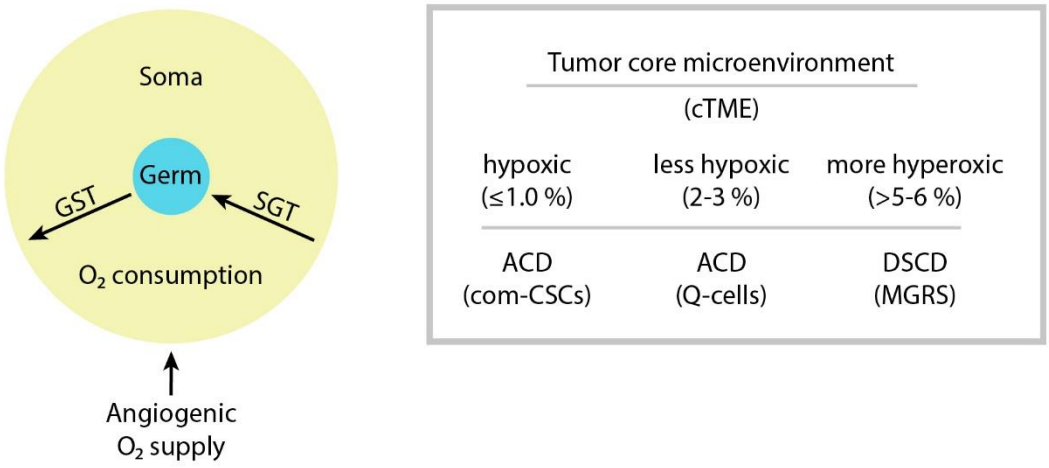


**Figure 1.** Intratumoral oxygen gradient and hypoxic versus hyperoxic core living conditions. The intratumoral oxygen gradient results from the balance between oxygen supply through angiogenic processes and oxygen consumption by the peripheral somatic bulk tumor layer (*yellow*). Adequate oxygen consumption maintains hypoxic conditions within tumor niches (core), which support proliferation of the stemgermline (*blue*) in a self-renewing mode, accompanied by differentiation into committed cancer stem cells (com-CSCs) or by non-differentiation leading to the formation of quiescent (Q) cells. Reduced oxygen consumption leads to hyperoxic conditions in the tumor core. Under these conditions, stemgermline cells proliferate in a DSCD mode and reconstruct dysfunctional genomes through activation of DNA damage response (DDR) circuits and the MGRS genome repair pathway. GST, germ-to-soma transition; SGT, soma-to-germ transition (EMT), DSCD, dysfunctional cells, which proliferate through symmetric cell division **Ch+.**

Conversely, when somatic bulk tumor cells at the tumor periphery are damaged by chronic intrinsic stressors or intensive chemotherapy, functional stemgermline cells within the inner compartment are rapidly signaled. Upon receiving these signals, stemgermline cells activate an extended GST program that *desensitize and protect all the sensitive genome sites* and generates a more resistant bulk-cell phenotype that are no longer susceptible to environmental stressors or chemotherapeutic agents. These resistant cells migrate as somatic replacement sublines toward the tumor periphery, where they establish a drug- and stressor-resistant boundary.

### *1.3. The Life Cycle of Cancer Stemgermline*

Evolutionary cancer cell biology (ECCB) redefines cancer stem cell populations within a system-centered framework. Rather than viewing cancer stem cells (CSCs) as a cell population in which each cell possesses both self-renewal and differentiation capacity, ECCB distinguishes between two fundamentally different functional entities: (i) self-renewing CSC lineages (stemgermlines) that lack differentiation capacity, and (ii) differentiated, committed CSCs (com-CSCs) that lack proliferative capacity.

Stemgermlines represent the principal evolutionary drivers of cancer progression. They form self-renewing lineages capable of indefinite propagation through asymmetric cell division (ACD). However, ACD does not expand the stemgermline pool; rather, it maintains a constant number of self-renewing stemgermline cells. In contrast, non-proliferating committed com-CSCs retain the capacity to expand through cyst-like cycles of polyploidization and depolyploidization. This process generates progenitors that form new sublines, thereby expanding the pool of self-renewing cells.

The life cycle of the stemgermline comprises multiple phenotypic states, including: (i) the strongly hypoxic ACD phenotype, which proliferates via asymmetric division and generates com-CSCs under severe hypoxia; (ii) a less hypoxic ACD phenotype, which produces non-committed

quiescent (Q) cells; and (iii) the defective symmetric cell division (DSCD) phenotype, which has lost functional stemness and proliferates through aberrant symmetric division.

Dysfunctional DSCD cells, having lost stemness, can activate a characteristic extended DNA damage response (DDR) program. Multiple DSCD cells may fuse to form a hyperpolyploid multinucleated genome-repair syncytium (MGRS), within which genome reconstruction occurs. The progeny generated from this structure (MGRS-derived buds or spores) give rise to replacement stemgermlines. These spores are stemgermline progenitors. In this way, stemgermline continuity is restored through regulated repair cycles. The exended DDR, including the hyperpolyploid MGRS pathway reintroduces the functionality of the germline genome (Nic 1-4).

This stemgermline -centered cancer cell system, which assigns the stemgermline a central evolutionary role, contrasts with the prevailing dualistic CSC model. By distinguishing functional stemgermline states from their committed derivatives, ECCB provides a more coherent framework for understanding cancer evolution, plasticity, and therapeutic resistance.

**Table 1. The cancer stem cell family** concept reflects the architecture of an ancestral unicellular system that evolved approximately one billion years ago in the common AMF ancestor (1000 Mya). Most of the stemgermline phenotypes represent stages within extended DDR circuits and germline repair cycles. Importantly, oxygen concentration critically regulates germ-to-soma transitions. When angiogenesis modestly increases intratumoral oxygen levels beyond ~2% $O_2$, stemgermlines lose their ability to differentiate com-CSCs. Excess oxygen levels approaching 5.7–6.0% $O_2$ irreversibly damage the stemgermline, leading to stemness-negative DSCD phenotypes. These, in turn, activate unicellular DDR circuitry and genome-reconstruction programs (MGRS) or PGCC (polyploid giant cancer cells) pathways, ultimately restoring genomic integrity in replacement stemgermlines, sublines and clones.

| Stemgermline phenotypes | Description |
|---|---|
| Strong-hypoxic productive ACD phenotype (≤1.0% $O_2$). | Capable of self-renewal and generation of com-CSCs under severe hypoxia. It represents the fundamental state of the stemgermline. |
| Non-proliferating com-CSCs | Unipotent cells capable of forming cyst-like polyploid structures that accumulate progenitors for new stemgermlines; primary com-CSCs retain tissue-specific profiles derived from their DSCD precursors |
| Q-cells | Intermediate germline phenotypes capable of either differentiating into com-CSCs or reverting to self-renewing ACD states |
| DSCD phenotypes | Dysfunctional, stemness negative phenotypes that proliferate through |

| | |
|---|---|
| | **defective symmetric cell division. They activate DDR and genome-reconstruction pathways, forming hyperpolyploid multinucleated genome-repair syncytia (MGRS).** |
| **Hyperpolyploid MGRSs** | **Stemness positiv giant cells - in cancer termed polyploid giant cancer cells (PGCCs). They have hyperpolyploid giant nuclei capable to reconstruct the functional stemgermline genome integrity** |
| **Spores or buds** | **Stemness positive progenitors generated via reductive division of giant MGRS nuclei** |

*1.4. Germ to Soma and Soma-to-Germ Plasticity (GST-SGT Cycles)*

Thus, tumor organization is characterized by mutual support and reciprocal protection between the inner and outer compartments mediated by complementary GST and SGT programs, in cancer better known as MET and EMT plasticity. Somatic cell populations, such as bulk tumor cells in cancer and trophozoite populations in parasitic amoebae, do not arise through classical differentiation from self-renewing stemgermline cells, but rather through germ-to-soma transition (GST). This process is more accurately defined as cell conversion rather than differentiation.

When cells of the hypoxic core become dysfunctional, bulk tumor cells compensate by generating replacement stemgermline clones. In this way, new stemgermline cells and replacement sublines can be generated through reverse soma-to-germ transition, highlighting the bidirectional plasticity of these cellular systems. Conversely, when peripheral bulk cells are damaged or eliminated, stemgermline cells generate new, increasingly resistant bulk-cell layers that reinforce protection of hypoxic stemgermline genome compartment.

All of these intratumoral dynamics are driven by non-genetic mechanisms inherited from the AMF ancestor and reveal a deep homology between the cancer cell system and its unicellular evolutionary predecessors. In the following sections, the ancestral mechanisms underlying cancer drug resistance are discussed in detail. This paper first reviews contemporary, non-evolutionary cancer research on drug resistance and subsequently reinterprets these findings within an evolutionary framework.

## 2. Conventional Cancer Research

*2.1.. Pre-Existing and Acquired Drug Resistance*

In contemporary cancer research, the term *drug resistance* refers to the capacity of malignant cells to become less responsive or refractory to chemotherapeutic agents, a phenomenon observed across

most cancer types and a major contributor to treatment failure and poor clinical outcomes. Accordingly, drug resistance arises through multiple mechanisms, including alterations in the tumor microenvironment, genetic mutations, changes in drug targets or receptors, and increased drug efflux capacity [5]

Drug resistance is generally described as a highly complex and multifactorial process involving numerous interacting determinants, which may function as inducers, drivers, or effectors of resistance [6] and lead to desensitization to chemotherapy. According to the researchers, these determinants include: (i) genetic diversity, (ii) mutations of drug targets, (iii) oncogene amplification, (iv) compensatory bypass signaling pathways, (v) epigenetic alterations that increase intratumoral heterogeneity, (vi) tumor cell plasticity, (vii) activation of the DDR pathways, (viii) epithelial–mesenchymal transition, (ix) tumor microenviron-mental influences, (x) cellular reprogramming, and (xi) genes directly implicated in the development of drug resistance [6–12]

More recently, drug resistance research has increasingly focused on identifying and targeting specific molecules and proteins that mediate resistance. Pharmaceutical strategies now emphasize the development of targeted inhibitors designed to counteract drug and multidrug resistance mechanisms and to overcome intrinsic or therapy-induced resistance to cancer cell death.

### *2.11. Pre- Treatment Resistance and Treatment- Induced Resistance*

Conventional cancer research classifies cancer drug resistance into intrinsic (primary) resistance and acquired (secondary) resistance [6–12] (Figure 2). In addition, Bell and Gilan further distinguish between reversible and irreversible drug resistance, as well as non-mitotic drug persistence [7]. Intrinsic (primary) drug resistance reflects pre-existing stress response mechanisms in untreated primary tumors, whereby intrinsic stressors exert effects comparable to those induced by endogenous tumor agents. Both stress conditions activate innate cellular defense programs against toxic insults, rendering tumor cells partially or fully refractory to subsequent chemotherapy.

By contrast, acquired (secondary) drug resistance develops during or after treatment as an adaptive response of the entire tumor cell population to therapeutic pressure therapy. It represents a progressive transition from an initially drug-sensitive state to therapy and is associated with the loss of treatment efficacy and clinical responsiveness [13–16]

In 2024, Khan *et al.* [9] proposed that cancer therapy resistance arises from a complex interplay between intrinsic (innate) and acquired (extrinsic) resistance According to this model, intracellular defense pathways acting in concert with oncogenic signaling networks can promote therapeutic desensitization and confer resistance. These coordinated responses may modify drug targets, enhance DNA repair capacity, and activate survival pathways, thereby enabling cancer cells to evade treatment-induced cytotoxicity [15]. Among the mechanisms conferring resistance, the authors highlight multiple oncogenic pathways, including (i) alterations in drug targets, (ii) enhanced DNA repair capacity, and the(iii) activation of survival pathways, which collectively enable cancer cells to evade the cytotoxic effects of therapy [15]

### *2.12. Loss of Responsiveness to Drugs and Tumor Drug Resistance*

Accordingly, reduced drug responsiveness (desensitization) [8] is understood to result from the combined actions of genetic, nongenetic, and environmental factors. Most commonly, resistance is attributed to a conglomerate of genetic diversity, acquired mutations in drug targets, oncogene amplification within compensatory or bypass signaling pathways, and epigenetic modifications. Together, these processes further shape intra-tumoral heterogeneity, tumor cell plasticity, DNA repair capacity, and susceptibility to cell death pathways, ultimately giving rise to multifactorial drug resistance [17–20]

Extrinsic tumor factors primarily include components of the tumor microenvironment (TME) that actively contribute to the ability of cancer cells to evade the cytotoxic effects of anticancer therapies [21,22]. These components encompass a wide range of TME elements, including alterations in the extracellular matrix (ECM), tumor-associated stromal cells, growth factors,

extracellular vesicles, immune cells, and cancer-associated fibroblasts (CAFs). Collectively, these factors play critical roles in tumor growth, metastasis, and resistance to cancer therapy by secreting diverse growth- and survival-promoting signals and by modulating therapeutic accessibility and immune surveillance [23,24]

### *2.13. Epigenetic Regulation and DNA Damage Repair*

As reported by Li et al (2025) [6] epigenetic regulation and transcriptional reprogramming play central roles in the emergence and modulation of cancer drug resistance [25,26]. Accordingly, drug resistance is closely associated with two interconnected processes: (i) epigenetic regulation, including DNA methylation and histone modification, which alter chromatin accessibility and gene expression, thereby enabling transcriptional plasticity; and (ii) transcriptional reprogramming, defined as the dynamic reorganization of transcription factor activity and regulatory gene networks in response to therapeutic and environmental stress. Together, these mechanisms allow tumor cells to establish stable yet adaptable gene-expression programs that sustain drug resistance and promote survival under selective pressure [27–32]

Histone modifications likewise play a critical role in the regulation of DNA repair pathways and in the development of acquired cancer resistance. Epigenetic alterations favor the DNA damage repair in cancer cells [33–35] while DNA hypermethylation has been shown to increase the accumulation of somatic mutations [36,37]. Epigenetically changed gene expression and transcription, can persist for multiple cell divisions that eventually develop nongenetic heterogeneity and drug non-responsiveness [34]

Consistent with this view, Lei et al. [8] proposed that epigenetic regulatory mechanisms can suppress the expression of DNA repair genes, thereby directly contributing to cancer drug resistance. This supports the idea of a functional interplay between DNA methylation, genome maintenance pathways, and therapeutic resistance, and suggests that targeting aberrant epigenetic regulation—particularly DNA methylation—may represent a promising strategy for overcoming drug resistance in cancer.

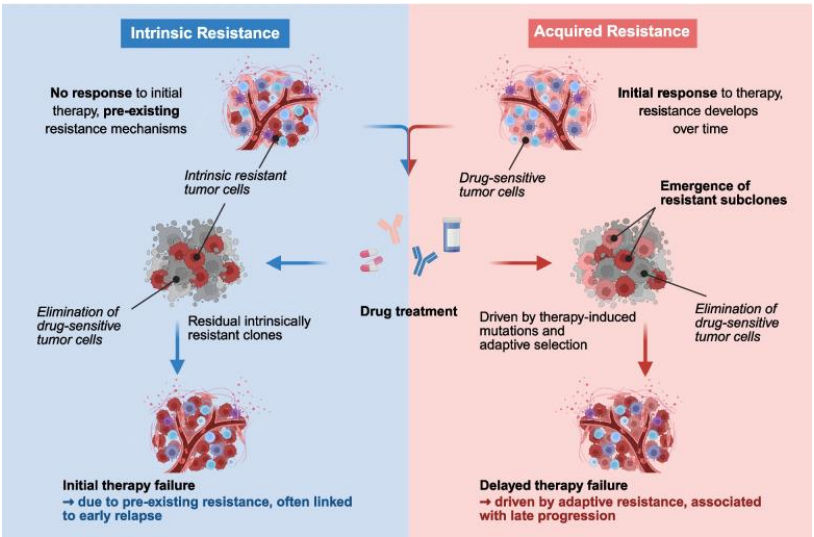


**Figure 2. Pre-existing and acquired drug resistance** as considered by Li J et al. **[6] (**Creative Commons Attribution 4.0 ).

### *2.2. Reversible and Irreversible Drug Resistance*

In a 2020 work, Bell and Gilan [7] described several heterogeneous cancer cell phenotypes with varying resistance potentials, ranging from reversible drug tolerance to stable, irreversible resistance as follows:

*2.21. Drug-Tolerant Phenotypes (DTPs)*

Drug-tolerant persistent (DTP) cells are genetically indistinguishable from the bulk tumor population, and their resistance is reversible upon drug withdrawal. These cells typically arise at low frequencies within tumor cell populations and are characterized by reduced proliferative capacity and altered metabolic states, which together would confer tolerance to therapeutic stress. Because of these properties, DTP cells are widely considered critical precursors of stable drug resistance [38–40]

*2.22. Unstable and Stable Drug Resistance*

Beyond transient persistence, cancer cells that acquire epigenetically mediated drug resistance that remains reversible and reverts to drug sensitivity following interruption of therapy [41,42]. Such phenotypic plasticity reflects dynamic state transitions that depend on the presence or absence of chemotherapeutic agents.

According to these researchers, unstable and stable resistance represent transient states along a resistance continuum. DTP cells and temporarily resistant phenotypes can progress toward stable resistance, thereby serving as reservoirs from which durable genetic or non-genetic resistance mechanisms may emerge [38,43]. In many cases, therapeutic withdrawal leads to partial or complete re-sensitization; however, such reversibility can be suppressed by cyclic or adaptive treatment regimens [41]

In contrast, sustained therapeutic pressure promotes the emergence of mitotically active, stably resistant phenotypes that persist independently of drug presence and display long-term resistance stability [44–48]. These stable resistance states are typically associated with fixed genetic alterations or heritable epigenetic programs that lock tumor cells into an irreversible drug-refractory phenotype

According to several researchers, the epigenetic mechanisms underlying drug resistance remain largely unknown and require further investigation [49,50]. There is currently no precise explanation for why reprogramming into resistant phenotypes occurs [7,45–55]. Earlier studies on drug resistance described the stable resistant phenotype as the final stage of an innate chemoresistance program that exists prior to chemotherapy. Subsequent treatment then selects pre-existing stable resistance clones from the heterogeneous intra-tumoral population; These clones survive therapy because they evade apoptosis and cannot be effectively eliminated [7]

*2.23. Multidrug Resistance*

As considered by Vaidya FU et al. [33], multidrug resistance (MDR) is a complex phenomenon involving multiple intricate pathways through which malignant cells become insensitive to various drugs and acquire the ability to survive repeated exposure to anticancer therapies. This includes the development of non-genetic drug resistance mechanisms.

According to Alhazza et al. [56] MDR includes noncellular mechanisms and overactivation of compensatory cellular pathways, ranging from inhibition of apoptosis to DNA damage repair [57]. Such adaptive responses increased DNA damage repair capacity and epigenetic reprogramming [58]

Persistent activation of DNA damage response (DDR) pathways plays a pivotal role in MDR by enabling tumor cells to repair therapy-induced DNA damage and survive under selective pressure [56,59]. Consequently, targeting DDR components could represent a promising strategy to overcome MDR.

*2.3.. Presumed Ancestral Origin of Drug Resistance Mechanisms*

Recently, several relevant non-evolutionary review articles support the evolutionary perspective, although without acknowledging unicellularization or the ancient origin of the cancer cell system [26,33,60]

These authors questioned whether the classical Darwinian model of evolution [52,60–63] - based on rigid, irreversible phenotypes caused by genetic alterations and natural selection - adequately explains cancer drug resistance. Instead, they proposed that drug-resistant cancer cells frequently

exhibit reversible phenotypes and involve non-Darwinian factors interconnected with phenotypic plasticity and epigenetic memory. They further emphasized the role of a *stressful microenvironment* capable of acclimating cancer cells to dynamic adaptive processes.

According to Menon et al. [26], *epigenetic regulation* modifies gene expression through mechanisms such as DNA methylation and histone modifications. Epigenetic modulation plays a central role in phenotypic plasticity and determines cell fate in multiple transition processes, including germ–soma transitions (evolutionary GST, SGT) as well as the induction and maintenance of senescence. Patterns of gene expression and epigenetic memory contribute to stabilizing cellular states or enabling transitions between them. These mechanisms are also crucial for the development and maintenance of cancer subpopulations with stem-like characteristics, including high tumor growth potential, drug resistance, and intratumoral heterogeneity [50,64]

The researchers argue that *epigenetic memory* plays an important role in stabilizing cellular states or enabling transitions from one state to another [65,66]. Compared with the bulk tumor population, these subpopulations that ar capable capable of transition are likely to exist in a dynamic state, characterized by continuous phenotype switching.

However, from a ECCB perspective, phenotypic plasticity, stemness plasticity, and epigenetic memory represent fundamental principles of evolutionary cancer cell biology. In this framework, these cancer cell hallmarks do not arise ad hoc within tumor cells but originate from the common AMF ancestor and its ancestral unicellular regulatory system

This evolutionary interpretation also applies to the *stress-induced premature senescence* (*SIPS*) phenotype [67–72], which is analogous to the defective symmetric cell division (DSCD) phenotype described in ECCB. Both SIPS and DSCD are stress-induced, senescence-like states triggered by intrinsic and extrinsic factors such as oncogenic stress, ionizing radiation, or hyperoxia. These ancestral programs activate the DNA damage response (DDR), enabling genome reconstruction, stemness recovery, and the generation of replacement stemgermlines.

The seemingly paradoxical correlation between *senescence and stemness* [48] is therefore not contradictory. Therapy-induced senescence can indeed promote cancer stemness through cellular reprogramming, as previously proposed, but this occurs within the framework of the DDR process. This phenomenon is not exclusive to cancer cells and its universality derived from the common AMF ancestor and its dual unicellular cell cycle, in which epigenetic modulation emerged as a central mechanism governing phenotypic plasticity and cell fate.

Finally, in 2021, Shlyakhtina et al. [73] proposed that non-genetic heterogeneity plays a critical role in shaping the evolutionary trajectory of cancer cell populations. This view is supported by multiple studies demonstrating that (i) cancers display extensive non-genetic heterogeneity, often manifested as phenotypic variability, and (ii) genetically identical subclones may adopt distinct and relatively stable phenotypic states that respond very differently to environmental stimuli [74–84]

In contrast, the author of the present work agrees that stress-induced phenotypic plasticity and epigenetic wrapping are primordial player in cancer drug resistance and significantly contributes to cell reprogramming. Likewise, strategies targeting phenotypic plasticity, epigenetic regulation, and metabolic adaptation represent promising therapeutic approaches.

## 3. Evolutionary Oncology: Drug Resistance Reactivates Survival Mechanisms of Ancestral Cell Systems

This work in evolutionary oncology provides a broader interpretative framework for previously reported findings on cancer drug resistance and their evolutionary origins, and explains why these ancestral non-genetic mechanisms of cancer drug resistance are retained specifically in dual, a unicellular patterned cell systems of cancer and parasitic protists.

The present analysis aligns with recent calls within conventional cancer research for a more integrative understanding of the mechanisms underlying cancer drug resistance and their cooperative interactions [8]

In this context, evolutionary cancer cell biology (ECCB) [1–4] offers a unifying framework in which cancer drug resistance is understood as the coordinated output of ancestral genome-protection and damage-prevention networks. These systems function to minimize DNA double-strand break (DNA DSB) formation, safeguard genomic integrity, and sustain stemness-related capacities.

Moreover, ECCB analysis suggests that the cancer cell system is genomically structured into distinct functional sub-compartments, some of which are selectively shielded by epigenetically regulated protective envelopes. The activation of these ancestral protection programs is orchestrated through expanded DDR circuitry that integrates hyperpolyploid genome reconstruction and repair with coordinated programs of cellular plasticity and epigenetic reinforcement [1–4]

### *3.1. Cancer Cell Sensitivity to Stressors and Desensitization from the Perspective of the Evolutionary Cell Biology*

Cancer drug resistance (desensitization) can be interpreted as an evolutionary extension of ancient genome-protection and damage-prevention networks that originated in the common AMF ancestor and its strong hypoxic Ur-germline. From an evolutionary perspective, two ancient oxygenation events acted as key stressors that triggered the emergence of protective mechanisms shaping the coordinated genome-protection networks of unicellular and unicellularized cell systems [1–4]

***The first evolutionary oxygenation event*** - here designated the *α-moment*-imposed selective pressure that favored the emergence of an oxygen-resistant somatic ancestral lineage capable of shielding the hypoxic genome compartment from the damaging effects of elevated oxygen levels. This GST (germ-to-soma transition) process resulted in a *constitutive soma program* and generated a dual-cell system in which oxygen-resistant somatic cells shielded an epigenetically inactivated hypoxic genome compartment (the Ur-germline genome) from the deleterious effects of oxygenation.

During the transition from the hypoxic Ur-germline to the oxygen-adapted somatic line - conceptualized here as the germ-to-soma transition (GST) – oxygen-sensitive regions of the hypoxic Ur-germline genome became tightly packaged around histone proteins, resulting in the epigenetic silencing and inactivation of parts of the hypoxic genome that controlled stemness and self- renewal. In this way, DNA methylation ensures the preservation fundamental stemgermline hallmarks.

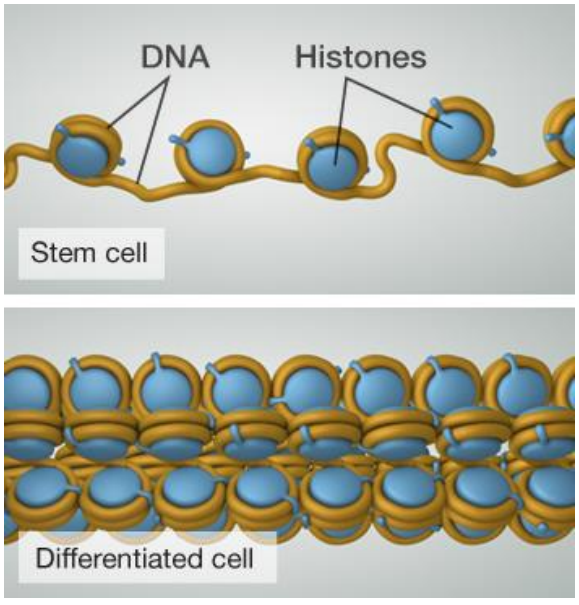


**Figure 3.** In stemgermline cells that are converted into oxygen-resistant somatic cells through the process of germ-to-soma transition (GST), DNA (gold) is loosely wrapped around histone proteins (blue). In somatic cells that do not exhibit stemness, the stem cell genome compartment is hidden and protected from hyperoxic stress.Reproduced from https:// learn.genetics.utah.edu/ content/stemcells/ips.

Such methylation-dependent mechanisms have also been implicated in therapy resistance across diverse treatment modalities [37]. Hypermethylation-mediated silencing of tumor suppressor genes, together with modulation of DNA damage response pathways, represents a recurrent evolutionary strategy for cellular adaptation to stress.

This epigenetic inactivation by wrapping - conceptualized here as the *α- protection shield* - protect the functionally inactivated vulnerable genomic loci of the hypoxic stemgermline genome during oxygenic life phases. This protective α-configuration ensured the long-term integrity and function of the hypoxic stemgermline genome.

***The second evolutionary oxygenic event*** - here designated the *ß-moment* - marked a critical evolutionary transition in which sustained increases in surrounding oxygen exposed residual vulnerabilities that were not safeguarded by the *α-protection shield*. These vulnerable sites – evident in the somatic cells exposed to chronic hyperoxia - became targets of intensified oxidative stress. The second oxygenic crisis necessitated expansion of the evolutionary epigenetic system into a more comprehensive *ß-protection program* capable of securing all sensitive genomic loci to ß-stressors and apoptotic death.

The cancer cell system appears to have retained both of these ancestral genome-protection programs. In conventional cancer research, activation of the ß- protective program is commonly described as *pre-treatment resistance* or intrinsic multidrug resistance—a condition in which tumors exhibit broad refractoriness to various therapeutic agents.

Historically, the roots of the cancer cell plasticity cycles (EMT/MET cycles) lie in the ancestral *α-* and *ß-GST* cycles that evolved millions of years ago, at the evolutionary turning points marked by the first and second oxygenic events as the increase in environmental oxygen destabilized the hypoxic stemgermline genome, abolishing differentiation and stemness functions through irreversible genomic damage and dysfunction of DNA repair systems (Nic 1,2).

### *3.2. The First Evolutionary Oxygen Crisis, the α-Protection Program and the Hallmarks of the Newly Constituted Soma*

The emergence of an additional oxygen-resistant soma lineage provided an adaptive solution to the first evolutionary crisis. This newly evolved soma was capable of shielding the hypoxic Ur-germline genome from irreversible oxygen-induced damage, thereby preventing genomic destabilization. During the GST process, the hypoxic genome compartment was transferred into the oxygenic somatic cell line in an epigenetically protected cell state. The protective epigenetic wrap, which originated in the first evolutionary oxygen crisis, is conceptualized here as the alpha *α protecting shield*.

Soma serves multiple objectives of the dual cancer cell system. First, it enabled more efficient utilization of tumoral resources, thereby supporting sustained symmetric proliferation and population expansion (bulk tumor cell proliferation). Second, it secured the hypoxic genome compartment during the transition to oxygenic life. Third, the oxygen-resistant soma functions as an effectively unlimited reservoir for the generation of replacement stemgermlines. And fourthly, when the damaged stemgermline signals irreparable DNA damage soma activated a restorative soma-to-germ transition producing progenitors for stemgermline replacement. Somatic cancer cells closed the ancestral GST/SGT (MET/EMT) cycle and ensured the evolutionary persistence of functional stemgermlines (Figure 4).

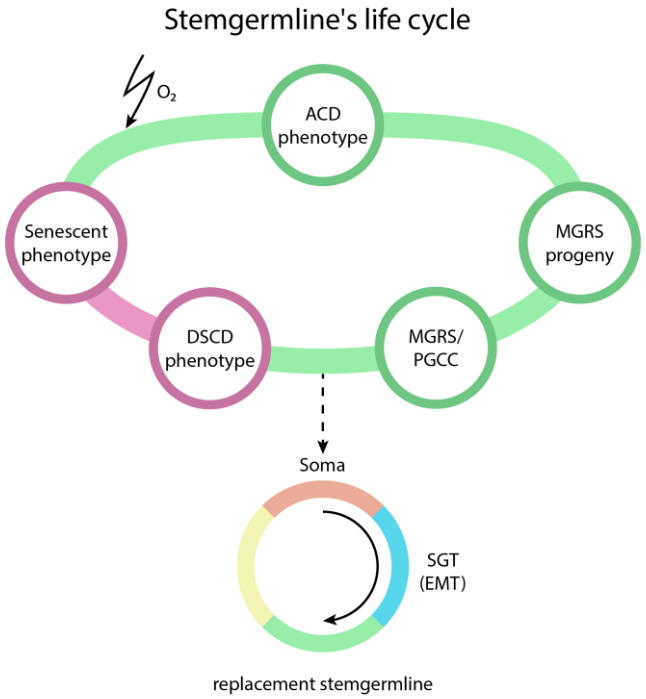


**Figure 4.** Stemgermline life cycle, genome reconstruction, and generation of replacement clones and sublines. Upper left: Hyperoxia-stressed ACD stemgermline cells undergo severe DNA damage (DNA DSB), enter senescence, and evade apoptosis (red). These amitotic, senescent cells persist and give rise to DSCD exiters, which proliferate via defective symmetric cell division (red). Genome repair is mediated through stemness-positive multinucleated genome repair structures (MGRS) and polyploid giant cancer cells (PGCCs), whose progeny serve as precursors for replacement clones and sublines (green). Lower panel: An additional soma-to-germ transition (SGT) pathway contributes to the generation of replacement stemgermlines. At the terminal stage of the SGT process, transiting cells (blue-to-green transition) undergo progenitor accumulation through cyst-like polyploidization, ultimately giving rise to new stemgermline lineages.

In cancer, the constitutive soma occurs from the nascent cancer stemgermline and not from mitotic cell differentiation. The constitutive GST program establishes a *somatic phenotype* that secured the primordial functions of the stemgermline - stemness and ACD potential - during the oxygenic life phase. However, this epigenetic $\alpha$-wrap did not encompass all sensitive genome compartments, leaving *much sensitive sites* unprotected and vulnerable to stressors. It produced merely a *state of partial genome protection:* while it effectively safeguarded the core functions of the stemgermline, somatic bulk tumor cells remain sensitive to chronic stress and chemical stressors such as drugs and chemotherapeutic agents.

### *3.21. DNA Damage Response and the Dual Strategy for Achieving Replacement Stemgermlines*

Both unicellular and unicellularized cell systems have evolved a dual strategy **to** cope with evolutionary *α-stressors***,** enabling the replacement of damaged stem–germline and/or somatic cell populations by newly generated stemgermline or somatic sublines. When stem–germline cells are genomically compromised by $\alpha$-stressors, specific molecular signaling pathways activate SGT processes. During this process, the intact, intra-somatically protected stemgermline genome is reactivated and generates replacement stemgermline cells capable of self-renewal and polyploid cyst-like differentiation. Conversely, when somatic sublines undergo apoptotic damage as a result of a $\alpha$-stressor activity, the remaining intact stemgermline cells generate new fully functional somatic clones and replacement sublines through *α-GST* processes (Figure 4).

In cancer, stemgermline cells located in the vicinity of newly formed capillaries may experience acute oxygenic stress. This hyperoxic exposure can compromise the stemgermline through severe DNA DSB damage. Such angiogenesis-associated damage (Figure 1) may have profound consequences. The stemgermline may become dysfunctional, aborting ACD proliferation and com-CSC differentiation. Under these circumstances, multiple molecular signaling pathways are activated, initiating a dual strategy for the development of replacement stemgermlines.

3.21.1. First Strategy: The Genome Reconstruction Program

The first DDR pathway represents an attempt by injured stemgermline cells to restore their dysfunctional genome through a hyperpolyploid genome-reconstruction program (Figure 4). Cancer stemgermline cells harboring irreversible DNA DSB damage may resist apoptotic death and continue proliferating as dysfunctional DSCD cells. These cells can undergo fusion and form multinucleated genome-repair structures MGRS' (PGCCs) (Figure 4).

Within hyperpolyploid MGRS syncytia, damaged genomic parts are selectively eliminated, reorganized, or reduced, while intact stemgermline genomic configurations are progressively reconstructed. The progeny emerging from these structures give rise to replacement stemgermline sublines. Thus, this first DDR pathway functions as a direct regenerative response of the damaged stemgermline compartment.

3.21.2.. Second Approach: The $\alpha$-STG Process

The second DDR pathway targets somatic tumor cells carrying the epigenetically inactivated stemgermline compartment (Figure 5). A subset of these somatic cells is stimulated to undergo *α-SGT* processes. During this transition, both the repressive epigenetic configuration as well as the accumulated somatic mutations (SM) are selectively eliminated, and the functional stemgermline genome is reactivated.

Progenitor cells resulting from by both DDR pathways generate replacement stemgermlines that assume the functional role of the damaged stemgermline. In this manner, the cancer cell system preserves its regenerative stemgermline core not only through direct genome reconstruction of the injured stemgermline genome, but also through activation of the protected germ genome embedded within soma cells.

*3.3. The Second Evolutionary Oxygen Crisis: ß- Stressors and Tumor Cell Desensitization*

Cancer drug sensitivity arises from multiple sensitive genomic loci that persist in somatic cells derived from *α-GST* processes. However, these vulnerabilities can be progressively overcome through recurrent *β-GST* processes and repeated epigenetic shielding (Figure 5), ultimately rendering somatic cells resistant to *ß-like stressors*, such as sustained, chronic hyperoxia and therapeutic agents.

In cancer, this evolutionary *ß-resistance* typically becomes detectable during the course of therapy and is accompanied by recurrent GST processes that expand the *ß-epigenetic protection shield*. In some tumors, however, *β-resistance* may also arise in the absence of therapy through chronic intrinsic stressors.

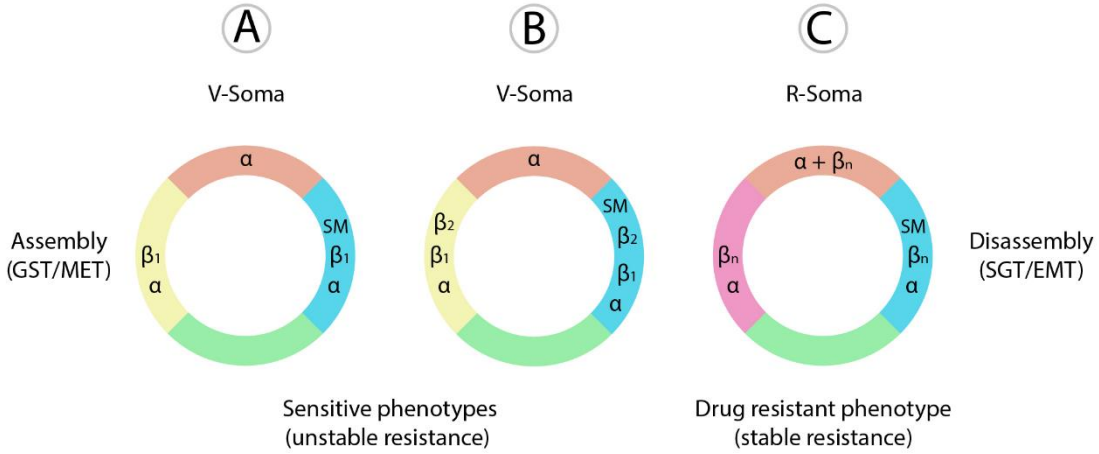


**Figure 5.** Reversible and irreversible somatic bulk tumor resistance Panels A and B illustrate reversible resistance phenotypes, whereas panel C represents an irreversible resistant state. V-soma denotes somatic cells with vulnerable genomic sites, while R-soma denotes resistant somatic cells capable of sustained proliferation under drug-resistant conditions. During SGT-mediated disassembly processes, α- and β-wrapping structures, as well as somatic mutations (SMs), are progressively removed, enabling phenotypic transitions between resistant somatic cells and new stemgermline clones. Optionally, following the dissolution process, an environment-dependent, hidden process of precursor accumulation may occur through cyst-like polyploidization and depolyploidization.

This evolutionary resistance mechanism, inherited from the common AMF ancestor, suggests that *ß-resistance* represents a specialized adaptive feature of early non-genetic evolution conferring a substantial survival advantage to the drug-resistant somatic cells. Corresponding *ß-GST* processes enabled the formation of somatic resistant clones and sublines long before cancer appeared as a pathological condition.

## 4. Reassessing Drug Resistance from an Evolutionary Perspective

When the findings of conventional cancer research on reversible and irreversible drug resistance [38–48] are considered in light of the previously analyzed α-protection program, four fundamental principles emerge:

(i) Drug desensitization unfolds progressively throughout recurrent *ß-GST* processes.

(ii) The desensitization process is completed only when somatic, *ß-GST*-derived bulk tumor cells reach an irreversible state of drug resistance;

(iii) Complete epigenetic protection against therapeutic agents generally occurs when all evolutionarily sensitive genomic regions are epigenetically silenced through *β-wrapping*.

(iv) along the trajectory toward this terminal resistant state, tumor cells pass through several intermediary configurations, that represent reversible, non-permanent states of drug resistance.

It becomes evident that tumor cell desensitization comprises a hierarchy of transitional substages. During extrinsically induced, recurrent *ß-GST* phenotypes must sequentially identify drug-sensitive genomic loci and epigenetically shield them. Only when *all* stress-sensitive loci are inactivated and epigenetically protected is the transition to a permanent resistant somatic phenotype completed. These fully protected somatic cells correspond to the resistant bulk tumor cell population observed clinically [38–48]

If, however, exposure to drugs or chemotherapeutic agents is too brief or insufficiently intense, the epigenetic *β-protection* remains incomplete and reversible. Under such conditions, intermediary phenotypes *($\beta_1$, $\beta_2$ … $\beta_n$)* emerge (Figure 5) that are neither fully desensitized nor permanently resistant. In cases of therapy withdrawal, partially protected *β-clones* may subsequently lose their epigenetic shielding and revert to drug-sensitive states or regress toward a germline-like condition.

In contrast, fully protected somatic cells that have achieved permanent and irreversible resistance retain this expanded protective configuration during subsequent mitotic proliferation and recurrent plasticity cycles. During reverse SGT processes, the protected genome compartments may be transiently dismantled. However, the resulting stemgermline clones and sublines preserve the once-activated full protection program as an epigenetic memory. Upon re-entry into a new GST process, this memory facilitates the rapid re-establishment of the complete protective configuration. Consequently, the derived somatic bulk tumor population remains stably resistant or becomes multidrug resistant.

This full epigenetic *ß-protection* enabled the emergence of desensitized somatic phenotypes that were resistant to oxygen and chemical stressors, including therapeutic agents. Unlike the earlier restricted This *protection* wraps on hypoxic stemness compartments, this ß-protection program ensures full genome protection and prevent genome destabilization occurring in conditions of, chronic, permanent, stress.

The unicellularized cancer cell system has adopted both ancestral genome-protection programs together with the corresponding GST/SGT cycles (Figure 5). While the first, restricted epigenetic protection program underlies repair-based survival through recurrent activation of DNA damage responses and replacement SGT processes, activation of the second, comprehensive epigenetic protection program gives rise to intrinsically resistant somatic clones and sublines that no longer depend on repeated repair cycles.

This framework indicates that cancer drug resistance is fundamentally rooted in evolutionarily conserved programs designed to preserve the genomic integrity and functional capacity of the cancer stemgermline. In conventional cancer research, activation of this second ancestral program is commonly described as intrinsic or pre-treatment multidrug resistance - a state against which a broad spectrum of therapeutic agents proves ineffective.

Significantly, the comprehensive protection program does not prevent the occurrence of somatic mutations. However, such mutations exert limited impact on long-term genomic integrity within the genome-preserving GST/SGT cycles. (Figure 5) During SGT processes, the epigenetic wrap structures characteristic of the resistant somatic phenotype is dismantled, and somatically acquired mutations are largely eliminated or rendered functionally irrelevant. The newly generated hypoxic replacement stemgermlines are thus restored to full functionality with respect to stemness, asymmetric cell division (ACD), and differentiation capacity. Subsequent GST processes re-establish the comprehensive epigenetic *β-wrap*, thereby reinstating resistance of the somatic progeny to environmental stressors and therapeutic agents.

## 5. $\alpha$- and ß-Phenotypes in Parasitic Amoebae

Parasitic amoebae such as *Entamoeba* provide compelling biological evidence that evolutionary $\alpha$- and β-genome protection programs operate beyond the cancer cell system. The parasitic life cycle of *Entamoeba* encompasses the very same phenotypes as the unicellular parasitic system of cancer, both in vivo and in vitro. The most significant difference between the two life cycles lies in how they form stemgermline precursor cells. In cancer, this occurs through a hidden, cyst-like polyploidization at the end of the SGT process. In contrast, *Entamoeba* exhibits a species-specific polyploid cyst that can interrupt the GST/SGT cycle for an extended period.

Different *Entamoeba* culture conditions characterized by varying oxygen gradients selectively favor either hypoxic stemgermline populations (formerly referred to as “Minuta”) or more oxygen-resistant somatic cell types (trophozoites, or “Magna”). Culture growth is regulated by oxygen gradients comparable to those found in host organs, blood, and tumor environments. Hypoxic growth conditions, achieved in sediments containing metabolically suppressed, oxygen-consuming bacteria (OCB) [84,85], promote the emergence of somatic $\alpha$-phenotypes. In contrast, synthetic, bacteria-free hyperoxic conditions (axenic cultures) favor the development of somatic hyperoxia-resistant β-phenotypes alongside the formation of defective stemgermline cells (DSCD phenotypes).

Somatic cells arise through the GST and not through differentiation processes from self-renewing stemgermline phenotypes. In vivo, GST cells can invade the intestinal mucosa and migrate via the bloodstream to more oxygenic host organs, particularly the liver, where they cause abscesses. During migration, the cells undergo the $\alpha$-transition. Hepatic *Entamoeba* cells are somatic cells that protect their hypoxic stemgermline genome with a suitable $\alpha$-wrap.

*Hepatic trophozoites* typically represent somatic $\alpha$-phenotypes that can proliferate extensively until host death. They are genomically homologous to unicellularized, drug-sensitive bulk tumor cells. These cells lose stemness and ACD potential, while their hypoxic stemgermline genome remains protected by $\alpha$-wrapping. Although hepatic trophozoites no longer form cysts, they represent an epigenetically stabilized $\alpha$-phenotype that preserves the capacity to regenerate stemgermline clones and sublines via reverse SGT processes.

Axenic trophozoites arise in synthetic, hyperoxic, bacteria-free cultures originally developed by Diamond (1961) [86]. Due to their simplicity and high yield of somatic cells, these cultures became standard reference systems for *Entamoeba*. However, this artificial environment imposes strong oxigenic stress. As a result, both somatic trophozoites and stressed, defective stemgermline cells (DSCD) fail to form cysts under hyperoxic culture conditions. Encystation can only be induced under extreme stress, typically when cells leave the culture environment and are exposed to hypoosmotic stress and nutrient deprivation.

Axenic trophozoites arise through the process of axenization, defined as the transfer of *Entamoeba* from the microbiota-rich intestinal environment into bacteria-free synthetic culture media. Adaptation to this extreme environmental shift and hyperoxic chronic stress is slow and demanding, typically requiring up to 30 days. This transition is enabled by extensive hyper-polyploidization and the emergence of multiple intermediary cell states [87,88]

Polyploidization and hyper-polyploidization are hallmarks of both parasitic unicellular systems and unicellularized cell systems in amoebae and cancer. These processes not only facilitate progenitor accumulation during the late stages of reverse SGT processes (Figure 6), but also restructuring and functional restoration of dysfunctional DSCD genomes into a functional stemgermline genome. (Figure 4)

Genome reconstruction proceeds in two sequential phases [3,89]. Following DSCD cell fusion and MGRS formation, each individual MGRS nucleus undergoes cyst-like polyploidization, reaching an amplification ratio of up to 1:16. This genetic amplification process is observed in both *protist- and cancer-associated MGRS* structures. In the second phase, the amplified but still dysfunctional genome copies are merged and reorganized into one or more hyper-polyploid giant repair nuclei. During genome reconstruction, DNA DSBs parts are resolved, and intact genomic regions are selectively assembled to generate a new, functionally competent stemgermline genome. MGRS were described in *Entamoeba* 150 years ago by Craig [90]

Under sustained hyperoxic stress, intestinal amoebae convert into a resistant somatic β-phenotype, homologous to drug-resistant somatic bulk tumor cells. In these synthetic culture conditions, chronic oxidative stress progressively eliminates genomic vulnerabilities through successive rounds of hyper-polyploidization, multiple culture passages [91,92], and multiple intermediary β-phenotypes, culminating in the establishment of a stable somatic β-phenotype that is homologous to the drug-resistant bulk tumor cells. Both *Entamoeba* and *tumor phenotypes* are thus shaped by chronic hyperoxia and persistent stress, leading to the stabilization of a β-genome program that is propagated to subsequent cell generations (Figure 6).

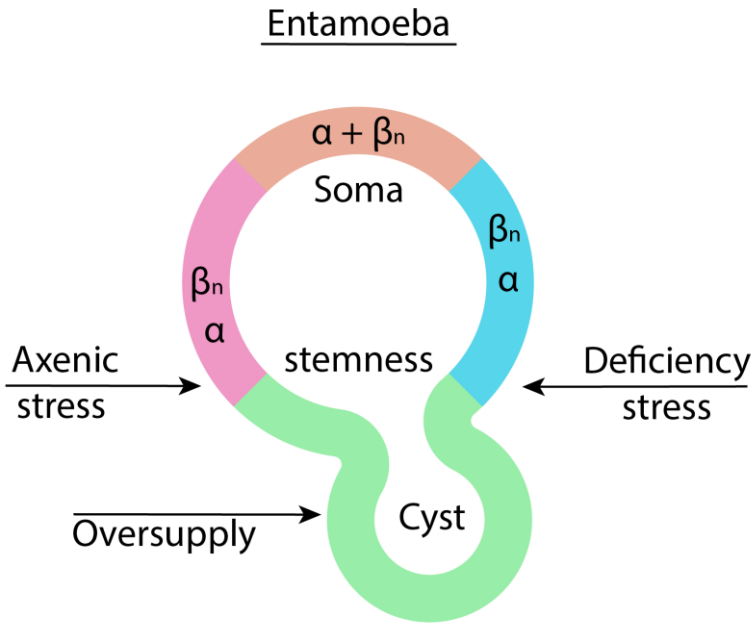


**Figure 6.** β- plasticity cycles in axenically grown somatic Entamoeba before and after exposure to encystment conditions. Upon transfer to encystment media (deficiency stress), soma phenotypes derived from axenic cultures interrupt proliferation and initiate soma-to-germ transition (SGT), followed by encystation, which promotes polyploidization, genome amplification, and stemness recovery. Cyst formation is essential for species survival and dissemination. Following uptake by secondary hosts or exposure to culture media, cysts encounter nutrient- rich conditions (oversupply) that trigger excystation and the release of stemgermline progenitors (amoebulae). These haploid progenitors are capable of re-establishing functional stemgermlines, which subsequently continue both α- and β-GST processes. [83,84,90,91].

It cannot be excluded that hepatic *Entamoeba* clones may undergo partial conversion toward β-like phenotypes. Notably, different stains of *Entamoeba* exhibit significant differences in terms of their pathogenicity and virulence; Whether these differences are related to the preventing genomic vulnerability through β-shielding processes remains to be determined.

## 6. Conclusions

Taken together, these observations indicate that the cellular organization and stress-response strategies of parasitic amoebae and cancer cells are not merely analogous but reflect deeply conserved evolutionary programs. The α- and β-phenotypes observed in *Entamoeba* likely represent modern manifestations of ancient genome-protection strategies that emerged during the two major evolutionary oxygen transitions (α- and β-events) in early eukaryotic history.

Within the ECCB framework, these programs correspond to distinct germ-to-soma transition modules that regulate genome protection, cellular plasticity, and adaptive survival under fluctuating oxygen conditions. The striking parallels between *Entamoeba* and cancer therefore suggest that the unicellularized cancer cell system [92] exploits ancestral survival programs originally evolved in the common ancestor of Amoebozoans and Metazoans. These programs, which combine genome reconstruction with reversible cell-state plasticity, may underlie the remarkable resilience of both parasitic amoebae and cancer cells to environmental and therapeutic stress.

This work in evolutionary oncology provides a broader interpretative framework for previously reported findings on cancer drug resistance and their evolutionary origins, and explains why these ancestral non-genetic mechanisms of cancer drug resistance are retained specifically in dual, unicellular patterned cell systems of cancer and parasitic protists. It demonstrates that the majority of non-genetic cancer mechanisms reflect conserved evolutionary programs that emerged in the

common AMF ancestor under selective pressures imposed by the first and second historical oxygen increases (the *α- and β- crises*), when the Urgermline of the AMF ancestor was exposed to rising oxygen levels.

Together, multiple reparative plasticity cycles, stemness loss and recovery dynamics, expanded DNA damage response (DDR) processes, and polyploidy–hyperpolyploidy cycles constitute a complex and highly resilient life cycle. This integrated system enables cells to withstand diverse stressors and therapeutic challenges. All of these processes are encoded within conserved compartments of the ancestral human genome.

The evolutionary plasticity programs, which can still be observed in cancer and parasitic protists today, function to protect the hypoxic stemgermline genome of both ancestral cell systems. They ensure that sensitive stemgermline genome compartments are inactivated under hyperoxic stress or drug exposure and preserved as epigenetically protected genomic reserves within the somatic bulk cancer cell population.

These mechanisms comprise: (i) the intrinsic *α-GST* program generating somatic bulk tumor cells; (ii) the extrinsic, induced *β-GST* program, which extends α-wrapping and identifies additional sensitive β-genome sites for epigenetic β-wrapping; (iii) the GST/SGT cycles—better known as MET/EMT cycles—where the SGT (EMT) process removes epigenetic wrapping and somatic mutations, thereby reactivating genomically intact replacement stemgermlines; and (iv) epigenetic memory mechanisms that maintain the *β-wrapping* configuration during proliferation of the somatically resistant population. *α-GST* and *β-GST* are distinct genome-protection programs that emerged during germ-to-soma transitions in response to the first (*α-)* and second (*β-)* oxygenic crises.

In cancer, all of these evolutionary processes are activated by hyperoxic damage to the hypoxic cancer genome compartments, which in turn initiates unicellular DDR circuitries of DNA damage repair involving MGRS/PGCC-mediated genome repair and reconstruction.

From a historical perspective, extrinsic environmental factors approximately 1.0 billion years ago (1Gya) initiated the evolution of the common AMF ancestor, its Urgermline, and the associated non-genetic mechanisms for genome protection, genomic damage prevention, and genome integrity maintenance.

From an evolutionary standpoint, cancer drug resistance represents a cellular state that arose long before the emergence of cancer in multicellular host organisms. The chemoresistance of dual cell systems—observed in both protists and cancer—reflects a universal principle of evolutionary cell biology.

Both forms of cancer drug resistance described in conventional research as intrinsic and extrinsic are rooted in ancestral chemoresistance mechanisms established during the second oxygen event (*β-crisis*). Cancer drug resistance represents a modern manifestation of this ancestral *β-resistance program*, which inactivates and epigenetically shields sensitive hypoxic genomic regions of the common ancestor as well as of cancer and protist genomes.

During *β-GST* (MET) processes, drug-triggered sensitive genomic sites are progressively identified, inactivated and epigenetically protected. This results in a series of intermediary β phenotypes *($\beta_1$- $\beta_2$- … $\beta_n$)* with variable resistance capacity. Only when *β-wrapping* is fully completed and no sensitive genomic sites remain unprotected is the stable somatically resistant phenotype achieved. If exposure to extrinsic stressors or chemotherapeutic agents is insufficient in duration or intensity, epigenetic remodeling remains incomplete, and intermediary GST phenotypes are neither fully desensitized nor irreversibly resistant.

These intermediary resistant phenotypes, described in conventional cancer research, may lose their incomplete epigenetic protection and remain vulnerable to subsequent stressors or therapies. It remains unclear whether such clones maintain their intermediary state over extended periods or revert to a germline-like state with reactivation of the ACD phenotype. (Figure 5)

In contrast, cells that achieve a fully established epigenomic protective shield (*$\beta_n$-phenotype*) retain this configuration during subsequent *$\beta_n$-SGT* cycles and somatic proliferation. They preserve molecular memory of the *β-program* and autonomously maintain *β-wrapping*.

Insights from evolutionary oncology indicate that future successful therapies must target the vulnerable nodes of these ancestral non-genetic mechanisms, including both senescent phenotypes and DDR circuitry and the GST/SGT plasticity cycles. Even more promising may be molecular strategies directed against the hypoxic nascent cancer stemgermline and the unicellularization process of the cell of origin, potentially including vaccine-based approaches. An evolutionary framework may therefore substantially enhance the effectiveness of future molecular cancer therapies.

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## References

1. Niculescu VF. From multicellular constraint to unicellular control: Ancient mechanisms of genome reconstruction, repair, and expansion in cancer evolution. Cancer Plus. 2025;7(3):42-72
2. Niculescu VF. Non-genetic mechanisms in cancer evolution: senescence, unicellularization, and cycles of stemness recovery. Academia Molecular Biology and Genomics 2025;2. https://doi.org/10.20935/AcadMolBioGen7755
3. Niculescu VF. Reevaluating cancer stem cells and polyploid giant cancer cells from the evolutionary cancer cell biology perspective. Cancer Plus. doi: 10.36922/cp.3970
4. Niculescu VF. Understanding cancer from an evolutionary perspective: high-risk reprogramming of genome-damaged stem cells. Academia Medicine 2024;1. https://doi.org/10.20935/AcadMed6168
5. https://www.sciencedirect.com/ topics/medicine-and-dentistry/drug-resistance-in-cancer)
6. Li J, Hu J, Yang Y, Zhang H, Liu Y, et al. Drug resistance in cancer: molecular mechanisms and emerging treatment strategies. *Mol Biomed*. 2025;6:111.
7. Bell CC, Gilan O. Principles and mechanisms of non-genetic resistance in cancer. *Br J Cancer*. 2020;122:465-472.
8. Lei ZN, Tian Q, Teng QX, Wurpe JND, Zeng L, et al. Understanding and targeting resistance mechanisms in cancer. *MedComm*. 2023;4:e265.
9. Khan SU, Fatima K, Aisha S, Malik F. Unveiling the mechanisms and challenges of cancer drug resistance. *Cell Commun Signal*. 2024;22:109.
10. Turajlic S, Furney SJ, Stamp G, Rana S, Ricken G et al. Whole-genome sequencing reveals complex mechanisms of intrinsic resistance to BRAF inhibition. *Ann Oncol*. 2014;25(5):959-967.
11. Ma Y, Wang L, Neitzel LR, Loganathan S, Tang L et al. The MAPK pathway regulates intrinsic resistance to BET inhibitors in colorectal cancer. *Clin Cancer Res*. 2017;23(8):2027-2037.
12. Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. *Nat Rev Immunol*. 2020;20(1):25-39.
13. Wicki A, Mandala M, Massi D, Taverna D, Tang H et al. Acquired resistance to clinical cancer therapy: a twist in physiological signaling. *Physiol Rev*. 2016;96(3):805-829.
14. Saleh R, Elkord E. Acquired resistance to cancer immunotherapy: role of tumor-mediated immunosuppression. *Adv Cancer Res*. 2020;13-27.
15. Meador CB, Hata AN. Acquired resistance to targeted therapies in NSCLC: updates and evolving insights. *Pharmacol Ther*. 2020;210:107522.
16. Lu J, Li J, Lin Z, Li H, Lou L, Ding W, et al. Reprogramming of TAMs via the STAT3/CD47-SIRP$\alpha$ axis promotes acquired resistance to EGFR-TKIs in lung cancer. *Cancer Lett*. 2023;564:216205.

17. Hu X, Zhang Z. Understanding the genetic mechanisms of cancer drug resistance using genomic approaches. *Trends Genet*. 2016;32(2):127-137.
18. Kara A, Ozgur A, Nalbantoglu S, Karadag A. DNA repair pathways and their roles in drug resistance for lung adenocarcinoma. *Mol Biol Rep*. 2021;48(4):3813-3825.
19. Damia G, Broggini M. Platinum resistance in ovarian cancer: role of DNA repair. *Cancers (Basel)*. 2019;11(1):119.
20. Dzobo K, Senthebane DA, Thomford NE, Rowe A et al. Not everyone fits the mold: intratumor and intertumor heterogeneity and innovative cancer drug design and development. *OMICS*. 2018;22(1):17-34.
21. Labrie M, Brugge JS, Mills GB, Zervantonakis IK.. Therapy resistance: opportunities created by adaptive responses to targeted therapies in cancer. *Nat Rev Cancer*. 2022;22(6):323-339.
22. Nussinov R, Tsai CJ, Jang H. Anticancer drug resistance: an update and perspective. *Drug Resist Updat*. 2021;59:100796.
23. Senthebane DA, Rowe A, Thomford NE, Shipanga H, Munro D, et al. The role of tumor microenvironment in chemoresistance: to survive, keep your enemies closer. *Int J Mol Sci*. 2017;18(7):1586.
24. Khan SU, Fatima K, Malik F, Kalkavan H, Wani A. Cancer metastasis: molecular mechanisms and clinical perspectives. *Pharmacol Ther*. 2023;250:108522.
25. Adhikari S, Bhattacharya A, Adhikary S, Singh V, Gadad SS, et al. The paradigm of drug resistance in cancer: an epigenetic perspective. *Biosci Rep*. 2022;42(4).
26. Menon DR, Hammerlindl H, Torrano J, Schaider H, Fujita M. Epigenetics and metabolism at the crossroads of stress-induced plasticity, stemness and therapeutic resistance in cancer. *Theranostics*. 2020;10(14):6261-6277.
27. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell*. 2012;150(1):12-27.
28. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell*. 2013;152(6):1237-1251.
29. Aydin B, Mazzoni EO. Cell reprogramming: the many roads to success. *Annu Rev Cell Dev Biol*. 2019;35:433-452.
30. Poetsch AR, Plass C. Transcriptional regulation by DNA methylation. *Cancer Treat Rev*. 2011;37(Suppl 1):S8-S12.
31. Zhao LY, Song J, Liu Y, Song CX, Yi C. Mapping the epigenetic modifications of DNA and RNA. *Protein Cell*. 2020;11(11):792-808.
32. Bushweller JH. Targeting transcription factors in cancer—from undruggable to reality. *Nat Rev Cancer*. 2019;19(11):611-624.
33. Vaidya FU, Chhipa AS, Mishra V, Gupta VK, Rawat SG, et al. Molecular and cellular paradigms of multidrug resistance in cancer. *Cancer Rep (Hoboken)*. 2022;5(12):e1291.
34. Wilting RH, Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. *Drug Resist Updat*. 2012;15(1-2):21-38.
35. Shah K, Rawal RM. Genetic and epigenetic modulation of drug resistance in cancer: challenges and opportunities. *Curr Drug Metab*. 2019;20(14):1114-1131.
36. Mazloumi Z, Farahzadi R, Rafat A, Asl KD, Karimipour M et al. Effect of aberrant DNA methylation on cancer stem cell properties. *Exp Mol Pathol*. 2022;125:104757.
37. Zhao A, Xu W, Han R, Wei J, Yu Q, et al. Role of histone modifications in neurogenesis and neurodegenerative disease development. *Ageing Res Rev*. 2024;98:102324.
38. Brock A, Chang H, Huang S. Non-genetic heterogeneity—a mutation-independent driving force for the somatic evolution of tumours. *Nat Rev Genet*. 2009;10:336-342.
39. Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med*. 2016;22:262-269.
40. Bai X, Fisher DE, Flaherty KT. Cell-state dynamics and therapeutic resistance in melanoma from the perspective of MITF and IFNγ pathways. *Nat Rev Clin Oncol*. 2019;16:549-562.
41. Knoechel B, Roderick JE, Williamson KE, Zhu J, Lohr JG, et al. An epigenetic mechanism of resistance to targeted therapy in T cell acute lymphoblastic leukemia. *Nat Genet*. 2014;46:364-370.

42. Sun C, Wang L, Huang S, Heynen GJJE, Prahallad A, et al. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. *Nature*. 2014;508:118-122.
43. Li S, Garrett-Bakelman FE, Chung SS, Sanders MA, Hricik T, et al. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. *Nat Med*. 2016;22:792-799.
44. Bell CC, Fennell KA, Chan YC, Rambow F; Yeung MM, et al. Targeting enhancer switching overcomes non-genetic drug resistance in acute myeloid leukaemia. *Nat Commun*. 2019;10:2723.
45. Shaffer SM, Dunagin MC, Torborg SR, Torre EA, Emert B, et al. Rare cell variability and drug-induced reprogramming as a mode of cancer drug resistance. *Nature*. 2017;546:431-435.
46. Kim C, Gao R, Sei E, Brandt R, Hartman J, et al. Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing. *Cell*. 2018;173:879-893.e13.
47. Sharma A, Cao EY, Kumar V, Zhang X, Leong HS, et al. Longitudinal single-cell RNA sequencing reveals drug-induced infidelity in stem cell hierarchy. *Nat Commun*. 2018;9:4931.
48. Milanovic M, Fan DNY, Belenki D, Dabritz HM, Zhao Z, et al. Senescence-associated reprogramming promotes cancer stemness. *Nature*. 2018;553:96-100.
49. Paksa A, Rajagopal J. The epigenetic basis of cellular plasticity. *Curr Opin Cell Biol*. 2017;49:116-122.
50. Wainwright EN, Scaffidi P. Epigenetics and cancer stem cells: unleashing, hijacking, and restricting cellular plasticity. *Trends Cancer*. 2017;3:372-386.
51. Pisco AO, Brock A, Zhou J, Moor A, Mojtahedi M, et al. Non-Darwinian dynamics in therapy-induced cancer drug resistance. *Nat Commun*. 2013;4:2467.
52. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell*. 2010;141:69-80.
53. Liau BB, Sievers C, Donohue LK, Gillespie SM, Flavahan WA, et al. Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. *Cell Stem Cell*. 2017;20:233-246.e7.
54. Fong CY, Gilan O, Lam EYN, Rubin AF, Ftouni S, et al. BET inhibitor resistance emerges from leukaemia stem cells. *Nature*. 2015;525:538-542.
55. Zawistowski JS, Bevill SM, Goulet DR, Stuhlmiller TJ, Beltran AS, et al. Enhancer remodeling during adaptive bypass to MEK inhibition is attenuated by targeting the P-TEFb complex. *Cancer Discov*. 2017;7:302-321.
56. Alhazza A, Oyegbesan A, Bousoik E, Montazeri Aliabadi H. Multidrug resistance: are we still afraid of the big bad wolf. *Pharmaceuticals (Basel)*. 2025;18:895.
57. Emran TB, Shahriar A, Mahmud AR, Rahman T, Abir MH, et al. Multidrug resistance in cancer: molecular mechanisms and therapeutic approaches. *Front Oncol*. 2022;12:891652.
58. O'Connor MJ. Targeting the DNA damage response in cancer. *Mol Cell*. 2015;60:547-560.
59. Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. *Science*. 2017;355:1152-1158.
60. Roesch A, Vultur A, Bogeski I, Wang H, Zimmermann KM, et al. Overcoming intrinsic multidrug resistance in melanoma by blocking the mitochondrial respiratory chain of slow-cycling JARID1B(high) cells. *Cancer Cell*; 2013;23:811-825.
61. Das Thakur M, Salangsang F, Landman AS, Seller WR, Pryer NK, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature*. 2013;494:251-255.
62. Becker A, Crombag L, Heideman DA, Thunissen FB, van Wijk AW, et al. Retreatment with erlotinib: regain of TKI sensitivity following a drug holiday for NSCLC. *Eur J Cancer*. 2011;47:2603-2606.
63. Mittal K, Derosa L, Albiges L, Wood L, Elson P, et al. Drug holiday in metastatic renal-cell carcinoma patients treated with VEGFR inhibitors. *Clin Genitourin Cancer*. 2018;16:e663-e667.
64. Snyder V, Reed-Newman TC, Arnold L, Thomas SM, Anant S. Cancer stem cell metabolism and potential therapeutic targets. *Front Oncol*. 2018;8:203.
65. Poljsak B, Milisav I. Clinical implications of cellular stress responses. *Bosn J Basic Med Sci*. 2012;12:122-126.
66. Etchegaray JP, Mostoslavsky R. Interplay between metabolism and epigenetics: a nuclear adaptation to environmental changes. *Mol Cell*. 2016;62:695-711.
67. de Magalhaes JP, Passos JF. Stress, cell senescence and organismal ageing. *Mech Ageing Dev*. 2018;170:2-9.
68. Takahashi A, Ohtani N, Yamakoshi K, Iida S, Tahara H, et al. Mitogenic signalling and the p16INK4a-Rb pathway cooperate to enforce irreversible cellular senescence. *Nat Cell Biol*. 2006;8:1291-1297.

69. Koch CM, Reck K, Shao K, et al. Pluripotent stem cells escape from senescence-associated DNA methylation changes. *Genome Res*. 2013;23:248-259.
70. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*. 1997;88:593-602.
71. Bielak-Zmijewska A, Wnuk M, Przybylska D, Grabowska W, Lewinska A et al. A comparison of replicative senescence and doxorubicin-induced premature senescence of vascular smooth muscle cells isolated from human aorta. *Biogerontology*.2014;15:47-64
72. Sakaki M, Ebihara Y, Okamura K, Nakabayashi K, Igarashi A, et al. DNA methylation in the initiation and establishment of replicative senescence. *PLoS One*. 2017;12:e0171431.
73. Shlyakhtina Y, Moran KL, Portal MM. Genetic and Non-Genetic Mechanisms Underlying Cancer Evolution. *Cancers* 2021; 13; 1380. https:// doi.org/10.3390/cancers13061380
74. McDonald OG, Li X, Saunders T, Tryggvadottir R, Mentch SJ, Warmoes MO et al. Epigenomic reprogramming during pancreatic cancer progression links anabolic glucose metabolism to distant metastasis. *Nat. Genet.* 2017; 49; 367–376.
75. Sharma A, Merritt E, Hu X, Cruz A, Jiang C et al. Non-Genetic intra-tumor heterogeneity is a major predictor of phenotypic heterogeneity and ongoing evolutionary dynamics in lung tumors. *Cell Rep.* 2019; 29; 2164–2174.e
76. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: A looking glass for cancer? *Nat. Rev. Cancer* 2012; 12; 323–334
77. Quintanal-Villalonga Á, Chan JM, Yu HA, Pe'Er D, Sawyers CL et al. Lineage plasticity in cancer: A shared pathway of therapeutic resistance. *Nat. Rev. Clin. Oncol.* 2020; 17; 360–371.
78. Gaiti F, Chaligne R, Gu, H, Brand RM, Kothen-Hill S et al. Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia. *Nat. Cell Biol.* 2019; 569; 576–580.
79. Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS et al.; Phenotypic Heterogeneity among Tumorigenic Melanoma Cells from Patients that Is Reversible and Not Hierarchically Organized. *Cancer Cell* 2010; 18; 510–523
80. Roesch A, Fukunaga-Kalabis M, Schmidt EC, Zabierowski SE et al. Temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. *Cell* **2**010; 141; 583–594.
81. Marjanovic ND, Hofree M, Chan JE, Canner D, Wu K et al. Emergence of a High-Plasticity Cell State during Lung Cancer Evolution. *Cancer Cell* 2020; 38; 229–246.e13
82. Brock A, Chan H, Huang S. Non-genetic heterogeneity—A mutation-independent driving force for the somatic evolution of tumours. *Nat. Rev. Genet.* 2009; 10; 336–342
83. Pavet V, Shlyakhtina Y, He T, Ceschin DG, Kohonen P et al. Plasminogen activator urokinase expression reveals TRAIL responsiveness and supports fractional survival of cancer cells. *Cell Death Dis.* 2014; 5; e1043
84. Niculescu VF. The stem cell biology of the protist pathogen *Entamoeba invadens* in the context of eukaryotic stem cell evolution. *Stem Cell Biol Res.* 2015;2:2.
85. Niculescu VF. Growth of *Entamoeba invadens* in sediments with metabolically repressed bacteria leads to multicellularity and redefinition of the amoebic cell system. *Roum Arch Microbiol Immunol.* 2013;72(1):25-48.
86. Diamond LS. Axenic cultivation of Entamoeba hitolytica. Science. 1961; 4;134(3475):336-7. doi: 10.1126/science.134.3475.336
87. Mukherjee C, Clark CG, Lohia A. Entamoeba shows reversible variation in ploidy under different growth conditions and between life cycle phases. PLoS Negl Trop Dis.2008;2:e281.
88. Mukherjee C, Majumder S, Lohia A. Inter-cellular variation in DNA content of3 Entamoeba histolytica originates from temporal and spatial uncoupling of cytokinesis from the nuclear cycle. PLoS Negl Trop Dis. 2009;3(4):e409.
89. Salmina K, Huna A, Kalejs M, Pjanova D, Scherthan H, et al. The cancer aneuploidy paradox: In the light of evolution. *Genes* (*Basel*). 2019;10:83.
90. Craig CF. Studies upon the amebae in the intestine of man. J Infect Dis. 1908;5:324–77. doi: 10.1093/infdis/5.3.324
91. Krishnan D, Ghosh SK. Cellular events of multinucleated giant cell formation during the encystation of *Entamoeba invadens*. *Front Cell Infect Microbiol.* 2018;8:262.

92. Hazra S, Kalyan Dinda S, Kumar Mondal N, *et al*. Giant cells: Multiple cells unite to survive. *Front Cell Infect Microbiol.* 2023;13:1220589.
93. Ashouri, A.; Zhang, C.; Gaiti, F. Decoding Cancer Evolution: Integrating Genetic and Non-Genetic Insights. *Genes* **2023**, *14*, 1856. https://doi.org/10.3390/ genes14101856