

Cancer as an evolutionary and ecological process

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Abstract | Neoplasms are microcosms of evolution. Within a neoplasm, a mosaic of mutant cells compete for space and resources, evade predation by the immune system and can even cooperate to disperse and colonize new organs. The evolution of neoplastic cells explains both why we get cancer and why it has been so difficult to cure. The tools of evolutionary biology and ecology are providing new insights into neoplastic progression and the clinical control of cancer.

Clone

A set of cells that share a common genotype owing to descent from a common ancestor. In some contexts a clone is more restrictively defined as a set of genetically identical cells.

Fitness

The average contribution of a genotype to future generations. Fitness is generally a function of both survival and reproduction.

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Cancer is a disease of clonal evolution within the body¹⁻³. This has profound clinical implications for neoplastic progression, cancer prevention and cancer therapy. Although the idea of cancer as an evolutionary problem is not new^{1,4}, historically, little attention has been focused on applications of evolutionary biology to understand and control neoplastic progression. That is now beginning to change⁵⁻¹³.

A neoplasm can be viewed from an evolutionary perspective as a large, genetically and epigenetically heterogeneous population of individual cells. Genetic and epigenetic alterations that are beneficial to a neoplastic clone, enabling it to expand, are generally deleterious to the host, ultimately causing death to both the host and the neoplasm. Because these somatic abnormalities have differing, heritable effects on the fitness of neoplastic cells, mutant clones might expand or contract in the neoplasm by natural selection and genetic drift, regardless of any negative effects on the organism. The fitness of a neoplastic cell is shaped by its interactions with cells and other factors in its microenvironment (its ecology), including interventions to prevent or cure cancer. Clonal evolution generally selects for increased proliferation and survival, and might lead to invasion, metastasis and therapeutic resistance.

Three decades of research have broadly supported Nowell's description of cancer, in 1976 (REF. 1), as an evolutionary system. Since 1976, researchers have identified clonal expansions¹⁴⁻¹⁷ and genetic heterogeneity^{5,8,13,18} within many different types of neoplasms. However, many promising opportunities for the application of evolutionary biology to carcinogenesis and oncology remain unexplored. What are the rates of genetic and epigenetic changes in a neoplasm? How can we alter those rates? How do clones expand and what can we do to control such expansions? What are the relative fitnesses of various carcinogenic alterations? What are the selective

effects of our therapies? Answering these questions will enable us to measure, manage and interrupt neoplastic progression and therapeutic relapse.

Here we examine cancer through the lens of evolutionary and ecological biology. We will review what is known about the evolution and ecology of neoplastic clones, examine the consequences of these dynamics and identify important missing pieces in the puzzle of neoplastic progression, its causes, prevention, and treatment of the resulting malignancies.

Levels of selection

Evolutionary forces work on many levels in biology¹⁹. Selection among somatic cells occurs on the timescale of a human lifetime. Selection on organisms, over millennia, has led to adaptations that constrain somatic evolution^{4,20}. An analysis of the trade-offs in the conflicting levels of selection helps to reveal not only our natural defenses against cancer, but also the nature of some remaining vulnerabilities to cancer^{2,21-24}. Organism-level and gene-level selection has led to the evolution of general tumour-suppression mechanisms (BOX 1) and oncogenic vulnerabilities in our genomes (BOX 2). This review will concentrate on selection and evolution in populations of cells, rather than individuals.

Mutation

Evolution requires heritable variation within the population. Various forms of mutation (defined broadly as any event that contributes to heritable variation between cells) have a role in neoplastic progression. Studies of heterogeneity in tumours clearly show that there is extensive cytogenetic, genetic and epigenetic variability in neoplastic cell populations, and the degree of variability can predict progression to malignancy^{8,13,18,25}. For example, every genetically distinct clone detected in a Barrett's

At a glance

- Neoplasms are composed of an ecosystem of evolving clones, competing and cooperating with each other and other cells in their microenvironment, and this has important implications for both neoplastic progression and therapy.
- Selection at the different levels of genes, cells and organisms might conflict, and have resulted in a legacy of tumour-suppression mechanisms and vulnerability to oncogenesis in our genomes.
- Most of the dynamics of evolution have not been measured in neoplasms, including mutation rates, fitness effects of mutations, generation times, population structure, the frequency of selective sweeps and the selective effects of our therapies.
- Many of the genetic and epigenetic alterations observed in neoplasms are evolutionarily neutral.
- Cancer therapies select for cancer stem cells with resistance mutations, although various evolutionary approaches have been suggested to overcome this problem, including selecting for benign or chemosensitive cells, altering the carrying capacity of the neoplasm and the competitive effects of neoplastic and normal cells on each other.
- Dispersal theory suggests that high cell mortality and variation of resources and population densities across space might select for metastasis.
- There is evidence of competition, predation, parasitism and mutualism between co-evolving clones in and around a neoplasm.
- We will need to interfere with clonal evolution and alter the fitness landscapes of neoplastic cells to prevent or cure cancer. Evolutionary biology should be central to this endeavor.

oesophagus pre-malignant lesion was associated with an increased risk of progressing to oesophageal adenocarcinoma by a factor of 1.4, and every 10% of genetic divergence between clones was associated with a further risk factor of 1.6 (REF. 8). Because the genetic instability that generates genetic heterogeneity is a ubiquitous characteristic of neoplasms, and is fundamental to the processes of neoplastic progression, it should be recognized as a hallmark of cancer²⁶. This heterogeneity poses a problem for the study and management of neoplasms because a biopsy sample might not be representative of the neoplasm, and the neoplasm continues to change after the biopsy sample is taken.

Genetic and epigenetic alterations are widespread in cancers. Stoler *et al.* estimated that there are at least 11,000 genomic alterations in the clone that generates a colon carcinoma, although many lie within non-coding regions²⁷. Widespread loss of heterozygosity might be fertile ground for recessive mutations to emerge. How cells survive and even flourish with losses as large as whole chromosomes remains unresolved²⁸, although the sheer number of changes suggests that most are effectively neutral for the clone, and many might even increase its fitness^{22,29,30}. Although it seems to be a relatively late event in neoplastic progression, the loss of *TP53* (the gene that encodes the tumour suppressor p53) normal cell cycle and apoptotic responses to chromosome breaks could confer such a large fitness advantage, by enabling cells to survive and divide, that the clone might be able to tolerate many deleterious mutations and still have a fitness advantage over p53 wild-type clones²⁹. It might be that most genes in the human genome are devoted to building and maintaining a multicellular body, and are therefore irrelevant to a neoplastic cell under selection for increased survival and proliferation^{22,31}. This might be analogous to organisms that switch from independent to obligate parasitic or mutualistic associations, like the ancestor of the mitochondrion, shedding genes that are no longer necessary for their new lifestyle³².

Changes in methylation patterns can alter the expression of genes and, as the methylation rate is thought to be faster than the genetic mutation rate, epigenetic mutations might be more likely to initiate neoplasms than genetic mutations^{33,34}. Hypermethylation has been shown to inactivate genes associated with DNA-damage response and repair, such as *MLH1*, *MLH3*, *MSH6* and *SFN*, in neoplasms^{35,36}. In these cases, epigenetic instability probably leads to genetic instability. Therefore, the effects of many forms of (epi)genetic instability are layered on top of one another as neoplasms progress.

Rates of different types of somatic mutation have not been measured *in vivo*, although the rates themselves

Box 1 | Control of somatic evolution

Uncontrolled somatic evolution is a fundamental source of neoplasia, but organisms have also found ways to exploit somatic evolution to their benefit. This is most evident in the adaptive immune system, which uses controlled clonal selection to defend against cancer¹³⁶. Somatic selection is also harnessed as a mechanism for efficiently eliminating (through apoptosis) any cells that are inappropriately proliferating or that have activated oncogenes^{43,55,137}.

Although some forms of somatic selection are harnessed by the organism for protection against cancer, the simplest and most wide-spread defense against cancer might be to suppress selection among cells where possible. Two primary mechanisms are thought to have key roles in the suppression of somatic selection: cellular senescence and cell differentiation.

If the number of cell generations is limited by senescence, this also limits the potential for multistage somatic evolution that underlies carcinogenesis. The dilemma faced by natural selection among organisms is how to enforce cellular senescence without creating organismal senescence that would reduce organismal fitness²³.

Similar to cellular senescence, cell differentiation limits the number of cell generations within any given cell lineage (FIG. 1). If a finite number of cell generations pass before the lineage ends in fully differentiated and non-dividing cells, this also limits the potential for multistage carcinogenesis¹³⁸. In addition, rapidly dividing epithelia, like the skin and gastrointestinal tract, continuously shed these cells from the body. To enable the renewal and maintenance of the organism, each tissue must also include non-differentiating somatic stem cells as a sustained source of new cells¹³⁹.

Given the existence within a tissue of both reserve cells and differentiating cells, the problem arises of how to organize these cell types in such a way as to minimize the risk of tumorigenesis. This optimization problem includes the relative number of reserve cells versus differentiating cells^{4,140,141}. It also involves the tissue architectures that subdivide cell populations and thereby help to limit clonal expansions^{4,7,140,142}.

Genetic drift

Random changes in allele frequencies over generations. This dynamic of random sampling has a greater effect in smaller populations.

Neutral mutation

A mutation that has no fitness effect (survival or reproductive effect).

Box 2 | **The evolution of cancer-susceptibility genes**

The maximization of fitness often involves trade-offs between different selective forces. In some cases, a germline oncogenic mutation, an allele that is particularly vulnerable to an oncogenic mutation, or an allele that disrupts tumour-suppressor gene networks, might spread in a population if the selective effects of cancer are overwhelmed by other fitness benefits of the mutation.

BRCA1 mutations seem to be more prevalent than would be expected given their carcinogenic effects on fitness and the generation of new BRCA1 germline mutations¹⁴³. Positive selection has been detected in the RAD51-interacting domain, which is important in the response of BRCA1 to DNA damage, although why there would be diversifying selection on DNA-damage response is unknown¹⁴⁴. BRCA1 alleles that predispose to breast cancer seem to have originated surprisingly recently, implying strong selection against them that probably cannot be explained by their carcinogenic effects¹⁴⁵. BRCA1 is involved in the spindle checkpoint, many cell-cycle checkpoints, the DNA-damage response and development^{146,147}. In addition, the high density of Alu repeats¹⁴⁸ increases the probability of somatic mutations in BRCA1, and might indicate conflicting selection between retrotransposons¹⁴⁹ and the host.

In development, cadherins contribute to epithelial differentiation, embryonic implantation and placenta formation, and in adults they form adherens junctions¹⁵⁰. Cadherins, particularly E-cadherin, are commonly lost in cancer and are associated with an invasive, metastatic phenotype. A comparison of cadherins between vertebrates suggests that some members of the cadherin family, those expressed during embryonic and/or fetal development, are subject to diversifying selection in humans¹⁵⁰.

A survey of evidence of recent selection in the human genome has implicated several genes that are associated with both cancer and spermatogenesis¹⁵¹. Crespi and Summers suggest that genes that are the subject of ongoing genetic conflict will both tend to show recent evolution and might be associated with cancer risk because the fitness effects of the genetic conflict overwhelm the selective effects of cancers that develop after reproduction¹⁵². These evolutionary conflicts might also play out through epigenetic imprinting, which has been shown to have dramatic carcinogenic effects¹⁵³.

would be fundamental biomarkers of progression and risk stratification, as well as tools to measure the effects of interventions. Knowledge of mutation rates would enable us to develop better surveillance protocols for high-risk patients. Mutation frequency studies and measurements in cell culture put the sequence mutation rate at 10^{-6} – 10^{-7} per locus per cell generation^{37,38}. Although genetic instability is a hallmark of cancer, an increase in mutation rate might not always be beneficial, as most non-neutral mutations are thought to be deleterious³⁹. In bacterial experiments, mutator phenotypes have emerged, although they did not evolve more quickly than non-mutator populations⁴⁰. Breivik has shown that the type of environmental insults (for example, methylating agents or bulky-adduct-forming carcinogens) select against the checkpoints that they trigger, because cells that lose those checkpoints can reproduce more quickly than those that stop to repair the damage²⁹. Therefore, the mutator phenotype might be selected owing to its effects on cell cycling rather than its generation of further advantageous mutations.

The number of mutations necessary and sufficient to cause cancer is unknown, even for retinoblastoma⁴¹. Estimations range from 3–12 mutations for different forms of cancer⁴². Organs with many cells and rapid turnover require more mutations^{42,43}. Loeb⁴⁴ argued that the spontaneous rate of somatic mutation is not high enough to generate so many mutations in a cell. To resolve this paradox, two hypotheses have been proposed: either a genetically unstable phenotype might arise that increases the mutation rate⁴⁴, or the expansion of clones generates

target populations large enough to produce the necessary subsequent mutations^{45,46}. The two hypotheses are not mutually exclusive⁴⁷, and we have shown that the clonal expansion of genetically unstable clones predicts progression to oesophageal adenocarcinoma⁴⁸. Determining exactly which mutations are necessary and sufficient to generate a cancer is important to help identify targets for cancer prevention, as well as biomarkers for risk stratification and early detection.

Neutral mutation and genetic drift

Changes in allele frequencies due to stochastic processes (BOX 3) might contribute to cancer progression. In small populations, chance might have an important role in altering allele frequencies. In general, parameters crucial for understanding the role of genetic drift in cancer progression have not been measured. These include the effective population size (the actual number of cells that contribute to future generations; N_e), cell generation times and cell turnover (the frequencies of cell division and apoptosis).

Genetic drift is intimately related to the selective advantage or disadvantage of a particular mutation and the size of the population of cells. Some mutations might have no selective effect and are considered neutral. If a particular mutation has a selective advantage much less than $1/N_e$, genetic drift is still the predominant force. Therefore, the definition of a neutral mutation is related to the type of mutation, the selective advantage and the population size⁴⁹.

Crucial to determining the effective population size, N_e , is an understanding of the role of cancer stem cells^{50–52} and normal stem cells⁵³ during carcinogenesis. Intestinal crypts seem to contain only a few stem cells, making the effective population size very small⁵⁴ (FIG. 1). Therefore, neutral and even deleterious (for example, genetic instability) mutations in stem cells might drift to fixation in a crypt⁵⁵.

The random loss or fixation of alleles might occur through reductions in cell population sizes ('population bottlenecks'). This can occur normally in the body, for example, through the apoptosis of breast epithelium during the menstrual cycle, in disease processes such as repeated wounding in ulcerative colitis and Barrett's oesophagus, and in cancer therapies. Mutations early in development can also generate large clones ('jackpots'), and these are predicted to have a significant affect on cancer incidence^{56,57}.

Many of the mutations seen in neoplasms seem to be neutral. There is evidence that many clones can coexist for a long time⁵⁶, suggesting that the mutations that distinguish these clones might be evolutionarily neutral, although there are many mechanisms that enable competitors to coexist (BOX 4). In addition, large numbers of neutral hitchhiker mutations⁵⁸ ('passengers') might be carried to fixation by adaptive mutations¹⁶.

Determining which carcinogenic mutations are neutral versus advantageous, depending on particular contexts of the microenvironment, will help predict clonal expansions and identify how we can change the microenvironment to make a carcinogenic mutation neutral or deleterious and prevent clonal expansion.

Fixation

When an allele (or in this case a clone) reaches 100% frequency in a population.

Hitchhiker mutation

An effectively neutral mutation that expands in a population because it is linked to a selectively advantageous allele. Sometimes called a 'passenger mutation' in cancer biology.

Box 3 | The theory of genetic drift

In genetic drift, individuals can leave different numbers of offspring by chance rather than fitness differences. Given enough time in a population of constant size, one clone will go to fixation and all others will go extinct. Therefore, if there are N cells, representing N clones, each clone has $1/N$ chance of reaching fixation. Furthermore, assuming a Moran model¹⁵⁴ in which cells divide and die asynchronously, the expected time it takes for a clone to expand from a single cell to fixation is $N(N-1)$ total cell divisions¹⁵⁵. Clinically detected neoplasms are often 10^9 – 10^{12} cells, so the chance of fixation by genetic drift is vanishingly small, and the time that would take is far longer than a human lifetime.

These results assume populations of constant size and overlapping generations (Moran model¹⁵⁴) with no recombination, no population sub-structure and no fitness effects of mutations. Populations that violate some of these assumptions often behave as idealized populations of a different size N_e , called the effective population size. In a neoplasm, the total number of cells can be much larger than the effective population size owing to differentiation, limited replicative potential, changes in population size and the occurrence of selective sweeps.

A mutant is likely to go extinct even if it has a selective advantage. Therefore carcinogenic mutations might appear and go extinct many times before one is lucky enough to attain a population size that is no longer in danger of going extinct by genetic drift alone. For example, a mutation in a stem cell with a 10% fitness advantage over its competitors (fitness advantage: $s = 0.1$) would have a 91% chance of going extinct by genetic drift before it could sweep to fixation in a population of $N_e = 10^6$ stem cells¹⁵⁵. In a neoplasm with $N = 10^9$ cells and $N_e = 10^6$ stem cells, there is only a 1 in 1,000 chance that the mutation occurs in a stem cell, and so the chance of extinction increases to 99.99%. If a new mutation has a relative fitness advantage, the chance of extinction is shown by equation 1 (adapted from REF. 155).

$$1 - P = 1 - \frac{1 - (1 + s)^{-1}}{1 - (1 + s)^{-N_e}} \left(\frac{N_e}{N} \right) \quad (1)$$

In addition, identifying neutral mutations might enable us to use them as a molecular clock to determine the time since the initiation of a neoplasm^{5,6}.

Natural selection

The heritable variation of reproductive success in a population is necessary and sufficient to cause natural selection⁵⁹. Natural selection occurs in neoplasms because (epi)genetic mutations generate heritable variation, and some mutations confer a selective advantage or disadvantage on the cell. All the hallmarks of cancer²⁶ lead to the differential reproductive success of a clone. These fitness advantages will be amplified in tissues with repeated wounding, in which repeated cycles of cell death and proliferation enable a mutant clone with a survival or reproductive advantage to expand.

The presence of proliferating and apoptotic cells in neoplasms implies that clones can expand and contract. Mutations that increase the fitness of a clone might lead to a selective sweep through the population of cells, eventually reaching fixation in the neoplasm (FIG. 2). In most cases, it is unknown how clones expand through a neoplasm and if there are population sub-structures that inhibit those expansions. Both clonal expansions^{14–17} and carcinogenic exposures might explain field effects in carcinogenesis⁶⁰. The expansion of a pre-malignant clone that seems histologically normal can predispose a large region to further progression and result in multi-focal and locally recurrent cancers¹⁵. Clonal expansions driven by epigenetic mutations have not yet been established.

If a clonal expansion is driven by the mutation of a tumour suppressor or oncogene (a hypothesis often tested *in vitro* but rarely *in vivo*^{14,16}), then those lesions are good candidates for biomarkers of progression because they are causally related to cancer outcome and can be easily sampled.

A crucial unresolved question is why patterns of gene loss and/or gain differ between cancers in different organs and cell types? It will be important to understand selective pressures in different organ environments. In addition, whether or not a gene is used in a normal cell type will affect the fitness of the cell with mutations in that gene⁶¹.

Mutations in some genes are only advantageous to the clone after there has been a lesion in another gene. For example, in Barrett's oesophagus, the inactivation of *TP53* is almost always observed after the inactivation of *CDKN2A* (the gene that encodes the tumour suppressor *INK4a*)¹⁶. It is possible, in a case like this, that a mutation that is neutral on its own could expand by genetic drift before a second mutation in that clone makes the first mutation selectively advantageous. However, it is more probable that the mutation that is selectively advantageous on its own (for example, in *CDKN2A*) will initiate a clonal expansion that creates many opportunities for the other mutation (for example, in *TP53*) to occur, sparking a second clonal expansion within the first. Such genetic dependencies (BOX 5) lead to regularities in the order in which mutations appear. Linear⁶² and tree models⁶³ of progression that implicitly rely on genetic dependencies and their predictive value⁶⁴ might be improved by testing the implied dependencies.

Artificial selection

Cancer therapies often select for resistance, caused by various mechanisms, which is the central problem in cancer therapy. At relapse, mutant clones have been discovered in lung cancer with point mutations in epidermal growth factor receptor (*EGFR*) that cause resistance to anilinoquinazoline *EGFR* inhibitors⁶⁵. In chronic myeloid leukaemia, an amino-acid change in *BCR-ABL* confers resistance to imatinib (Glivec)⁶⁶, and amplification of the thymidine synthase gene causes resistance to 5-fluorouracil in colorectal cancer⁶⁷. This shows that therapies do not simply select for cancer stem cells⁶⁸, but also cancer stem cells with resistance mutations⁶⁹.

The number of cell divisions (and the potential for mutational events) before therapy far outweighs those after therapy. A classic early experiment in evolutionary biology⁷⁰ tested whether the exposure of a bacterial population to a selective pressure (the presence of a phage) caused new mutations, or if applying the pressure selected for pre-existing mutants. The second case proved to be true. The same principle is expected to apply to cancer, although mutagenic therapies might generate resistance mutations⁷¹. There is evidence for resistance mutations before the application of Glivec⁷². The implication is that the earlier we intervene in progression, the less probable it is that a resistant mutant will emerge⁶⁹.

Cancers that develop without selection for genetic instability or enough time to produce much genetic heterogeneity should be unlikely to harbour a resistant clone⁷³.

Molecular clock

When mutations occur at a constant rate, the number of mutations that have accumulated between two different lineages is representative of the time since the lineages diverged.

Selective sweep

The process of an adaptive mutation spreading through a population, typically ending in fixation.

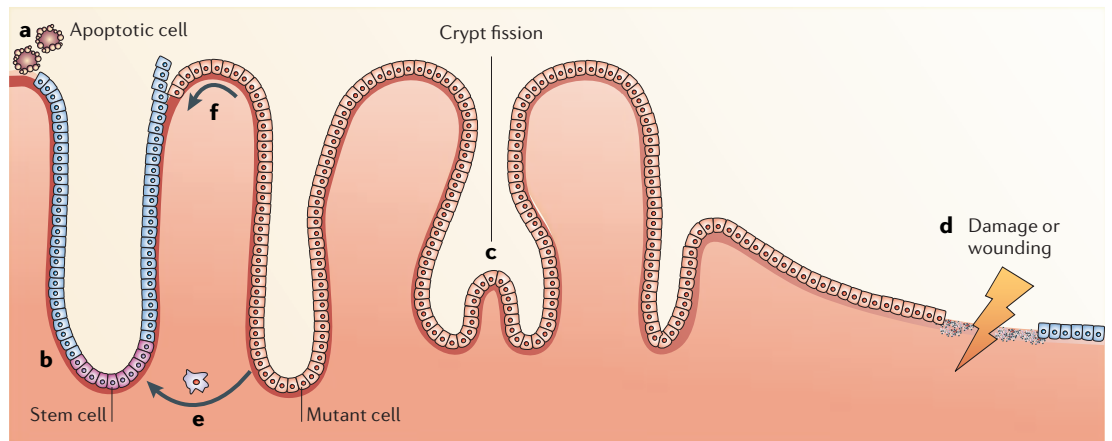


Figure 1 | Intestinal tissue architecture and sub-population structure. **a** | Cells differentiate as they move up the crypt, and eventually initiate apoptosis and slough into the lumen. **b** | Each crypt is continually renewed by a small number of long-lived stem cells that reside near the bottom of the crypt. Therefore, crypts sub-divide the epithelium into isolated sub-populations. In some conditions¹⁶, mutant clones (red) can expand over many crypts, although the mechanism of this expansion is unknown. **c–f** | Hypotheses include: crypt fission (**c**); wounding with epithelial restitution (**d**); dispersal through the basement membrane and stroma into the base of neighbouring crypts, perhaps through epithelial–mesenchymal–epithelial transitions (**e**); and, more speculatively, dispersal over the surface of the epithelium (**f**), along the basement membrane, and then down into neighbouring crypts against the flow of cells emerging from the crypt (which might require differentiation and then dedifferentiation).

This is probably the case for most pre-clinical models of cancer. If only a few mutations are required to produce a clinically detectable neoplasm, then the neoplasm is less likely to be genetically diverse, and so is less likely to harbour a resistant clone compared with a neoplasm that must accumulate many mutations before it is detected. Many childhood cancers seem to require few mutations^{42,74}. In cases such as retinoblastoma, there are few cells vulnerable to progression, and they are only vulnerable for a short period of time. Therefore, only a few tumour-suppressor genes are required to prevent retinoblastoma in most children⁴³. The importance of detecting a neoplasm before wide-spread genetic heterogeneity develops is consistent with clinical experience that shows increased survival with the detection of early-stage disease⁷⁵.

There are several possible evolutionary approaches to cancer therapy and prevention that could address the problem of therapeutic resistance. These include multi-drug therapies⁷⁶, therapies that work to alter competition between cancerous and non-cancerous cells by boosting the fitness of benign cells¹⁰, selection for chemosensitivity¹⁰, selection for genetic stability⁷⁷ and the induction of crippling bottlenecks. Of these strategies, only multi-drug therapies have been explored experimentally and/or clinically⁷⁶. The way a therapy is applied might also affect the evolutionary dynamics in a neoplasm. Evolutionary experiments show that the application of selective pressures in pulse versus continuous treatment can alter the outcome of competition⁷⁸. Traditional chemotherapies are applied in large pulsed doses, but evolutionary theory, and evidence from anti-angiogenic therapy⁷⁹, suggests that lower, continuous doses might work better. Neoplastic cell populations that expand between doses might generate new resistance mutations⁷⁹. In addition, under pulses of a therapy,

the fitness of a neoplastic cell is the average of its fitness during therapy and its fitness between doses, weighted by the duration of those conditions. This is likely to be higher than the fitness under a lower but continuous dose, although pulses of extremely high doses have also been shown to be efficacious in some cases⁸⁰.

The population bottleneck caused by cancer therapy might be able to cripple a neoplasm. Following therapy, many patients with leukaemia show minimal residual disease, in which a very small population of leukaemic cells remain as a stable subpopulation, and do not grow exponentially as would be characteristic of cancer⁸¹. One hypothesis for the population stability is that the characteristics selected by chemotherapy might also interfere with proliferation. For example, if a cancer drug only kills proliferating cells, then quiescent cells might survive the treatment and remain quiescent thereafter⁸¹. Alternatively, if the bottleneck is small enough, cells with fitness disadvantages can become fixed in the neoplasm by genetic drift. Because the rate of evolution is very slow in small populations, it might take a very long time before a leukaemic clone acquires mutations that enable it to expand again.

Dispersal and colonization

Allele frequencies can change (evolution can occur) through dispersal. There are at least three ways in which dispersal can be important in cancer: the movement of cells between the partially isolated sub-populations of proliferative units, local invasion of neighbouring tissues and emigration of metastatic cells from the primary tumour.

The epithelium of most organs is organized into proliferative units such as crypts in the intestine (FIG. 1), acini in liver and breast, proliferative units in squamous epithelium and so on. These proliferative units form

Box 4 | Mechanisms of coexistence

Cells in a neoplasm seem to compete for the same resources, space and nutrients, and so we would predict that a clone with a fitness advantage should drive other clones extinct as it sweeps to fixation. However, there is evidence that clones can coexist for many years^{5,6} (FIG. 2), and that clonal diversity might increase with progression⁸. How can more than one clone stably coexist in a neoplasm? Ecology and evolution suggest various mechanisms:

- Mutations might be evolutionarily neutral, providing no fitness advantage, and therefore no selective sweep.
- Fitness might be density dependent, so that as a clone becomes more frequent in the population, its fitness decreases. This might be caused by an immune reaction (predation), one clone gaining a fitness benefit by proximity to another clone (parasitism), or pollution of its environment by metabolic byproducts.
- Niches: clones might specialize on different resources or different microenvironments, and thereby reduce their competition¹⁰⁷.
- If the environment fluctuates faster than any one clone can reach fixation, then clones adapted to the different environments could coexist in non-equilibrium.
- Clones might be physically separated, and therefore unable to invade each other's territory⁴ (FIG. 1).
- The total population might be expanding, therefore reducing competition for space¹³.

Which, if any, of these mechanisms are at work in neoplasms is an important open question in cancer biology.

semi-isolated sub-populations, which typically include a small number of stem cells and a larger number of transient amplifying and fully differentiated cells^{4,54}. The observation of clonal expansions^{16,82,83} implies that some mutants can breach the barriers between proliferative units. In most cases, we do not know how clones expand (FIG. 1). In the skin, UV light can destroy proliferative units that might be reconstituted by neighbouring mutants¹⁴. Does clonal expansion always require some form of wounding, or is there normal turnover of proliferative units? Mutants might also spread by dispersal between proliferative units.

This 'local metastasis' hypothesis could explain genetically related multi-focal tumours in some tissues, but has not been rigorously tested⁸⁴.

Metastasis requires that cells leave the primary tumour, but few such cells successfully colonize a distant organ⁸⁵. This leads to a paradox: metastatic clones should have a fitness disadvantage relative to non-metastatic clones in the primary tumour owing to the loss of the progeny that emigrate. How could a metastatic clone expand and produce enough metastatic cells to successfully colonize a distant site^{86,87}? Early⁸⁷ or late in progression⁸⁸, mutations that confer a metastatic phenotype might also provide a fitness advantage within the primary tumour that can compensate for the loss of emigrating progeny. Alternatively, metastatic mutations might be hitchhikers on selective sweeps, and their phenotype might be triggered later by a change in the tumour environment. An analogous example of compensating pleiotropy can be found in the evolution of ageing, in which a mutation that increases fitness before reproduction might be advantageous to the organism even if it causes decreased fitness later in life⁸⁹. Hitchhiker mutations that reach fixation and then become deleterious with a change in environment are difficult to observe.

Within a single population of organisms, there is selection against dispersal. The main selective advantage of dispersal is the colonization of new populations⁹⁰. Colonizing individuals often have high fitness because they can escape from deteriorating local conditions caused by population growth and the over-consumption of resources. The high density⁹¹ and necrotic centres⁹² of most solid neoplasms suggest that space and nutrients are limited. This leads to fierce competition, so there might be selection for dispersal.

Other conditions also select for dispersal, including high mortality rates, the variation of resources across space (for example, because of neoangiogenesis) and time (for example, because of wounding), and even stochastic fluctuations in local population densities⁹³. If neoplastic cells, like many organisms, face trade-offs between local competition and dispersal, then local

Box 5 | How to study evolution in neoplasms

The study of evolution rests on measuring changes in the frequency of (epi)genetic variants in a population. This requires measuring different clones in a neoplasm, which in practice entails:

- Isolating the cell population of interest.
- Extracting and assaying DNA in the purified population.
- Measuring the frequency of (epi)genetic lesions in the DNA.

If clones in the neoplasm can first be separated (for example by flow cytometry), then patterns of (epi)genetic alterations can be associated with specific clones, and frequencies of those lesions can be measured by the frequencies of the clones in the neoplasm. The easiest way to separate clones is to take more than one biopsy from a neoplasm, separated by space, and to analyse each biopsy separately.

Analysing several biopsies from a neoplasm also enables the powerful but under-used technique of genetic-dependency analysis (clonal ordering) to be used, in which the order in which genetic lesions arose can be inferred from the spatial patterns of shared lesions¹⁵⁶. That is, if one biopsy has lesions in loci A and B, and another biopsy only has a lesion in locus A, we can infer that the lesion in locus A probably occurred first, was associated with a clonal expansion, and the lesion in locus B occurred later. If this pattern occurs in many neoplasms, it is evidence that lesions in locus B are only selectively advantageous in cells with the locus A lesion, and so there is a genetic dependency between the lesions.

Tracking clones as they evolve over time would be even better than clonal ordering from single time points. Such studies have already been reported from several conditions, including oesophageal squamous-cell carcinoma¹⁵⁷, Barrett's oesophagus^{8,158–160}, oral leukoplakia^{161,162} and ulcerative colitis^{163,164}. Serial biopsies can also be obtained during randomized trials to prevent or treat some cancers, for example, gastric¹⁶⁵ and prostate cancer¹⁶⁶. A randomized trial offers the opportunity to observe clonal adaptation to the intervention, which might provide valuable information in designing new trials even if the original was unsuccessful.

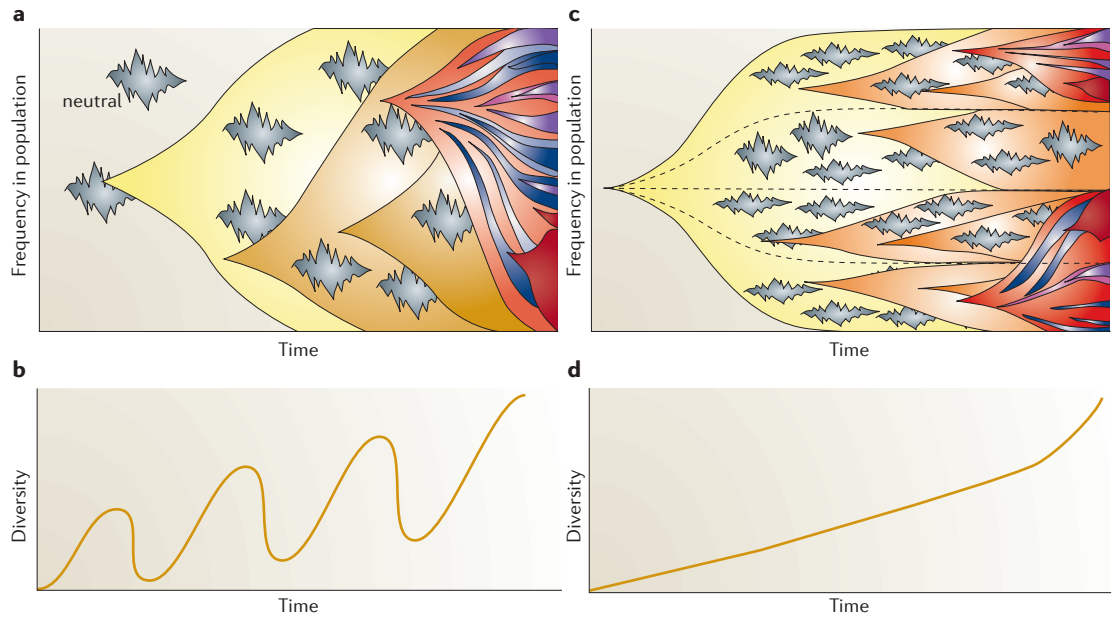


Figure 2 | Asexual evolution in neoplastic progression. Frequency within a neoplasm is shown on the Y-axis and time on the X-axis. **a** | If a neoplasm acts like a single population of cells, then an adaptive mutant can sweep through the population and become fixed (yellow, orange and red clones). Multistage carcinogenesis is thought to represent a series of such selective sweeps. The emergence of a clone with high levels of genetic instability (red) might accelerate the generation of new clones. **b** | Genetic diversity should fluctuate, increasing as genetic instability generates new clones and decreasing when a clone homogenizes the neoplasm in a selective sweep. **c** | If the neoplasm is divided into sub-populations (dashed lines) or there is a diversity of microenvironments that create different niches, then selective sweeps will tend to be constrained within a sub-population or niche, although they might occasionally invade a neighbouring sub-population. **d** | In a sub-divided population, total diversity might increase over time because selective sweeps cannot homogenize the entire population. Figure modified with permission from REF. 16 © (2004) American Association for Cancer Research.

therapeutic interventions that penalize cell proliferation, such as radiotherapy, will favour the ability to metastasize over the ability to compete within a neoplasm. It might even be possible to select against the emergence of metastasis (and resistance⁷³) by relaxing these constraints on a neoplasm, but this remains to be tested in preclinical models and might be difficult to translate to the clinic.

The seed and soil hypothesis⁹⁴ suggests that metastasis is analogous to the colonization of a new habitat. Success at colonization of an ecosystem seems to depend on the characteristics of the invader⁹⁵, the climate⁹⁶, available space and resources in the new ecosystem and the configuration of native organisms⁹⁷. A predictive model of metastasis might benefit from the identification and measurement of similarities in the ‘climate’ (microenvironment) between organs. There is some evidence that polyploidy in plants, and perhaps aneuploidy in neoplasms, is associated with an ability to invade new environments⁹⁸, perhaps owing to an increased opportunity for mutations, deletions and genetic rearrangements with the presence of extra alleles⁹⁹. Some ecological studies have supported the hypothesis that increasing species complexity in an ecosystem facilitates further invasions⁹⁷. The relationship between cell type complexity in an organ and its colonization by metastases has not yet been studied, but a recent experiment in *Escherichia coli* suggests that colonization by a superior competitor is more probable

in a genetically diverse population than a community with few genetic variants¹⁰⁰.

Ecology

Ecology studies the dynamics of communities of species and their interactions. Ecological interactions can be classified by their fitness effects on the interacting individuals (FIG. 3). Examples of many different ecological interactions can be found in neoplasms, and most of these deserve further study.

Competition. For neoplastic cells in a heterogeneous population, competition exists in the form of resource consumption (oxygen for example). However, neoplastic clones can also have direct negative effects on each other through unknown soluble factors^{101,102}. Neoplastic clones injected into opposite flanks of mice¹⁰³ and rats¹⁰⁴ can inhibit each other’s growth, although in some cases the inhibition only affects one of the clones, and so is an amensal interaction (FIG. 3). Apparent competition can also occur in neoplasms in which one clone can stimulate an immune response that clears other clones and the immunogenic clone.

Carcinogenesis models based on Lotka–Volterra competition equations define conditions under which cancerous cells might be driven extinct. These include reducing the number of cancer cells that can be supported in the tissue, reducing the negative competitive effects of cancer cells on normal cells and increasing the

Amensal

An interaction between individuals that decreases the fitness of one party but has no effect on the other.

Lotka–Volterra competition equations

The Lotka–Volterra model of competition is based on logistic growth equations of two populations that negatively affect each other’s growth.

negative competitive effects of normal cells on cancer cells¹². These and other models can help define the parameters that must be targeted by therapies and the most effective methods for drug treatment regimens^{11,105–107}.

Predation. Predator species have negative effects on their prey, and gain some growth and reproductive benefit in return. Models of predation might be applicable to the interaction of neoplastic cells with the immune system.

Neoplasms evolve various mechanisms to escape predation from the immune system, including downregulation of the major histocompatibility complex¹⁰⁸. The various mechanisms that a neoplasm can use to escape the immune system suggests that immune therapies are unlikely to work except in neoplasms with little genetic heterogeneity. Activated cytotoxic T lymphocytes do not directly benefit from the destruction of neoplastic cells, although they clonally expand in response to activation by antigen-presenting cells, and so the end result is the same. One dissimilarity here is that a predator will go extinct if its prey goes extinct. This is clearly not the case for T cells if they clear the neoplasm.

Minimal residual disease might be understood in terms of a predation model or a population bottleneck as discussed above. It is possible that residual neoplastic cells are not quiescent and are continually culled by the immune system¹⁰⁹. If activated lymphocytes and neoplastic cell populations fluctuate in a typical predator–prey dynamic, we might be able to drive the neoplastic cells extinct by amplifying the fluctuations, perhaps by increasing the time lag between neoplastic clonal expansion and immune response. In populations of organisms, chaotic population fluctuations can be an effective source of local extinctions¹¹⁰.

Parasitism. Parasitism is similar to predation, in that one species benefits at the expense of the other, although parasites often produce many offspring without killing their host. There is little evidence of clones within a neoplasm parasitizing each other. However, there is ample opportunity for clones to be free-riders on the metabolic investments of their neighbours, such as stimulating associated fibroblasts to release growth factors, stimulating neo-angiogenesis or the breakdown of the extracellular matrix and the release of growth factors contained within^{107,111}, and so on. Such parasitism between lineages is known in microbes¹¹² and viruses¹¹³, and can be referred to as a ‘cheater strategy’¹¹⁴ because the parasitic clones gain a fitness benefit from their neighbours at no cost to themselves.

Mutualism and commensalism. Little is known about cooperative (mutualistic and commensal) relationships (FIG. 3) within neoplasms. However, Heppner, Miller and others have shown that a mutant clone can increase the fitness of other clones in commensal interactions, and even confer a metastatic phenotype on an otherwise non-metastatic clone^{3,103,115}. Axelrod *et al.* have proposed that clones in a neoplasm could cooperate through diffusible factors, and thereby circumvent the requirement

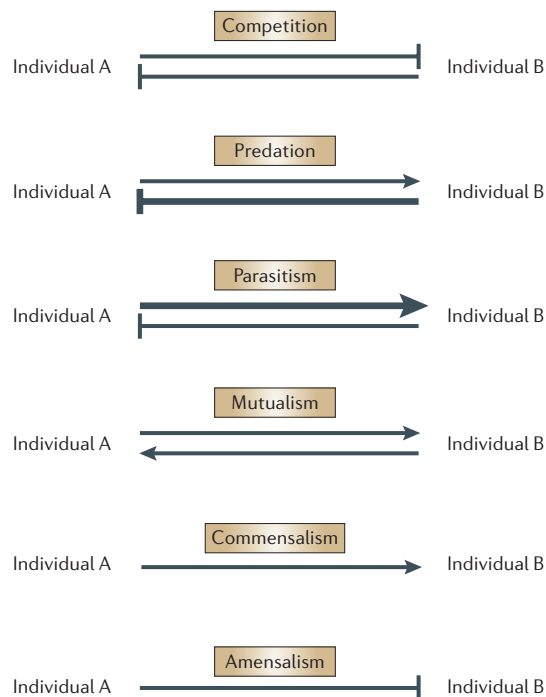


Figure 3 | Ecological interactions. Ecological interactions can be classified by the fitness effects of the individuals (neoplastic cells) on each other. Fitness effects can be positive (arrow) negative (closed arrow) or there might be no effect (no arrow). There are many mechanisms that can result in the different types of interactions¹⁶⁷, even in a neoplasm. For example, parasitism and predation are distinguished by the size not the type of the effects (sizes of the arrows), and clones might compete through the consumption of resources or by inhibiting each other through cell signalling¹⁰². Indirect interactions between clones might occur through direct interactions with a third type of cell, as in the case of a neoplastic clone reducing the fitness of another clone through the stimulation of an immune response^{101,103,104,168}.

that a single clone has to accumulate all the hallmarks of cancer¹¹⁶. To date, the only known case of mutualism in a human neoplasm is the relationship between neoplastic epithelium and activated fibroblasts, both of which get a fitness advantage from the association^{117–119} and seem to be co-evolving^{120–122}.

The environment

The microenvironment of neoplastic cells has a dramatic effect on progression¹²³. Placing teratocarcinoma cells in a mouse blastocyst is enough to suppress their carcinogenic phenotype¹²⁴. Metastasis can be suppressed by the injection of a metastatic cell line into a heterotopic site¹²⁵. Conversely, normal mammary epithelial cells can in some cases develop into invasive carcinomas in an environment that mimics activated stroma through the overexpression of hepatocyte growth factor (HGF) and/or transforming growth factor β 1 (TGF β 1)¹²⁶. Increased expression of HGF or stromal cell derived factor 1 (SDF1) by fibroblasts promotes epithelial

Mutualistic
An interaction between individuals that increases the fitness of both parties.

Commensal
An interaction between individuals that increases the fitness of one party and has no fitness effect on the other.

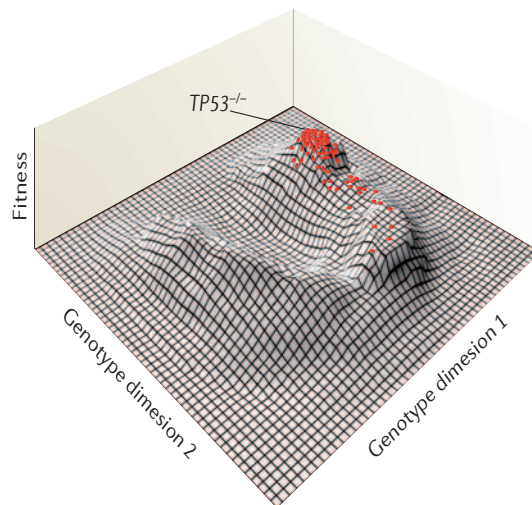


Figure 4 | Evolution of a neoplastic population. A highly simplified representation of a neoplastic cell population as a cloud of points evolving on a fitness landscape. Here, genotype is represented in the X and Y dimensions, and fitness is represented in the Z dimension. Locations are connected if a mutation (any possible genetic alteration) can change one genotype into a neighbour genotype. Evolving populations will typically move up fitness gradients by natural selection and only descend by mutation and genetic drift. Regions of neutral mutations are plateaux in a fitness landscape. Regularities in neoplastic progression reflect regularities in the fitness landscape. For example, if the points that represent genotypes missing *TP53* have high fitness, neoplasms will often evolve loss of *TP53*, although the paths they take to that region of the fitness landscape might differ. The fitness of genotypes, and therefore the topography of the fitness landscape, depends on the local microenvironment, including the ecology of other cells present. Interventions can be visualized as deformations of the fitness landscape.

neoplasms in mice^{127,128}. These experiments show that we can modulate progression by altering the neoplastic environment.

Repeated, moderate disturbance of cell populations might select for genetic diversity and progression. With too little disturbance the environment is relatively homogenous, and the best competitors drive weaker competitors extinct. Too much disturbance wipes out populations entirely¹²⁹. Perhaps chronic wounding promotes neoplasms by providing a diversity of micro-environments at different stages of recovery.

Differences from organismal evolution

The evolution of neoplasms differs in important ways from the ecology and evolution of organismal populations. Many of the formulae and phenomena analysed in evolutionary theory concern sexually reproducing species. Neoplastic cells are like asexual, single-celled organisms with limited horizontal transfer of genes within the neoplasm¹³⁰ and few life-history changes once differentiation has been abrogated. Asexual reproduction of neoplastic cells means there is no meiotic recombination,

no Hardy–Weinberg equilibrium of genotypes in the population and no sexual selection. Different cell types in the body are unlike species in that a stem cell can differentiate into any cell type, and non-stem cells might be able to trans-differentiate into different types¹³¹. The relatively short time frame, and the large-scale genomic alterations in neoplastic progression suggests that neoplastic cells will be unable to evolve complex adaptations to their environment. Most neoplastic mutations seem to remove pathways that suppress proliferation or trigger apoptosis^{26,31}, or co-opt pathways normally used in development and wound healing.

Parallels to organismal ecology also have their limitations. With a few important exceptions¹¹⁷, neoplasms do not contain many species or food webs. There is little diversity of resources in a neoplasm, so there are probably limited opportunities for specialization to different niches, except to the extent that there are different microenvironments in an organ.

Many of the differences between neoplasms and populations of sexual organisms simplify the study of evolution in neoplasms. Experimental evolution studies in bacterial systems have helped elucidate the roles of selection and drift in populations, the development of mutator phenotypes and the dynamics of adaptation¹³². Cancer systems share a similar empirical tractability. Asexual reproduction is easier to analyse than sexual reproduction. More importantly, we have access to the ancestral genotype in the normal tissues of the body, which enables us to study how the neoplasm has changed. Evolution in a neoplasm occurs on a timescale of years, not millennia. Life on Earth has provided us with a single example of how evolution can occur, making it difficult to distinguish regularities from historical accidents. By contrast, every new neoplasm is an example of how neoplastic evolution can proceed, modified by the genotype and exposures of a particular individual. Therefore, we might be able to map out the regularities of the fitness landscape that constrains neoplastic evolution^{106,133} (FIG. 4). In fact, efforts to develop models of the order of lesions in neoplastic progression are, in effect, the cartography of neoplastic fitness landscapes.

Conclusions and future directions

To understand cancer, we need to understand and measure the population dynamics and evolutionary parameters of neoplasms. These measurements provide biomarkers that can be used for risk stratification, intermediate endpoints and targets for new drugs. One study in HIV has shown that anti-viral therapy reduced the rate of HIV evolution by two orders of magnitude¹³⁴. Can this be shown in cancer? We need to understand the fitness landscapes of neoplasms to better predict how a particular neoplasm will evolve. We will also need to interfere with clonal evolution — change the fitness landscape and push the population of neoplastic cells down alternative paths to prevent and treat cancer¹⁰. To understand the evolutionary consequences of our therapeutic strategies, we need to assay the genetics of neoplasms both before and after interventions as part of clinical trials. The development of inexpensive,

Fitness landscape
A multi-dimensional space in which every point represents the genotype or phenotype of a cell and its fitness value. Points are connected if a mutational event can transform one genotype (or phenotype) into the other.

high-throughput, single-cell genomic assays will be important to all these endeavors.

Because neoplastic evolution tends to produce therapeutically resistant clones, one of the most powerful strategies is to prevent the initiation of a neoplasm in the first place, as might be achieved with the use of human papillomavirus vaccines to prevent cervical cancer¹³⁵. If initiation cannot be prevented, the early detection of a neoplasm, before it develops a high degree of genetic heterogeneity, will probably lead to increased cure rates⁷⁵.

The presence of clonal competition is an unavoidable fact of cancer biology. No matter how we intervene in

a neoplasm, some cells will grow back to fill that space. Whether those cells are normal or neoplastic will depend on the therapy and the mosaic of neoplastic cells that was present before therapy. We have highlighted that the fundamental problems of neoplastic progression and cancer therapy are also problems of evolutionary biology, and so it will be important to integrate evolutionary biologists into our cancer research teams. In addition, evolutionary biology should be a required part of the training of cancer biologists and oncologists. The application of evolutionary biology and ecology to cancer is already helping us to better understand, predict and control this disease.

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Competing interests statement

The authors declare no competing financial interests.

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