REVIEW

Evolutionary Determinants of Cancer

Mel Greaves

ABSTRACT Our understanding of cancer is being transformed by exploring clonal diversity, drug resistance, and causation within an evolutionary framework. The therapeutic resilience of advanced cancer is a consequence of its character as a complex, dynamic, and adaptive ecosystem engendering robustness, underpinned by genetic diversity and epigenetic plasticity. The risk of mutation-driven escape by self-renewing cells is intrinsic to multicellularity but is countered by multiple restraints, facilitating increasing complexity and longevity of species. But our own species has disrupted this historical narrative by rapidly escalating intrinsic risk. Evolutionary principles illuminate these challenges and provide new avenues to explore for more effective control.

Significance: Lifetime risk of cancer now approximates to 50% in Western societies. And, despite many advances, the outcome for patients with disseminated disease remains poor, with drug resistance the norm. An evolutionary perspective may provide a clearer understanding of how cancer clones develop robustness and why, for us as a species, risk is now off the scale. And, perhaps, of what we might best do to achieve more effective control. *Cancer Discov; 5(8); 806–20. ©2015 AACR.*

INTRODUCTION

Nothing in biology makes sense except in the light of evolution.
—Th. Dobzhansky, 1973

We have come a long way since the ancient Greeks asserted that cancer was a manifestation of black bile and constitutional melancholy. And there is much to celebrate, from preventative vaccines to high-resolution imaging diagnostics and genome-guided personalized medicines, and, in some cases, cures of otherwise lethal malignancies. But the reality is that cancer still exerts a massive burden on society worldwide with no immediate prospects for effective control (1). Major cancer research funding bodies, governments, and international agencies explicitly recognize the magnitude of the problem and have set priorities for prevention, early diagnosis, and improved treatment (2). But do we have an adequate grasp of the basic biology?

Is there a fully coherent explanation of why the lifetime risk of cancer is now so extraordinarily high? Do we really understand the covert process by which the progeny of one mutated cell may, or (more often) may not, after anything between one and 50 years, execute a lethal hijack of our tissues? Why do we still express surprise, as well as disappointment, when a heralded new drug prompts tumor regression followed, almost inevitably, by recurrence and resistance?

Dobzhansky's well-known remark is a truism not just for mainstream biology but, arguably, for much of medicine. In this review, I advance the argument that nothing makes sense in cancer except in the light of evolution. Cancer is, in its essence, a Darwinian dilemma. This should inform our attempts to control it.

INTRACLONAL DIVERSITY

Cancer has a multilayered, Russian doll–like diversity that affects all areas of clinical practice (Fig. 1). Arguably, the feature that provides the greatest challenge to therapeutic control is the dynamic genetic diversity, coupled with epigenetic plasticity, within each individual cancer (3, 4).

The concept that cancers are driven by genetic abnormalities, diversify, and evolve over time can be traced back to Boveri (5) and, later, in the mid-20th century, to astute pathologists interested in the natural history of cancer (6). However, the origins of our current evolutionary perspective of intraclonal genetic complexity in cancer lie in the 1970s with Peter Nowell's synthesis on clonal evolution (7) and John Cairns's view of intrinsic mutability of DNA in stem cells, paired with natural selection, as a liability (8).

In more recent years, the advent of cancer genomics (9), multiregional sequencing (10, 11), genetic screening of single cells (12–14) or single-cell–derived clones (15), and single-cell sequencing (16–19) has revealed the striking intraclonal genetic diversity in cancer. The result has been a vivid new portrait of genetic architectures and inferred clonal phylogenies or evolutionary trees in cancer. These are reminiscent, in broad view, of Charles Darwin's iconic "*I think*" tree drawing of 1837

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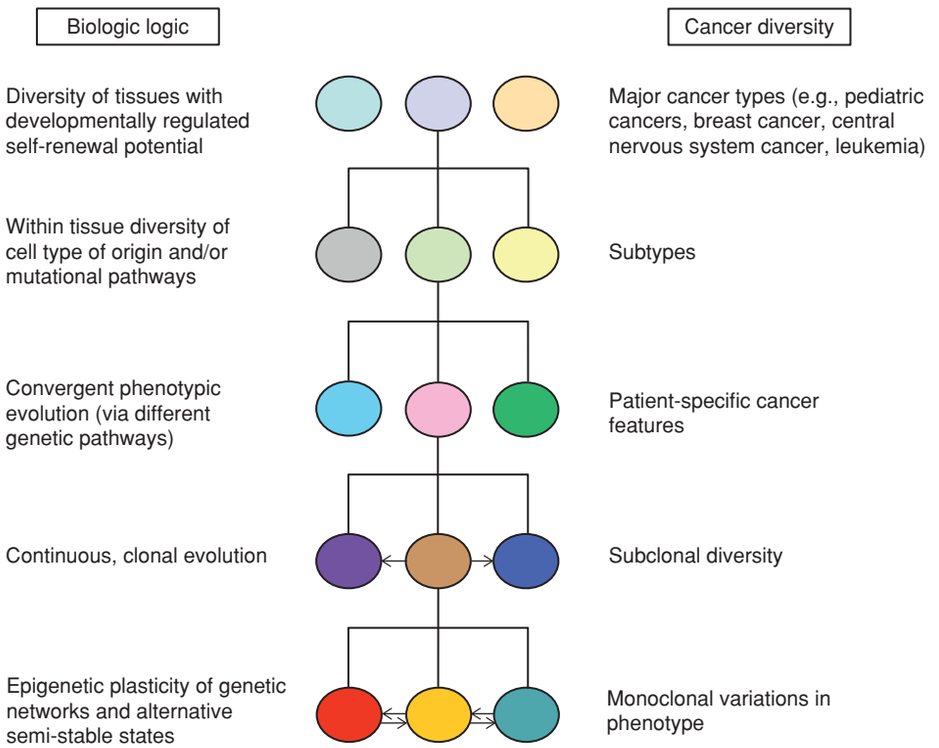


Figure 1. Cancer's Russian doll diversity. The marked differences in age-dependent incidence rates of different cancer types (or subtypes), particularly between children and adults, are likely to reflect developmentally constrained windows of risk from stem cell proliferation.

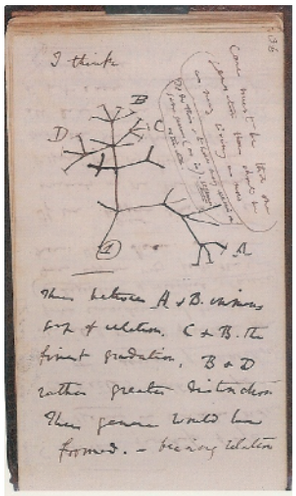
and parallel current phylogenomics research on species, with similar bioinformatic and computational challenges (20). Some strikingly consistent features of cancer clones have emerged that illuminate the underlying process, including branching or variegating genetic architectures, parallel or convergent evolution, and spatial segregation of subclones in primary sites and metastases (Fig. 2). This contrasts with the earlier view of cancer clone evolution as linear clonal succession. A few cancers might still appear to develop in this way, but deeper genetic integration is likely to reveal more complex and branching clonal structures. The variegated, genetic architecture of cancer is of some consequence for therapy. Actionable "driver" mutations may prove to be suboptimal targets for therapy if they are confined to subclonal branches of the cancer's evolutionary tree (21).

The clonal phylogenies we capture are mostly based on single time point snapshots. They allow us to infer a historical pattern of clonal evolution, but we may miss early clonal events, the dynamics, and underestimate complexity. To some extent, this is rescued by multiregional sampling coupled with the application of molecular clocks and spatio-temporal modeling (10), by serial sampling from different stages of disease (22, 23), or by "real-time" screening of tumor-derived DNA fragments in plasma (24–26). Phylogenetic trees based upon diagnostic sample snapshots may also fail to capture the order of early clonal events or ancestral clones. The order of driver mutations may be lost in subsequent selective sweeps or in regional samples with subclonal dominance (11, 27). Ancestral clones may become extinct or persist at very low frequencies. These early stages of clonal evolution can however be recovered, at least in some cancers. In childhood acute lymphoblastic leukemia, the early or initiating genetic events and covert preleukemic clones are discernible in monozygotic twins (28, 29) who share single premalignant clones spawned and shared *in utero* (30). In acute myeloid leukemia, preleukemic cells persist at low frequency but can be purified (31).

Charles Darwin imagined that evolution proceeds at a slow, steady pace. Others have argued that evolution is best characterized by long periods of stasis interrupted by explosive change—or punctuated equilibrium (32). There is increasing evidence for the latter in cancer (33), propelled by the onset of genetic instability (34), chromothripsis (35), or high-impact single mutations (36, 37).

At one point, the prevailing view or assumption was that cancer clone evolution was a matter of linear, sequential clonal succession, but it does not often look as simple as that. After initiation, multiple subclones often coexist, signaling parallel evolution with no selective sweep or clear fitness advantage. This can take the form of a starburst "big bang" (10) or branching clonal architectures (11, 13).

Nowell made no reference to Darwinian selection in ecosystems in his seminal 1976 review, but this is how we now see cancer clone development: diversification and selection in the context of tissue ecosystem pressures (38–42). The recurrent or "driver" mutations we identify via genomic sequencing have been distilled from a cacophony of random mutational noise and only qualify as "drivers" if they impart an ecosystem-dependent fitness advantage, i.e., they are adaptive. In this sense, cancer cells evolve by scaling higher or different fitness peaks as the ecosystem context changes. We sometimes designate "drivers" in a fashion that implies they have intrinsic or fixed fitness attributes. In reality, their functional impact is highly contingent upon both genotypic context, especially via epistasis (see Box 1), as for most mutational innovations in evolution (43), and



Clonal architecture and phylogeny in cancer

1. Extensive intraclonal diversity
2. Variegated genetics and branching architecture
3. Nonlinear dynamics
4. Reiterated (independent) mutations of same gene in branches
5. Every patient's clone unique
6. Early or founder mutation(s) identified (in trunk)
7. Subclones = multiple independent cancers
8. Subclonal territories in tissues
9. Backtracking origins of relapse, metastases, and drug resistance to minor clones in primary samples

Figure 2. 1. Revealed by single-cell genetic analysis or inferred bioinformatically from in-depth sequencing (9–13). The number of genetically distinct subclones identified depends upon the depth of genome sequencing, the number of cells interrogated, and the number and nature of mutations screened. Single-cell whole-genome sequencing suggests that every cell is unique (16), and therefore a tumor has, in one sense, as many subclones as there are cells. 2. Variegation of genetics and clonal phylogeny architecture inferred from single-cell genetics or multiregional sequencing using maximum parsimony, other phylogenetic methods, or probabilistic algorithms (192). Note that at present, cancer biologists use no single or uniform platform to infer and depict phylogenetic trees. There is much to learn here from phylogenomics of species (20). 3. Dramatic changes in clonal structure are reflected in clonal sweeps as in metastases or drug-resistant recurrence. These abrupt adaptive changes are prompted by stringent selection. 4. Reiterated or independent mutations in the same gene in subclonal branches (11, 13): a result of convergent evolution and strong selective pressure favoring those mutations. 5. Revealed by comparative clonal phylogenetics of cancers from multiple patients with the same subtype of disease (11–13). Branching clonal architectures, reiterated mutations (or convergent evolution) in different side branches, and overall uniqueness of each clonal architecture are all features of a complex adaptive or evolving biologic system. 6. Truncal mutations are defined as those shared by all cells in all extant subclones. When more than one is present, it is likely that they were acquired sequentially and some early "branching" may be lost. 7. Xenotransplantation studies using immune-deficient mice suggest that subclones have variable competitive ability to generate leukemias or cancers *in vivo*, but in most cases, this capacity resides in more than one or several subclones (13, 112). 8. Comparative genomics of matched relapse/recurrence and diagnostic samples made it possible to detect the drug-resistant mutations in the primary material (i.e., at low frequencies prior to selection; refs. 80, 81) as well to identify minor subclones that have spawned metastases or relapses (22, 23, 193). 9. Many cancers appear to show some topographical segregation of genetically distinct subclones revealed in tissue sections or microdissected regions of primary tumors (11, 194) and in metastatic lesions (22, 23), although admixtures are also common (10). Image from Charles Darwin's Notebook B, 1837.

contemporary selection pressures within the local cancer ecosystem (ref. 44; see Box 1). Cellular fitness is expressed, assessed, and selected principally via the phenotypic features registered as "hallmarks" of cancer (45), all of which ultimately affect fitness via survival and/or reproduction of cells.

In some cases, the match between mutational change, phenotypic consequences, and selective pressure is clear (Fitness test 3 in Box 1): *TP53* mutations are highly recurrent in cancer (~50% of cases), suggesting they provide a fitness advantage in the face of common selective pressures. The contextual or adaptive significance can be seen in the ubiquitous presence of *TP53* mutations in UVB (and genotoxic stress)–exposed skin (46), in response to genotoxic therapy (47) or oxidative damage (44, 48). As a remarkable ecological parallel, naked mole rats living under hypoxic subterranean conditions have a constitutive variant at Arg174 of *Trp53* that is the same as some acquired *TP53* mutations in cancer cells (49). The selective logic is in evasion of the *TP53*-dependent apoptosis pathway.

Matching of mutational adaptations in emergent subclones to particular selective pressures is also clearly evident with drug resistance mutations which are present in drug targets or response pathways (50, 51). And, similarly, when cancer cells are predated by foreign (allogeneic) immune cells, genomic deletion of mismatched HLA alleles selects for immunological invisibility (52, 53).

Evolutionary change operates not by radical innovation from a blank canvas but by tweaking what already exists. The emergence of mutational complexity, clonal trajectories, or architectures and clinical subtypes is therefore constrained by the cell type of origin (54) and by the early or initiating mutations that reset genotypic signaling networks and cellular phenotypes (10, 55). One interesting consequence of this is that some tumors may be "born to be bad" (10).

Ecosystem-selective pressures in cancer are diverse and imperfectly understood. They include exogenous exposures, multiple endogenous restraints, and, arguably the most potent selection pressure of all, cancer therapeutics. The nature and timing of these pressures will shape clonal architectures and dynamics and influence clinical response. Within tissue microenvironments, cancer subclones indulge in reciprocal dialogues with each other and with stromal, endothelial, and inflammatory cells, modulating each other in the struggle to maximize fitness. This can complicate analysis of fitness features, as subclones can cooperate as recipients of "public goods" provided by a subset of cancer cells or via paracrine loops (56–58). Production of a "public goods" signal comes at a cost, so the assumption is that there is likely to be some alternative or overall fitness benefit to the producer.

Cancer clone evolutionary progression and metastases involve multiple, reiterated, or distinctive selection hurdles (59) that can be negotiated only via randomly generated

Box 1. The "driving" test for mutations in evolution and cancer

Fitness test 1

Does it alter protein function (↑ or ↓)?[a]

✓ → Proceed to 2.

Fitness test 2

Does it alter cellular phenotype in a stable fashion endowing potential fitness advantage?

Context: *Genotypic network regulating phenotypes:* mutational impact minimized or re-equilibrated via negative feedback, redundancy, and buffering capacity.

Epistatic (nonadditive) interactions with other inherited and acquired gene variants in the network will determine potential fitness.[b]

Cell type: some mutations in cancer will alter phenotype only in particular cells.[c]

✓ → Proceed to 3.

Fitness test 3

Does the mutation-induced phenotypic change endow a sustained fitness advantage (via survival and reproduction)?

Context: *The prevailing ecosystem- or microenvironmental-selective pressures and restraints.* Is there a serendipitous match?

✓ → Proceed to 4.

Fitness test 4

Is the mutation encoding fitness advantage stably propagated?

Context: Is the mutation present in a cell that can propagate it extensively or indefinitely:
- any bacterial cell
- germ cells in sexually reproducing organisms
- self-renewing cells in cancer (see text).

✓ Passed. → Now repeat for each mutation.

[a]Only a few mutations in a gene can lead to a fitter protein (191). Loss of function is a more accessible change.
[b]The role of epistasis and genotype networks in influencing fitness in cancer cells is seen with mutations such as those in *BRCA1, MYC,* and *RAS*. These potentially powerful drivers affect fitness only if the apoptotic or senescence response they elicit from the signaling network is complemented by other mutations that block this default option (137, 183). The impact that epistasis and genotypic networks have on fitness precludes any straightforward measurement of the fitness advantage of individual "driver" mutations.
[c]E.g., *BCR–ABL1* only in hematopoietic stem cells, transcription factor mutations, and fusion genes, in particular lineages.

variants. Genetic and chromosomal instability in cancer cells increases the odds of a beneficial or "rescue" mutation arising (60, 61) and can be considered an adaptive response to genotoxic or other stresses, as in bacteria (62). But the probability of success is still limited. Genetically unstable clones can be less fit and reside closer to the edge of collapse or error catastrophe, as in hypermutable bacteria and viruses (58). Hence, most initiated cancer clones or tumors never maximize fitness as malignant derivatives. Which is fortunate because it is very likely that as we age, we all accumulate mutant clones and covert, incipient cancers (63–65).

Cancer and ecosystems broadly are sufficiently similar that concepts and computational mathematical models derived from ecology, such as evolutionary game theory (66), are now being profitably harnessed to investigate cellular interactions and clonal dynamics in cancer (67, 68), and to explore novel evolutionary approaches to therapy (see below).

CANCER AS A COMPLEX, ADAPTIVE SYSTEM

As in evolution in ecosystems, it is not all mutation-driven natural selection in cancer clones. Early phases of clonal expansion in particular may well involve neutral drift and fortuitous advantage (69), or disadvantage (44). Epigenetic plasticity may, in some contexts, be critical (ref. 70; see further below). A somewhat broader way to encapsulate the evolutionary complexity of cancer and its resilience is as a complex, adaptive system (71, 72).

The idea of complex systems derives from chaos theory and mathematics and has wide applications, including engineering, meteorology, and economics (73). Evolution of complex systems is common in nature, with examples being the single cell, neural networks and the brain, the immune system, whole ecosystems, and cancer. The essence of biologic complex systems is their nonlinear dynamics and, especially, their adaptability or resilience, maintaining functionality in the context of stress or challenge. They execute this via a few seminal features, including highly networked signaling with built-in redundancy, negative feedback, buffering, and modularity (72). And, additionally, they can re-equilibrate into alternative steady states via phenotypic plasticity. For complex systems with built-in genetic diversity—species in ecosystems, the immune system (74), and cancer—there is the adaptive tactic of differential survival and reproduction, or clonal selection, to maximize fitness in relation to the prevailing challenges. Complex adaptive systems generally evolve in

Table 1. Evolutionary origins of selection for drug resistance in cancer

	Mechanism	Origins
Genetic	• Selection of preexisting, stochastic mutations in drug response pathway	The "classic" escape route: the inevitable consequence of mutation rates and clone size.
	• Segregation of drug targets in subclones	For targeted therapy. The consequence of genetic variegation.
Epigenetic	• Quiescent stem cells	An ancient survival trait—"hunkering down" to protect against stress. Evident in bacteria and normal stem cells.
	• Signal block bypass	For targeted therapy. A consequence of signal network complexity, redundancy, and multiplicity of microenvironmental signals. An early evolutionary innovation, as seen in bacteria and yeast.

the direction of increased robustness, which equips them to withstand multiple destructive challenges, whether they be climate change, nutrient loss, predators, or infections (72). Or, in cancer, to withstand or bypass the inherent evolutionary restraints on cells becoming cheating or selfish replicators and, as a bonus, evade cancer therapeutics (75).

We see how robustness of a complex system underpins resilience in cancer by considering the multiple routes adopted by cells for therapeutic escape or drug resistance (ref. 76; Table 1). The "classical" or mutational routes to resilience are the inevitable consequence of a numbers game with intrinsic mutability of cellular DNA, as with antibiotic resistance in bacteria. And, as with evolutionary innovations in general, mutations in cancer arise by a stochastic process that may leave mechanistic footprints (77) but is entirely independent of, or blind to, functional consequences or utility (78). A vivid demonstration of this principle comes from the finding of resistance mutations to modern antibiotics in bacteria frozen in permafrost for 30,000 years (79). And in cancers, we now have the anticipated evidence that emergent drug mutations in disease recurrence or relapse can be backtracked to a time preceding drug exposure, i.e., before they were positively selected (80, 81).

The epigenetic routes to therapeutic escape in cancer have a similar evolutionary antiquity. Dormancy is a common tactic (82). Bacteria, as well as normal stem cells, adopt a dormant state—or "hunker down"—when under stress (83). It is a very old trick, but it works. How it works in normal (84) and malignant stem cells (82) is not entirely clear, but it can involve cell-cycle quiescence, sanctuary niches, and active drug efflux. Signal block bypass, for some targeted therapies (85, 86), is a consequence of highly networked signaling for cellular metabolism and cycling with built-in redundancy as a failsafe. Blocked signal pathways can be rescued by alternative microenvironmental signals (87). Complex genetic and signaling networks were a very early evolutionary innovation in bacteria (88) and are very evident in yeast (89). Signaling network complexity and plasticity was a feature of totipotent or stem cells of early multicellular organisms, as in their current descendants (90–92), and has been elaborated over evolutionary time along with increasing diversity of form and longevity. It is therefore unsurprising that cancer cells, and perhaps especially cancer stem cells, have a robustness that allows them to exploit both genetic diversity or epigenetic plasticity when therapeutically targeted.

CANCER STEM CELLS AS "UNITS OF EVOLUTIONARY SELECTION"

Stem cells are integral to the developmental assembly and sustainability of multicellular life (91). Their fate options when dividing are to either produce replicas of themselves—to "self-renew"—or, alternatively, to spawn differentiating progeny. This epigenetic plasticity is regulated by environmental cues within tissue niches and is orchestrated by key intracellular signals. Genes encoding the latter are recurrently mutated in cancer, effectively trapping cells in a self-renewing mode (93–95). Normal cells can transiently switch to symmetrical self-renewal in response to regenerative demand, but in cancer, the balance of stem-cell fate decisions shifts to self-renewal, at the expense of differentiation, in a stable fashion. Experimental evidence with both xenotransplants of human cancers and lineage tracing in murine models confirms that subpopulations of self-renewing cells sustain tumor growth (96–98) and establish metastases (99). Depending upon the mutations involved and the stage of disease, the normal hierarchical or differentiation-linked lineage structures will be maintained or collapse.

There is an argument that clonal evolution and cancer stem cell–generated cellular hierarchies are alternative models of cancer clone diversity and progression (100). An alternative view is that the two are inextricably linked (42, 98). The notion here is that self-renewing stem cells—irrespective of their variable phenotypes and frequencies—and the presence or absence of hierarchical lineage structures, are the focus of evolutionary selection in cancer, generating clonal architectures (Fig. 1), metastases, and drug-resistant recurrence, i.e., they are the predominant "units of selection" and cellular drivers of cancer (101).

The definition of units of selection is a contentious issue among evolutionary biologists, one with semantic and philosophical overtones (102, 103). An evolutionary perspective on hierarchical levels of selection may be helpful. Evolution by natural selection has been in business for some several billion years on planet Earth and will happen whenever a few simple conditions are met (Fig. 3). And, over very protracted periods of evolutionary time, the nature of the individual entities or "units" that are selectable has shifted into increasingly higher orders of hierarchical complexity—from molecules to cells to multicellular organisms to societies or groups of

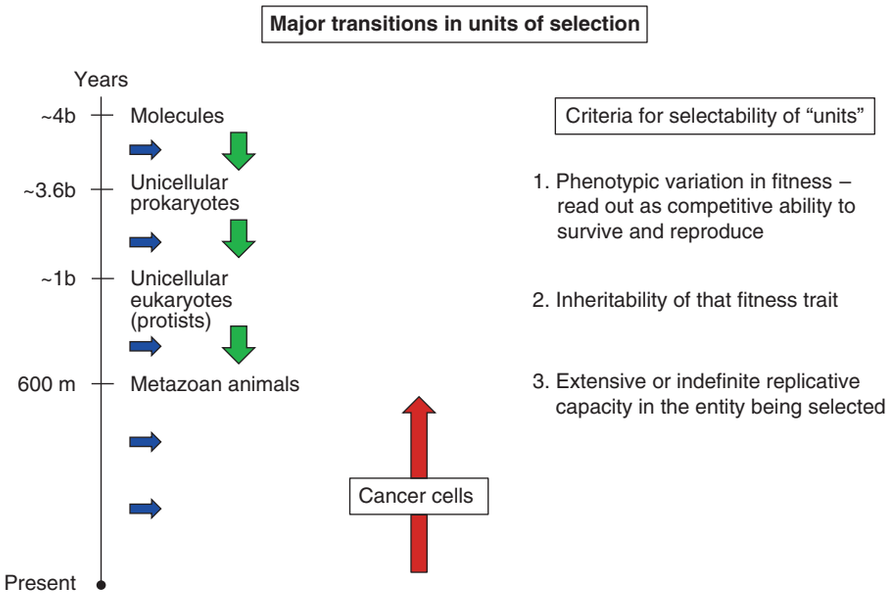


Figure 3. From an evolutionary perspective, clonal cheating or cancer is atavism, a return to a former lifestyle in cells with a 600-million-year memory of unicellular selfishness. Cancer cell behavior is more than a superficial parallel to atavism if protists or early stem cells in the first multicellular animals had the inherent potential to express many or most of the essential hallmark (or "fitness") features of cancer. Cancer mutations, from this evolutionary perspective, do not so much innovate novel phenotypes but rather decouple ancient and normally transient phenotypes from appropriate networked control (108). The physicist Paul Davies independently arrived at a similar conclusion, although he coupled it with the view that evolutionary atavism was incompatible with clonal selection models of cancer (195). It is not. Blue arrows: At each of these levels, individual entities may aggregate into conglomerates to optimize fitness (e.g., molecular complexes, colonial bacteria and protists, social insects), in which case selection may act at the group level.

individuals (refs. 104, 105; Fig. 3). At each of these major evolutionary transitions, the capacity of the smaller entities to selfishly replicate (and be available for selection) is rendered subservient to the fitness of the higher-order entities, though multilevel selection is still possible. However, at each transition point, the criteria for qualifying as effective units of selection are the same (Fig. 3).

The multicellular condition required conflict resolution and management of risky trade-offs, as the "lower-order" entity—stem cells in multicellular organisms—contributes to fitness of the individual via replicative ability. There are opportunities for cheating, particularly when this is fueled by mutational diversity, as in other social groupings in biology (106, 107). Cancer is an outcome of mutation-fueled cheating and, from an evolutionary and phenotypic perspective, is atavistic in nature (refs. 108–110; see Fig. 3).

In cancer, selection is for fitness of somatic cell variants to survive and reproduce. But can it be any cancer cell? Any dividing cell within a cancer clone can acquire, say, a drug-resistance mutation, because this is an entirely stochastic process. But in practice, the most effective units of selection in cancer will be cell populations that have sufficient genetic diversity (to provide a phenotypic substrate for selection) and with extensive self-renewal (to propagate any selected trait). The genetics of cancer stem cell populations have not been adequately explored, but there is evidence from xenotransplants that these cells are genetically diverse in individual patients (13, 15, 111, 112).

A complication in this argument comes from the fact that self-renewal or "stemness" is an epigenetic state, not a fixed entity (113). This is true of normal as well as cancer stem cells (114). Normal stem cells are prime targets for the initiation of malignant transformation (115), but downstream progenitors, prior to terminal differentiation, can acquire self-renewal capacity by mutational changes (116) or microenvironmental pressures, as in zones of hypoxia (117) or with metastatic spread and epithelial–mesenchymal phenotypic transition (118). Such progenitor conversion to a stem cell state could be either an initiation event that might or might not lead to overt malignancy, or a secondary selective event (31). The frequency of self-renewing cells or cells that can regenerate cancers in xenotransplant assays varies over several orders of magnitude, from one in a million up to almost 100%, between different cancers but also within a cancer with progression of disease (119–121). This huge variation, coupled with phenotypic plasticity, and concerns over the efficiency of *in vivo* xenotransplantation assays for stem cells, confounds examination of stem cell function in cancer (122, 123). In addition, genetically homogeneous cancer stem cells show functional variability in propagating activity and drug sensitivity (70). But these biologic complexities do not contradict the notion that cells with self-renewal are the principal units of evolutionary selection. Mutations that confer drug resistance can only have an impact with any clinical consequence if they are present in cells with self-renewal potential. Metastatic spread will be minimal unless it is seeded by cells with inherent or inducible self-renewing capacity.

The pivotal role of self-renewing cells as the prime selectable entity in clonal evolution is strongly endorsed by two sets of findings. First, that the quantitative burden of stem

cells in several cancer types assessed to date is associated with disease progression or clinical failure (121, 124–128). And, second, that self-renewal appears to be a tractable target for effective therapeutic control (129–131).

EVOLUTIONARY RISKS AND RESTRAINTS

There is a sense in which a baseline or intrinsic cancer risk can be seen as a trade-off or legacy of how life has evolved, with the necessity for both mutability of DNA and stem cell proliferation (108). It is unsurprising that cancer is found, or can be induced, in most classes or clades of multicellular animals, including those near the base of the metazoan phylogenetic tree in Cnidaria (132, 133).

But risk to reproductive success does not go unchecked in evolution. The transition to multicellularity, some 600 million years ago, required acquisition of controls to maintain the cellular integrity of tissues and restrain clonal cheating and cancerous proclivities (134). It is revealing then that most cancer suppressor genes arose at this time (135), though regulation of regenerative proliferation by stem cells may have been their primary function rather than cancer prohibition (92). Similarly, *Trp53*, as an orchestrator of stress responses, emerged in the common ancestors of unicellular protists and metazoan animals (136). DNA repair capacity, as might be expected, emerged much earlier with bacterial cells (135). Restraints on cellular escape are multiple, including intracellular-negative signaling feedback controls, triggering default pathways of apoptosis or senescence in cells with compulsive oncogene-driven proliferation (137). Microenvironmental controls include space or architectural constraints (138) and metabolic limitations (e.g., oxygen diffusion, pH). Because long-lived, essential, and numerically limited stem cells are the most "at risk" cell population, they should have been afforded special protection via evolutionary adaptation. This we see reflected in their residencies in epithelia, distal to external exposures, and in relatively hypoxic niches that may minimize DNA damage (117). Perhaps counterintuitively, stem cells reduce risk by proliferative quiescence. Most of the proliferative expansion required in steady-state tissue turnover is executed by transitory progenitor or precursor cells where mutations will have less impact (139). Risk of clonal escape by mutant stem cells is also constrained by proliferative dynamics within the confines of niche architectures. Stem cells "take turns" at exiting quiescence to proliferate. As a consequence, mutant and potentially malignant stem cells may be flushed out by normal stem cells before they can establish clonal dominance (44). Drug efflux pumps are very active in most normal and cancer stem cells, not to frustrate therapy (which they do) but as a long-standing adaptation to protect the limited pool of essential normal stem cells, and other critical tissue sites (the placenta and blood–brain barrier), from xenotoxic damage (140).

As multicellular animals became more complex, larger, resilient, and longer lived, risk of clonal escape will have escalated. The beneficial, adaptive traits of wound healing and angiogenic capacities, an invasive trophoblastic placenta, and mutagenic recombinases in lymphoid cells all come at a potential trade-off price in cancerous currency. But then tight regulation and additional restraints will have accrued—as an adaptive response to increased risk (141). Overall, risks and restraints will have been balanced at a level that minimizes life-threatening malignancy during reproductively active lifespans—the period during which natural selection has an opportunity to operate.

Long-lived and very large animals are interesting and challenging in this respect. Subterranean mole rats can live for approximately 30 years and appear to be cancer resistant. The bowfin whale lives for 200 or more years and has 1,000 times more cells than humans but appears to suffer very little cancer. How is it possible if cancer risk has a simple relationship to stem cell division and errors in DNA? The conundrum is referred to as Peto's Paradox (142). And the solution may be close to hand from recent comparative genomics, which suggests that long-lived species have adopted, we assume via natural selection, a variety of mechanisms to restrain increased cancer risk, operating via DNA repair efficacy, cell-cycle regulation, growth factor receptor signaling, and contact inhibition of cells (143, 144).

Which raises an obvious question. Has evolution passed us by?

THE EVOLUTIONARY DIMENSIONS OF CANCER CAUSE

Aging, nonhuman primates (in captivity) have low cancer rates. The lifetime risk of cancer for humans in Westernized or developed societies is now at around 1 in 2. To some extent, this grim statistic is the flip side of our success of avoiding predators, both microbial and human. But the retort that cancer is the inevitable price to pay for longevity and aging (145) is to shortchange the argument, although clearly there is a strong association between aging and cancer risk.

Figure 4 illustrates a simplified view of a causal network for cancer clone initiation and evolution. The roulette wheel of stochastic, potentially cancerous mutations instigating and driving clonal evolution in stem cells is the proximate causal mechanism in each and every cancer. But this game, underpinned by evolutionary legacies, does not have fixed odds. Mutation rates and stem cell pool size and cycling frequencies are not intrinsic physicochemical properties like radioactive decay. They are variables subject to extrinsic as well as mutational modulation. Data from genome-wide association studies (GWAS) indicate that risk of every cancer type evaluated to date is modulated by inherited gene variants (146). And epidemiologic data on incidence rates of all common cancers suggest that exposures or lifestyle-associated factors can influence risk substantially, 2- to 100-fold (1). Chance pervades all of these parameters, so for each and every individual patient with cancer it certainly is "bad luck."

Epidemiologists have explicitly recognized the multifactorial nature of causation in cancer (147), but as pragmatic scientists, we often focus on selected components of causal networks which we may then label as "*the* cause." This feeds into public expectations of singular and simple explanations and distorts our understanding of causal complexity.

Darwinian medicine adopts a different perspective on causation, through the lens of evolution. It addresses the question of how our historical past has shaped our vulnerability to diseases, such as cancer (148), both as a species and with

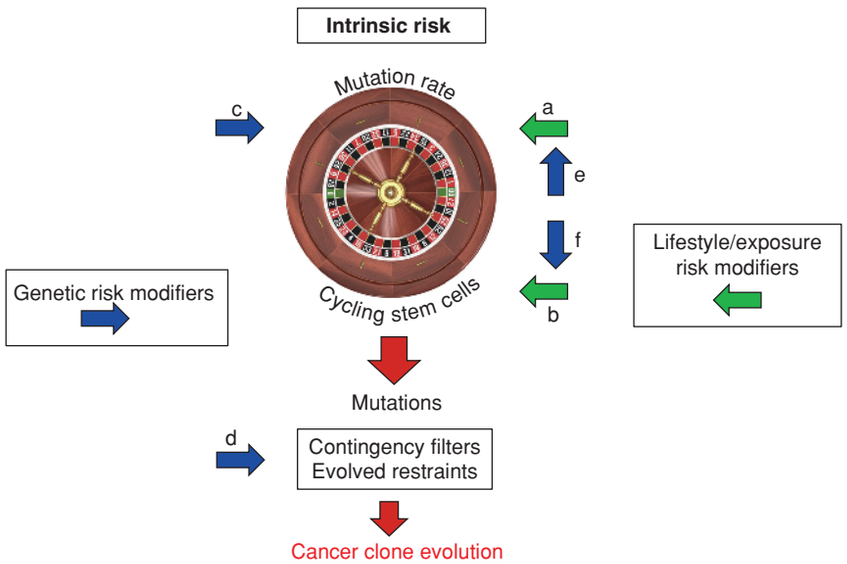


Figure 4. Lifestyle exposures (green arrows) can (a) directly affect mutation rate, e.g., genotoxic ionizing radiation or genotoxic chemicals, such as benzo(a)pyrene in cigarette smoke, integrated viruses, or chronic inflammation and oxidative stress. Alternatively, (b) they can indirectly increase mutation probability via replicative stress on stem cells (e.g., via toxic damage–stimulated regeneration, or persistent or cyclical proliferative stimulation, e.g., hormonal stimulation or microbial infection). Excess calories (diet/exercise balance) can feed extra proliferative cycles via IGFI levels. Inherited genetic variants (blue arrows) can directly affect mutation rate (c) via, for example, diminished DNA repair, or can influence cancer risk "downstream" by epistatic interaction with somatic mutations (d; see text and Fig. 2). Inherited variants can also affect intrinsic risk (e and f) via their effect on exposures/lifestyle, for example, via skin pigmentation (and UVB impact), nicotine addiction (and cigarette carcinogen impact), or efficacy of immune response to infections (146).

respect to variation between individuals. Why do we carry inherited allelic variants that increase the risk of cancer? Why should common lifestyles, behavioral factors, or exposures increase intrinsic risk? And what can we do about it?

FAST TRACK TO A DARWINIAN BYPASS?

Humans are unique in our fast-track and exotic social evolution, the product of a sapient brain, which has bequeathed many benefits but also unintended consequences for cancer risk. First, we live for decades after ceasing reproduction. The abrupt female menopause, uncoupled to general senescence, is uniquely human (149), and andropausal decline in men means that only a few aging men will have offspring. Hunter-gatherers, despite their low average lifespans, did and still do survive postreproductively into their seventies or beyond (150), but it is only in relatively recent human history that this has been the norm. Natural selection works effectively only up to and during reproductively active life, as J.B.S Haldane and Peter Medawar both argued many decades ago. Unlike mole rats and whales that reproduce for most of their protracted lifespans and have had millions of years to adapt, we have engineered longer postreproductive lives in a rapid way that reduces opportunity or time for adaptive evolutionary processes to operate. A caveat is that some adaptive selection for postreproductive longevity may have occurred via the "grandmother" effect which effectively transfers fitness benefits to offspring. But that aside, the extra time available is then more spins of the roulette wheel of stem cell division, mutations, and increased risk of cancer clone initiation or promotion. But the epidemiologic incidence data tell us that it cannot just be extra time for mutational accidents to accumulate.

THE EVOLUTIONARY "MISMATCH"

Evolution has no eyes to the future.
—George Williams, 1966

Context is everything in evolutionary adaptations. A winning fitness trait can become a loser if circumstances, or selective pressures, change. As they do. The vast majority of once winning or successful species on the planet no longer exist.

We have reshaped our ecology and lifestyles in a way that has resulted in a mismatch with our inherent genetics, the latter profiles reflecting adaptations to earlier and very different environmental circumstances (108, 148, 151). And the mismatch ratchets up intrinsic or baseline risk by directly or indirectly increasing mutation rates and/or stem cell turnover (Fig. 4).

Needless to say, nothing in our evolutionary history as a species could have adequately adapted our lungs or livers to the chronic barrage of carcinogenic chemicals in cigarette tar. But, that major cancer cause to one side, what were at one time beneficial adaptations have now become liabilities, increasing risk of cancer.

The clearest case is with the evolution of skin pigmentation and risk of cancer (148). Dark, or highly melanized, skin evolved some approximately 1.2 million years ago via purifying selection of a very active melanocortin receptor 1 allele (152). The selective pressure was solar UVB damage to the skin with consequent skin cancer (152), folate loss (153), or other lethal pathologies. The reverse, depigmentation,

was an evolutionary adaptation acquired in migrants from Africa to higher latitudes in Europe some 40,000 to 60,000 years ago. The benefit is thought to reflect a reduced need for melanin to protect against solar UVB damage and an increased need for UVB-induced vitamin D synthesis (though other adaptive benefits including resistance to frostbite may have been involved; refs. 152, 153). White-skinned individuals are orders of magnitude more at risk for the three major types of skin cancer, but, critically, overwhelmingly in the context of our mismatched behavior in relation to sun and UVB exposure. We were, historically, latitude adapted but are now maladapted in this geographic context.

Another potent example of evolutionary mismatch is with breast cancer (108, 151). Nonseasonal, monthly estrus was a relatively early human innovation, and the fecundity benefit, underpinned by gene variants, would have been subject to positive selection. This will have carried a trade-off risk with more proliferative stress to breast (and ovary) epithelial cells. But this in turn was diminished by early pregnancy, protracted breastfeeding, and frugal diets. Those brakes on the spins of the roulette wheel of proliferating stem cells have been taken off in modern, affluent societies. And contrarywise, the risk further escalated by dietary/exercise changes that fuel higher IGFI and estrogen levels, earlier menarche, delayed menopause, and more cell cycles.

Plainly, our register of calorie intake versus usage with physical activity, or our metabolic rate, is out of kilter with our ancestral phenotypes and with the relationship seen across mammalian species between size, longevity, and metabolic rates (154). And, experimentally, it is clear that dietary calorie restriction, or genetic manipulation of IGFI levels in mice, reduces cancer risk substantially (155). Humans with genetically determined low growth hormone receptor levels have very low cancer rates (156). Diet and calorie expenditure is difficult to study epidemiologically, but it is possible that its overall impact on cancer risk in developed societies is second only to that of smoking. We do not need to adopt Paleolithic diets to fix this.

Other aspects of social evolution may have resulted in mismatches with our ancestral past that escalate intrinsic risk. Infections are causal factors in some 3% to 35% of cancers in different societies and are particularly prevalent in less developed societies (1). The higher-density and unhygienic living that arose after the agricultural revolution, starting some 12,000 years ago, combined with proximity to domestic animals, would have greatly increased the opportunities for microbial transmission. Endemic, early and persistent, or chronic infection is a feature of most cancers associated with specific infections (157). Paradoxically, the opposite trend may have increased risk of a few cancers in more developed or affluent societies. The greatly improved hygiene and markedly reduced opportunities for infectious spread may have resulted in increased risk of some cancers in the young via immune dysfunction. The immune system has evolved to both recognize and require some infectious exposure early in life to balance or shape its subsequent regulation and repertoire (74). Absent or delayed infection in the very young, a feature of modern societies, may result in later dysregulated responses to infections that can trigger childhood acute lymphoblastic leukemia in susceptible individuals (158).

There is a striking parallel, or validation, of the evolutionary mismatch view of cancer causation. When we artificially and rapidly manipulate the lifestyle and bodies of domesticated animals, as with perpetual egg laying battery hens (159) and the breeding of large, heavy dogs (160), the same thing happens—cancer rates shoot up, in tissues where you would expect them to: in ovaries and long bones, respectively. Darwin would surely have recognized that clue.

WHAT IS THE EVOLUTIONARY RATIONALE FOR INHERITED RISK VARIANTS?

Any modeling of the impact of exposures or lifestyle factors in increasing cancer risk via evolutionary mismatches and the stem cell mutational lottery needs to accommodate the variation in risk within human populations that has a constitutive or genetic basis. Family studies, sibling and twin risk estimates, and GWAS (146) all testify to the fact that inherited susceptibility affects cancer risk in humans, and in some cancers, this appears to make a substantial contribution to variation in overall risk—in prostate and breast cancers, for example, with high monozygotic twin concordance rates (161). A few highly penetrant but relatively rare mutant genes can contribute to this risk, *BRCA1* and *BRCA2* and other genes involved in DNA repair being the prime examples. But most of the 200 or more SNP or allelic variants so far described that increase cancer risk are common in Caucasian populations (5%–80% frequencies). The increased risk attributable to each allelic variant is modest, with OR in the range of 1.01 to 2. Most of the variants at present do not have clearly attributable biologic functions, but many SNP variants are in putative or defined regulatory, noncoding regions, in some cases regulating the same genes that harbor somatic mutations in cancer (146, 162). A plausible rationale for this is that many of these inherited variants are integrated into the genotype networks in cells, and in this way, epistatically, they can increase the impact that acquired mutations will have (via the filtering effect of genotypic networks; see Box 1).

But what could be the evolutionary rationale, if any, for the high frequencies of an allele that increases cancer risk? It is possible that at least some of the inherited variants increasing cancer risk were positively selected in our ancestors for some benefit they endowed despite their now, in a different or mismatched context, deleterious trade-off (163, 164). Many human genes show signatures of historical positive selection, especially those involved in the immune response, skin pigmentation, signal transduction, olfaction, and fertility (165). Several of the SNP variants identified in GWAS for skin cancer are in the same gene region that underwent adaptive, allelic variation in relation to depigmentation of Caucasian skin (166). The "top hit" in testicular cancer GWAS (OR = 3.07), a SNP in the p53-binding domain of the *KITLG* enhancer, has a signature of prior positive, evolutionary selection (167). Whether that variant was of adaptive benefit for skin pigmentation or fertility remains unclear. But it is now a liability.

One testable proposition is that some of the many genes increasing susceptibility to prostate and breast cancers have been under historical positive selection because of the fertility benefits they once provided—via estrogenic or androgenic

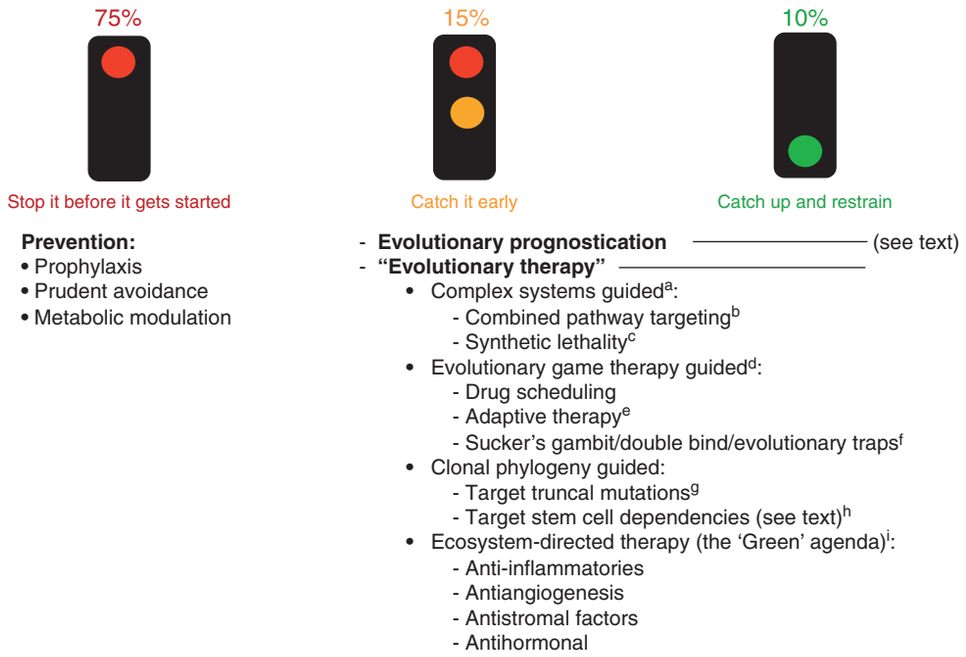


Figure 5. Modified from ref. 169. %: my very approximate estimates of the proportion of cancer deaths, worldwide, that might be avoidable via these three routes.
[a]See ref. 75.
[b]See refs. 85, 196.
[c]See ref. 183.
[d]See refs. 13, 66, 197.
[e]See ref. 198.
[f]See refs. 197, 199.
[g]See ref. 200.
[h]See ref. 201.
[i]See refs. 40, 110.

signaling pathways (164). If so, this places some individuals at greater risk of evolutionary mismatches.

AN EVOLUTIONARY FIX?

Waiting for natural, evolutionary processes to adapt to increased risk for cancer is not an option for us. Neither is manipulation of constitutive genotype (excepting embryo selection for high risk). The key question becomes: How can we best thwart the evolutionary resilience of cancer clones? In many ways, the challenge is similar to that we face with infectious parasites, bacteria, and viruses: It is a race against evolutionary progression and acquisition of drug resistance (168). Figure 5 illustrates a pragmatic, framework prescription highlighting the contribution that might come from evolutionary considerations (169).

The most effective way to thwart evolution—of microbial infections or cancer cells—is to stop it from happening in the first place, i.e., by prevention (see Fig. 5 legend). If Tomasetti and Vogelstein were correct, this would be a lost cause for most types of cancers, which they ascribe to chance mutation alone (170). The range of incidence rates for common cancers (1) suggests, however, that a high fraction is potentially preventable (108).

Effective prevention of cancer is difficult for a variety of logistical, social, and economic reasons. Even in the best-case scenario, we will be left with a significant cancer burden. One challenge then is for systematic surveillance and early intervention when clone diversity, robustness, and probabilities of drug resistance and metastases are less than maximized. Where early intervention is invasive (e.g., Barrett's esophagus, prostate cancer, and breast cancer), there is an urgent need for prognostic markers, particularly because only a minority of detected tumors are likely to progress (63, 141). The question then becomes: Can we predict evolution in cancer clones before it happens?

The stochastic or random nature of mutations, the impact of drift, the diversity, and idiosyncratic patterns of clonal architecture in cancer (Fig. 2) might suggest that evolution is not predictable in advance. Both complex system theory and evolutionary theory argue otherwise. Emergent fitness landscapes are heavily constrained by starting conditions, epistatic gene networks, and the prevalence of particular ecologic selection pressures. As a consequence, options are limited and evolutionary trajectories are frequently convergent in terms of phenotypic innovation and the recurrency of underlying genotypic changes (171).

In cancer clonal evolution, the key convergent phenotypes are metastases and drug resistance. Experimental modeling of evolution suggests that, provided there is some understanding of the fitness landscapes for these features, it should be possible to predict the likelihood or probability of future occurrence (172, 173). This has been tested with respect to predicting antibiotic drug resistance (174).

Pragmatically, developing predictive tests for progression of disease and drug resistance might best be achieved via surrogates for fitness. Genetic diversity and size of the stem cell pool are likely to be key variables reflecting the substrate available for selection. Progression of disease, or poor clinical outcome after treatment, has been found to be significantly associated with higher levels of stem cell activity (see above), as has genetic diversity within whole tumor populations (175, 176). Mathematical modeling and ecologic theory suggest that the harshness or diversity of the ecosystem of cancer, outside of the tumor cells themselves, should also be associated with progression of disease (177). This is endorsed by computational imaging of cellular diversity in tumor sections (178), expression signatures of stromal fibroblasts (179), and whole-tumor imaging (180). These data encourage the notion that evolutionary prognostication may assist patient management in the future.

EVOLUTIONARY THERAPEUTICS?

Even with optimized prevention, early diagnosis, and intervention, there will remain cancers that develop in a covert fashion and present late in their evolutionary trajectories with metastatic lesions or drug-resistant subclones on board. We see this currently with pancreatic cancer, glioblastoma, lung cancer, and many ovarian cancers for which the challenge of sustained control is considerable. It is well recognized that drug combinations will be required to thwart resistance (181), but the design of therapeutic strategies should be informed by a sounder grasp of the underlying biology (110) and explored by appropriate *in vitro* and *in vivo* modeling. There is a largely untapped opportunity here to exploit ideas derived from computational analysis of signal networks, teasing out inherent fragilities (75, 181–183), evolutionary game theory (184), and ecology (ref. 185; Fig. 5). Some of the tactics available may not bring about elimination of all cancer cells but rather slow down the evolutionary process, as with aspirin in gastrointestinal tumors (186, 187), or redirect and sustain stem cell dormancy, as with ABL kinase inhibitors in chronic myeloid leukemia (188). Serial monitoring is key to control of evolving populations (189), and in cancer, this is now possible in patients via tumor-derived DNA in plasma (24–26) and in model systems by high-resolution cell lineage tracking (190).

Because the resilience and robustness of cancer clones resides, to a large extent, in its branching or variegated evolutionary character, a better understanding of how this process is driven might provide therapeutic routes to reduce adaptiveness or fitness. Cancer is replete with evolutionary legacies. It might well yield to an evolutionary fix.

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No potential conflicts of interest were disclosed.

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