OPINION

Evolutionary dynamics of carcinogenesis and why targeted therapy does not work

Robert J. Gillies, Daniel Verduzco and Robert A. Gatenby

Abstract | All malignant cancers, whether inherited or sporadic, are fundamentally governed by Darwinian dynamics. The process of carcinogenesis requires genetic instability and highly selective local microenvironments, the combination of which promotes somatic evolution. These microenvironmental forces, specifically hypoxia, acidosis and reactive oxygen species, are not only highly selective, but are also able to induce genetic instability. As a result, malignant cancers are dynamically evolving clades of cells living in distinct microhabitats that almost certainly ensure the emergence of therapy-resistant populations. Cytotoxic cancer therapies also impose intense evolutionary selection pressures on the surviving cells and thus increase the evolutionary rate. Importantly, the principles of Darwinian dynamics also embody fundamental principles that can illuminate strategies for the successful management of cancer.

Evolution is a tinkerer. Francois Jacob (1977)[127]

In 1976, Peter Nowell proposed a model for somatic evolution in carcinogenesis, which was based on both his own prior work and the work of others[1]. Despite a lack of detailed genetic data, this model developed a prescient description of later data that demonstrated mutational heterogeneities in cancer[2,3]. More recently, insightful and profound evolutionary models of carcinogenesis have been developed, but they have not addressed the exact microenvironmental selection factors that direct cancers to evolve more malignant phenotypes[4,5]. In this Opinion article, we integrate microenvironmental factors that are at work during cancer progression, specifically environmental stressors such as hypoxia and acidosis. These commonly observed factors not only select for malignant phenotypes, but also affect genomic stability itself. Thus, this potential 'unifying theory' places the evolution of the genome within a dynamically changing adaptive landscape, the outcome of which is genotypic and phenotypic heterogeneity, which both negatively affect the ability of targeted therapies to exert cancer control.

Although cancer is conventionally defined as a disease of the genes, we propose that a teleological understanding of cancer will not necessarily emerge from cataloguing the vast number of genetic changes observed in clinical tumours. We[6], and others[4,5], have proposed that a unifying analytical framework can be found in evolutionary theory. Interestingly, Darwin knew nothing of genetics. As he described it, the dynamics of evolution simply required a mechanism of inheritance. Indeed, the successful characterization of evolution and ecology proceeded for nearly a century prior to the development of robust molecular methods. This success reflects two often neglected first principles of natural selection: nature selects for phenotype, not genotype, and population changes are dependent on local environmental selection forces. In multicellular organisms, many key traits are polygenetic so that the mapping of genetics to phenotypes is often imprecise. Thus, it is well recognized that common phenotypes in both cancer and normal cells can have myriad genetic causes[7]. In cancers, evolution is fundamentally driven by environmental selection forces that interact with individual cellular strategies or phenotypes, which supervene cell genetics. Understanding cancer as a disease starts with identifying crucial environmental forces and corresponding adaptive cellular strategies. Characterizing evolving populations solely by their genetic changes prior to understanding these fundamental evolutionary forces is likely to be futile[6].

Even if we accept evolution as a unifying paradigm, substantial limitations in our current application of these principles must be recognized. Specifically, although cancers are widely described as heterogeneous, it is commonly assumed (and hoped) that tumours are well-mixed and synchronous. Thus, tumours are commonly described by single attributes of drivers, such as ER-positive, triple-negative, mutant BRAF-expressing and so on. However, selection in cancers is explicitly local in nature, and the resulting phenotypic heterogeneity within individual tumours is germane to therapy response. Each cancer cell competes within its immediate environment to form an ecological and evolutionary horizon. Thus, tumours can be thought of as 'continents' that are populated by multiple cellular species that adapt to regional variations in environmental selection forces. It may be postulated that the greater this diversity of niches is, the poorer the prognosis[8]. Although this apparent chaos is daunting, tumours nonetheless remain governed by evolutionary principles and hence, specific patterns of selection and adaptation can be predicted, identified and exploited. In earlier work, we proposed intratumoral hypoxia and acidosis as strong evolutionary selective pressures that lead to common metabolic phenotypes of cancers[9,10]. In this article, we further this thesis by showing that hypoxia and acidosis may function both as regional selection forces and as promoters of rapid adaptation by inducing genomic alterations, which we contend is an atavistic response to environmental stress.

Mutator phenotypes

It is acknowledged that cancers are associated with profound alterations in the genome at multiple levels, including epigenetic regulation, point mutations, deletions, duplication and wholesale chromosomal rearrangements. What is less commonly appreciated is that these changes occur heterogeneously within a single tumour. Hundreds of gene mutations can be found in tumours. This may occur by the emergence of a mutator phenotype[11], which can be induced by heritable genetics, viral infections, or variations in microenvironmental conditions. Although mutation rates in cancers may not be different from those of normal tissues[2], it is undisputed that mutations accumulate, often to high levels, possibly owing to the abrogation of cell cycle and DNA integrity checkpoints. Genetic alterations can be directly induced by an inhibited or a reduced DNA repair response or by external genotoxic stressors. Genetic alterations can also indirectly accumulate by the inhibition of apoptosis, or even more indirectly by the induction of hyperplasia, leading to the important environmental sequelae of hypoxia, the generation of reactive oxygen species (ROS) and acidosis. These are combined in a unifying model (FIG. 1). Notably, the accumulation of mutations will only emerge

when there is strong evolutionary selection and the current local phenotype is not at a fitness maximum[12]. Hence, the mere existence of diverse mutations and chromosomal translocations in cancers at presentation implies a high degree of environmental selection in growing tumours.

Heritable mutators. At least 5% of all cancers can be attributed to inherited mutations (reviewed in REFS 13,14). A comprehensive list of genes that are known to lead to heritable cancers is provided in REF. 13, and it is illuminating in many ways. More than 50% of these genes are mutations or deletions in either DNA damage response (DDR)-associated pathways or inhibition of apoptosis, which, respectively, lead directly or indirectly to the accumulation of genetic alterations[15]. Other genes that are associated with inherited mutations and tumour development modulate growth factor-independent or adhesion-independent proliferation[13], both of which can lead to hyperplasia. An unanswered question is the effect of these heritable mutations on normal tissues in humans, for which there are few data. In mice, hyperplasia has been observed in response to *Pten* knockout, which can eventually lead to neoplasia[16]. Similarly, mice that express the proto-oncogene *Ret* consistently develop hyperplasia[17]. We speculate that humans inheriting these mutations might also develop hyperplasia prior to the predisposed incidence of cancer. Hyperplastic epithelia outgrow their blood supply and can become hypoxic and acidic[9,10], and these environmental sequelae amplify genomic instability, as described below. Thus, the vast majority of heritable cancer genes lead directly or indirectly to genomic alterations. Notably, these data are biased for non-lethality, as genes that control tightly regulated processes cannot be deregulated without being embryonic lethal[18].

Microenvironmental mutators. An important component of the current model is that somatic evolution occurs on an adaptive landscape that is entirely local. Thus, cells are responding to direct microenvironmental influences and are not susceptible to systemic perturbations unless these, in turn, alter the local microenvironment. At a single-cell level, genomic instability occurs in the presence of environmental stress. These stressors can be lethal and thus provide strong selective pressure along with genome instability. Those that do not die are winners in the evolutionary game (BOX 1). This increase in genome instability with environmental stress is an atavistic response, as it is observed in microorganisms such as yeast and bacteria[19], and can be observed in mammalian cells under stress[20,21].
The physical microenvironment of a nascent tumour is constantly changing, often in response to inflammation. Chronic inflammation is associated with the majority of sporadic cancers[22,23], and is the product of an immune response to infection, environmental factors and diet[24]. Inflammation is associated with cytokine-induced hyperplasia and ROS-induced cell death and genotoxicity. Cells in hyperplastic epithelia can grow into ductal lumens making them exist further from their blood supply, leading first to episodic intraluminal hypoxia, selection for a glycolytic phenotype (the Warburg effect) and consequently increased acidity, as well as nutrient and growth factor deprivation. These harsh conditions can also be viewed as an altered adaptive landscape with a significant increase in the slope of the fitness function (BOX 1). This altering adaptive landscape selects cells that are able to overcome microenvironmental barriers, such as hypoxia and acidosis. This is consistent with the observations of increased mutational frequency of reporter genes in xenografts compared with *in vitro* cultures, which can be ascribed to microenvironmental stressors of hypoxia and/or acidosis[25–27].

At the systemic level, genetic anomalies can also be directly acquired. Viruses can directly affect genome stability through insertion mutagenesis or p53 inactivation[28,29]. Additionally, there are environmental mutagens, such as those found in tobacco, coal tar and ultraviolet radiation[30–33]. As with inflammation, however, environmentally induced mutations will not lead to the outgrowth of cancers in the absence of local environmental selection that is mediated by an altered adaptive landscape.

Hypoxia and ROS. Hypoxia can be present early in carcinogenesis, even in *in situ* cancers[34–36]. In invasive disease, tumour hypoxia is a strong predictor for the presence of metastasis (reviewed in REF. 37). Hypoxia can lead to genomic instability through multiple mechanisms, such as ROS-induced DNA damage, replication restart errors and decreased activities of the DDR machinery, including mismatch repair and methylation silencing of BRCA1 (REFS 38–40). Re-oxygenation after hypoxia, or the presence of free iron during haemolysis, can induce ROS production and the activation of the DNA damage-associated kinase ataxia telangiectasia mutated (ATM)[41]. Genes that encode proteins that are involved in homologous DNA repair (such as RAD51 and RAD52) may also be downregulated, forcing cells to repair double-stranded breaks with the error-prone non-homologous end joining (NHEJ) pathway. Severe chronic hypoxia can select for apoptosis resistance or mutated p53 (REFS 42,43), further contributing to the accumulation of mutations. Intermittent hypoxia can lead to gene duplication or to wholesale chromosomal rearrangements[44–46]. From an evolutionary standpoint, gene duplication provides cells with the ability to interrogate new evolutionary trajectories at a minimal cost, as the original gene function is preserved[47]. This has been well established for the evolution of species, and we speculate that this powerful mechanism might also be true for cancer cells.

Acidosis. Through a combination of increased metabolism and poor perfusion, the extracellular pH of solid tumours can

Glossary

Atavistic
Reverting to or suggesting the characteristics of a remote ancestor or primitive type. In the current context, atavism is the expression of behaviours in cancer cells that are not normally observed in normal metazoan cells, but that are observed in prokaryotes and/or protozoa.

Clades
A taxonomic group of organisms classified together on the basis of homologous features traced to a common ancestor. In the current context, groups of cancer cells evolve in physically distinct niches, and exhibit local genetic homogeneity.

Nuclear grade
Breast cancers are assessed for the appearance of nuclei within the tumour cells and assigned a grade from 1 (small uniform cells) to 3 (marked nuclear variation).

Supervene
Describes a mathematical and philosophical formalism that characterizes the relationship between two sets — in this case phenotype (or more broadly adaptive strategies) and genotypes. In the subvenient set (genetics) each point will map to a point in the phenotype set, and in the supervenient set (phenotypes) each adaptive strategy can map too many different points in the genotype set.

Teleological
Describes a doctrine that final causes exist, and thus that purpose is a part of nature. In the current context, teleology dictates that cancers exist for a (self-serving) purpose. Thus, we ask why, and not how, cancers behave the way they do.

Theory
A coherent group of general propositions that can be used as principles of explanation and prediction for a class of phenomena. A proposed explanation the status of which is still conjectural and subject to experimentation.

reach values as low as 6.5 (REFS 48,49). Acidosis alone can be clastogenic, inducing chromosome breakages and translocations in both rodent and human diploid lines[50]. Although the mechanism of acid-induced genomic instability is unknown, low pH can induce double-strand breaks through ROS[51] and/or inhibition of topoisomerase II[52]. An unanswered question in studies of extracellular pH is how environmental pH affects intracellular events, as the intracellular pH is tightly regulated[53,54]. There are numerous mechanisms for cells to sense environmental pH, such as pH-sensitive G proteins and ion channels, and these may be involved in acid-induced signal transduction[55–57]. A low pH also results from a high rate of glycolysis, and the combination of acidosis with the resultant glucose deprivation may also provide a strong selection for activated oncogenes[58].

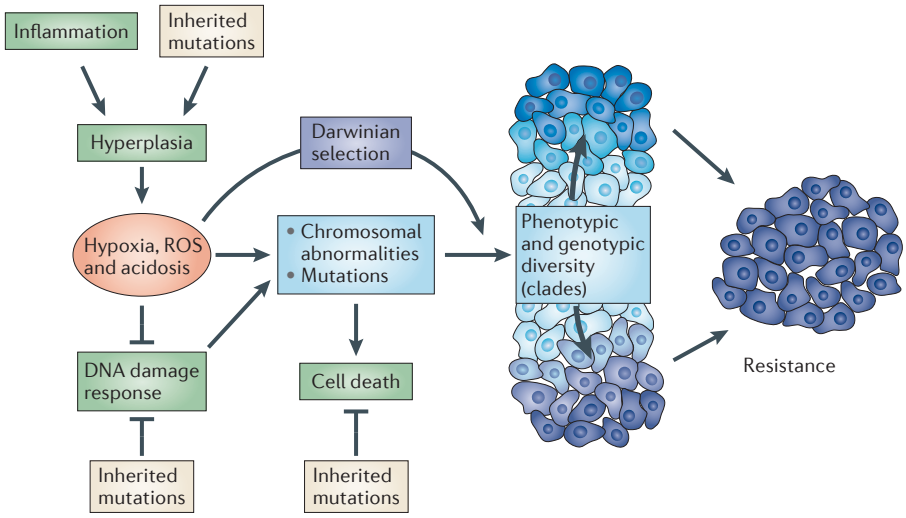

Figure 1 | **A unifying model of carcinogenesis.** Inflammation is implicated in most sporadic cancers and induces both hyperplasia and the release of reactive oxygen species (ROS), which are genotoxic. Hyperplastic epithelia grow intraluminally and have regions of chronic and intermittent hypoxia, which leads to the inhibition of the DNA damage response (DDR) machinery, as well as the induction of ROS. The combination of increased genotoxicity through ROS and decreased DDR increases the accumulation of mutations, which will normally cause cell death, but which can accumulate if cell death response pathways are inhibited. Hypoxia also selects for cells with a glycolytic phenotype (the Warburg effect), and an important sequela of glycolysis is intratumoral acidosis. Acidosis is clastogenic and leads to chromosomal abnormalities. Inherited mutations are indicated by cream boxes and include those that induce hyperplasia and metabolic defects, defects in the DDR machinery itself, and diminished efficiency of the cell death machinery. Notably, hypoxia, acidosis and ROS can also impart strong evolutionary selection, as well as increase genomic instability. The combination of genome instability along with Darwinian selection increases the rate of evolution and leads to the growth of distinct clades within tumours. The resulting genotypic and phenotypic diversity of nascent tumours leads to malignancy, and in the context of therapy, resistance.

Spatiotemporal heterogeneity

The induction of genomic alterations and localized selection by heritable and/or environmental factors will result in phenotypic heterogeneity. Heterogeneity can be viewed radiographically, in which a non-uniform pattern of enhancement or attenuation ('tumour texture') can be associated with poor outcome[59,60]. Even in pre-invasive, ductal carcinoma *in situ* (DCIS) breast cancers, a large number of microenvironmental niches can be identified histologically[8]. In this study[8], 112 DCIS cases were analysed for nuclear pleomorphism across multiple sections. Notably, more than 40% of tumours with more than one nuclear grade assigned to them were positive for mutated p53, suggesting that defects in this tumour suppressor led to an increased incidence of nuclear, and thus, genetic heterogeneity.

Physiological heterogeneity. Each tumour is an ecosystem that is inhabited by physical, physiological and metabolic factors, normal cells, inflammatory cells and the actual populations of tumour cells. An important physiological factor is tumour perfusion, which is often characterized as heterogeneous, or even chaotic. This became an established and measurable quantity with the advent of dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), which measures the time-dependent distribution of contrast agents[61,62]. The heterogeneity can be quantified and has been shown to be a powerfully negative prognostic factor[63,64]. Perfusion heterogeneity causes periodic and chronic deficits in metabolic substrates, particularly oxygen[65,66], and pH[48]. It is thus not surprising that regions of tumours with different perfusion patterns also have substantially different gene expression[67,68] and proteomic profiles[69]. Although expression changes may be reversible, they are also associated with genetic changes at the chromosomal and genome levels, which are not reversible.

Genetic heterogeneity. In 1930, Winge induced cancers in 80 mice with coal tar, and examined each tumour histologically. When possible, he counted chromosomes in multiple individual cells in the same tumour. In doing this, he documented that cells in the same tumour contained 35–138 chromosomes (normal diploid = 40)[70]. Although aneuploidy is a well-known hallmark of cancer[71], this study documented that a wide variation in chromosome number can occur in a single tumour. This is recapitulated in nuclear structure, as fractal and texture analyses of nuclei have also been shown to have high prognostic significance[72]. It has long been appreciated that this chromosomal instability is matched by a genetic instability[11,73]. Thus, it is not surprising that similar intratumoral heterogeneities in the genetic code are also observed. In 2010, Vogelstein's group sequenced the genomes from 11 different regions in the same pancreatic tumour and observed multiple constellations of mutations[74]. Notably, these patterns were not random, so that an evolutionary map of clades could be developed for this particular cancer, which probably evolved in distinct environmental niches. More recently, Gerlinger *et al.*[3] have carried out profound genomic analyses of four renal cell cancers and have reached the identical conclusion: that morphological heterogeneity is recapitulated in genomic heterogeneity with identifiable evolutionary trajectories. Notably, in the work of Gerlinger *et al.*[3], multiple instances of convergent evolution were observed, reinforcing the axiom that nature selects for phenotype, not genotype.

Individual cancers can accumulate and heterogeneously express many dozens of exomic mutations. It has become convention to classify some of these as 'drivers' that directly affect cancer cell proliferation or survival; and others are 'passengers' that are assumed to be phenotypically silent. This strict segregation is misleading because, as noted above, gene mapping to phenotype can be imprecise and environmental selection forces will vary in time and space.

Box 1 | **Evolutionary game theory**

The existence of a harsh environment and genotypic heterogeneity can be formally combined in evolutionary game theory, which can be summarized in a basic equation governing evolutionary rate[125]:

$$\partial\mu/\partial t = \sigma^2(\partial G/\partial\mu)$$

$\partial\mu/\partial t$ is the evolutionary rate at which the strategy (phenotype) (μ) of a population varies with time (t). In this context, strategy represents the phenotypes that control proliferation in the local environment. σ is the phenotypic diversity, which generally reflects genetic diversity. However, the genotype–phenotype relationship is non-stoichiometric, as genetic mutations may be phenotypically silenced through the action of molecular chaperones[126]. Notably, this equation states that the rate of evolution increases with the square of phenotypic diversity. $\partial G/\partial\mu$ is the slope of the fitness function, which relates the sensitivity of fitness, (G), to changes in phenotype, (μ). A harsh environment generally produces a high slope, meaning that even small changes in phenotype can cause large variations in fitness. This relationship explicitly links evolving cancer populations to both intracellular and environmental properties. Specifically, cancer populations that are phenotypically heterogeneous or that live in harsh, cytotoxic environments will evolve rapidly if they are below their fitness maximum. Importantly, environment and phenotypic diversity are also fundamentally coupled in that a stressful environment (hypoxia and acidosis) will lead to increased diversity (genetic alterations) via atavistic mechanisms. Administration of cytotoxic agents will convert even a stable tumour environment into one that is more selective, with a high value of $\partial G/\partial\mu$. This fundamental principal must be taken into account when devising therapeutic approaches.

Thus, genetic mutations that are crucial to survival in one environment may have a minimal role at another time under different conditions. Furthermore, although they may not provide an obvious growth advantage, passenger mutations have been shown to result in subtle phenotypic variations[75], further resulting in intratumoral phenotypic heterogeneity. Passenger mutations may be phenotypically silent until exposed to a specific selective condition, under which the mutation may confer a selective advantage, such as drug resistance[76]. The relationship between phenotypic diversity, local selection and evolutionary rate can be combined in evolutionary game theory formalism (BOX 1).

Evolutionary approaches to therapy

The past few decades have witnessed tremendous increases in our knowledge of the complex web of molecular signals that are deregulated in cancer and the development of specific agents to target these pathways. However, even when there is a well-known target and a highly specific drug, increased survival is generally measured in months, not years[77]. Although there are some long-term survivors[78–80], for most advanced cancers and most patients, response to therapy is fleeting, owing to the inevitable evolution and proliferation of a resistant population[81]. Because of large-scale genomic alterations and consequent diversity, the emergence of resistance is predictable as a fundamental property of carcinogenesis itself. This fundamental fact is commonly ignored in the design of treatment strategies[82]. Although challenging, the application of evolutionary principles can illuminate alternative therapeutic approaches.

It is 'chess', not 'whack-a-mole'. The emergence of drug resistance is rarely, if ever, dealt with until it occurs. We contend that it should be anticipated in an effort to develop patient-specific long-term therapeutic strategies. For example, populations that respond to an initial treatment will pass through an evolutionary bottleneck, which would render them transiently and extremely susceptible to a secondary therapy[4]. The choice of this therapy should be anticipated.

It has been claimed that combination therapies, analogous to those used in HIV, will provide sustained remissions[83]. However, HIV has five essential and four accessory genes, whereas cancer cells have thousands of genes and controlling elements that can have an influence. Although this is an intimidating thought, the number of possible resistance mechanisms seems to be finite. For example, the resistance of non-small-cell lung cancer (NSCLC) to tyrosine kinase inhibitors, such as erlotinib, can occur by 12 known mechanisms[81]. Although this is a large number, it may be tractable. As the most common mechanism of erlotinib resistance is a T790M point mutation in the epidermal growth factor receptor (EGFR), combination with an EGFR-specific antibody would be expected to forestall this type of resistance. Such an approach has been tried in ERBB2 (also known as HER2)-positive breast cancer with a combination of an ERBB2-specific antibody, trastuzamab, and a small-molecule inhibitor, lapatinib, that has resulted in some sustained responses[84]. Also in breast cancer, there is an apparent inverse relationship between oestrogen receptor (ER) levels and growth factor signalling pathways[85]. Hence, the increased expression of growth factors or growth factor receptors may allow continued proliferation of breast cancers in the absence of ER. This does not seem to be growth factor-specific, as epidermal growth factor (EGF), insulin-like growth factor 1 (IGF1), transforming growth factor-β (TGFβ), fibroblast growth factor (FGF) and heregulin can all down-regulate ER protein expression. Downstream, these growth factors can activate common pathways, such as the mTOR pathway. These signalling dynamics provide opportunities for applying evolutionary principles to targeted therapy. Recent clinical trials have shown that adding an mTOR inhibitor (everolimus) in combination with an anti-oestrogen aromatase inhibitor (exemestane) significantly increased progression-free survival[86]. Although combination therapies such as this are appealing, one could also use an evolutionary approach by starting with one therapy (anti-oestrogen, for example) that is both toxic and that provides selection forces promoting increased expression of growth factor receptors. Tumour cells expressing growth factor receptors can then be treated in a manner that promotes the increased expression of ER. This represents an 'evolutionarily futile' cycle that would effectively allow prolonged tumour control.

The future development of similar approaches can use several paradigms. First, it might be possible to develop biomarkers that would predict which resistance mechanisms will be favoured in a given patient. Such resistance mechanisms could be targeted (or pretreated) in combination with the standard treatment regimen, or they could be alternated. Each of these approaches can be modelled *in silico* prior to the commencement of therapy, to generate an interactive and individualized treatment strategy[87]. Second, biomarkers to detect resistance mechanisms early during recurrence need to be developed to define an adaptable treatment schedule that accounts for and overcomes these mechanisms. Third, such approaches can be used adaptively.

Adaptive therapy. It is a mantra of modern therapy that we need to treat the right drug, in the right patient, at the right time. Although considerable effort has been expended to define the right drug–right patient paradigm, there have been few, if any, advances in complex dosing schedules that

would identify the right time. Such dosing should exploit evolutionary principles to prolong tumour control by suppressing the proliferation of resistant populations.

In the absence of drug, one can infer that resistant cells are less fit than sensitive cells, as untreated cancers generally have a preponderance of cells that are sensitive to primary therapies. In controlled studies, it can be observed that some resistance mechanisms do indeed have a fitness penalty in which the resistant clones grow slower than the parental sensitive cells[88–90]. This is probably related to resource allocation to resistance mechanisms (such as upregulation and function of *p*-glycoprotein), which would reduce the energy available for proliferation. However, a fitness penalty for resistance cannot be assumed. In some cases, the resistant clones appear to grow just as fast as the parental cells. This may be the case for T790M-mutated EGFR[88] or for cells expressing the 190 kD multidrug resistance protein MRP1 (REF. 91). Nonetheless, if there is a penalty for resistance, treating tumours with sub-lethal doses of targeted therapy, and only treating when faced with quantifiable tumour growth, has the potential to prevent the emergence of a resistant population[90]. Such a paradigm is standard-of-care for some liquid cancers that can be easily monitored, and has recently been applied to hormone-sensitive solid cancers. With the advent of anti-androgens (such as abiraterone), men with prostate cancer are often treated periodically, primarily to reduce side effects. Although these therapies are not used adaptively, drug holidays can delay the emergence of a lethal, androgen-independent phenotype[92]. In hormone-sensitive breast cancer, periodic, compared with continuous, tamoxifen may delay the emergence of an oestrogen receptor-negative phenotype[93]. To our knowledge, such an approach has not been attempted in patients with an evolutionarily informed dosing schedule, or with non-hormonal pathway-specific targeted therapies.

Targeting phenotypes and selection forces. Phenotypes, rather than specific gene products, of cancer can be attractive as therapeutic targets. This is an old concept, as most early chemotherapeutics (such as anti-folates) were developed to inhibit a common metabolic phenotype associated with proliferation[94]. Angiogenesis inhibitors are often viewed as targeting a phenotype[95] as they interrupt vascular development and supposedly kill tumour cells through substrate deprivation. However, when viewed through an evolutionary lens, this is simplistic because angiogenesis inhibitors also alter the environment (through increased hypoxia and acidosis), which produces strong Darwinian forces that rapidly promote adaptive strategies, including increased invasiveness. Not surprisingly, anti-angiogenic therapy has shown little benefit as a monotherapy[96]. However, we note that the ability to predictably alter the adaptive landscape of a tumour remains a powerful evolutionary tool and, thus, combinations of anti-angiogenics with follow-on drugs that target the adapted phenotypes are likely to be successful[97,98]. More recently, the concept of targeting the phenotype has been expanded to target altered glucose metabolism and its sequelae. Agents targeting glucose metabolism have been developed at all levels of the metabolic pathway, including glucose transport, its metabolic intermediates and end products, and these have shown effects preclinically in combination with other targeted therapies[99–102]. Tumour acidosis follows from increased glycolysis and can lead to increased invasion and metastasis[103]. This acidity can be neutralized using buffers, such as sodium bicarbonate, imidazoles or lysine, which can inhibit the formation of spontaneous or experimental metastases[104–106]. Buffers have also been shown to increase the efficacy of weak-base chemotherapeutics through the reduction of ion trapping, which will increase the intracellular distribution of drugs[107].

Cancers are often characterized as diseases of proliferation, but it can equally be claimed that cancer is a disease of cell death. It may be that almost all malignant, drug-resistant cancers are deficient in apoptosis. Thus, rational targeting to re-stimulate sensitivity to apoptosis could have general applicability. However, this is daunting, as nature selects for phenotype (apoptosis resistance) and not for the myriad mechanisms that are available to cells to evade suicide[108]. Thus, as with targeted therapy, the efficacy of an apoptosis-inducing agent will depend on the specific mechanisms that are expressed by a specific patient's tumour. Additionally, the selective pressure to reduce apoptosis is strong and thus evolutionary game theory predicts that resistant clones would rapidly emerge. Nonetheless, it can be argued that apoptosis is rarely a component of normal physiology in adults and thus remains an attractive target, and that combination therapies to prevent the occurrence of resistance may be well tolerated. Apoptosis-promoting therapies that have shown some success include bortezemib to inhibit proteosomes[109], dichloroacetate to restore mitochondrial function[101,110], cell death cytokines such as TRAIL[111] and mTOR inhibitors such as rapamycin or everolimus[112–114]. Therapy based on the premise of tumour evolution suggests that these agents should be effective if used rationally in combination with drugs that should stimulate an apoptotic signal, and in combination with each other to prevent the emergence of a resistant phenotype.

Smart bombs, not magic bullets. As an alternative to targeting agents against specific signal transduction pathways, cancer control can theoretically be achieved through the delivery of regionally toxic agents to kill target cells with some collateral damage to surrounding cells, both cancerous and supporting stroma. This is the promise of radioimmunotherapy, which has been effective in managing liquid cancers in their bone marrow niche[115] and are being increasingly developed for solid tumours[116]. There is growing interest in the use of α-emitters, as compared with β-emitters, as they maximize collateral damage and are less susceptible to radioresistance[117,118]. As an alternative to molecular targeting, agents are also being developed to selectively deliver high dose chemotherapeutics or radionuclides to the unique tumour pathophysiology; that is, regions that are hypoxic or acidic, with the rationale that these conditions are not present in normal tissues. A maturing concept is to develop pro-drug carriers that will release their 'warheads' only under hypoxic or acidic conditions. For hypoxia, these pro-drug agents are generally based on 2-nitroimidazoles that are irreversibly reduced in the absence of oxygen. This involves an electronic rearrangement that results in the cleavage of the bond between the nitroimidazole and the drug[119,120]. Some of these agents have shown tumour-specific effects across a variety of cancers in Phase I/II clinical trials, either as monotherapy or as a combination with standard chemotherapeutics[121,122], and are now in Phase III trials. Although earlier in development, acidic regions can be targeted by small drug-carrying nanoparticles that dissolve at low pH, releasing their contents[123,124]. It may be possible, through the judicious use of these agents, that the most evolutionary selective regions of cancers can be periodically targeted, leading to the long-term management of this disease. However, from an evolutionary standpoint, such approaches will have to be strictly monitored, as the local delivery of high-dose therapy will add a further selection pressure to an already highly selective niche.

Conclusions

We propose a unifying model in which malignant cancers, regardless of aetiology (spontaneous, infectious or heritable) emerge following Darwinian dynamics. It is important

to recognize that somatic evolution is generated by complex local interactions between environmental stressors, adaptive strategies and genomic instability. Epigenetic alterations, mutations and chromosomal rearrangements contribute to continued cellular evolution but genetic changes, per se, are not sufficient for evolution to occur. Cancer cell development, like any Darwinian process, is governed by environmental selection forces and cellular adaptive strategies that are phenotypes or combinations of phenotypes. Attempting to characterize cancers through observed genetic changes and ignoring the adaptive landscape is most likely to be futile. Indeed, independent microenvironmental niches in a growing tumour have a high degree of physiological and genomic heterogeneity, leading to divergent phenotypes, which dramatically increase the potential evolutionary rate, instilling malignant cancers with an ability to be dynamically adaptable. Under the selective pressure of chemotherapy, resistant populations will invariably evolve. However, although the emergence of resistance is inevitable, the proliferation of resistant populations is not. It is important to recognize that cancer cells can only adapt to immediate selection forces — they cannot anticipate future environmental conditions or evolutionary dynamics. Importantly, we can anticipate; and this is our fundamental advantage in designing new therapeutic strategies. We can use our understanding of somatic evolution to strategically direct Darwinian processes to prevent the outgrowth of resistant cancer populations and so improve outcomes. Acknowledging this in therapy planning might lead to the sustainable management of cancers, and we have used this to enumerate a number of evolutionarily informed non-exclusive therapeutic strategies.

Robert J. Gillies, Daniel Verduzco and Robert A. Gatenby are at the Department of Cancer Physiology and Biophysics, H. Lee Moffitt Cancer Center and Research Institute, Tampa FL 33602, USA.
Correspondence to R.J.G.
e-mail: Robert.Gillies@Moffitt.Org

doi:10.1038/nrc3298
Published online 14 June 2012

1. Nowell, P. C. The clonal evolution of tumor cell populations. *Science* **194**, 23–28 (1976).
2. Jones, S. *et al.* Comparative lesion sequencing provides insights into tumor evolution. *Proc. Natl Acad. Sci. USA* **105**, 4283–4288 (2008).
3. Gerlinger, M. *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
4. Gerlinger, M. & Swanton, C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. *Br. J. Cancer* **103**, 1139–1143 (2010).
5. Greaves, M. & Maley, C. C. Clonal evolution in cancer. *Nature* **481**, 306–313 (2012).
6. Gatenby, R. A., Gillies, R. J. & Brown, J. S. Of cancer and cave fish. *Nature Rev. Cancer* **11**, 237–238 (2011).
7. Gillies, R. J., Robey, I. & Gatenby, R. A. Causes and consequences of increased glucose metabolism of cancers. *J. Nucl Med* **49** Suppl 2, 24S-42S (2008).
8. Allred, D. C. *et al.* Ductal carcinoma *in situ* and the emergence of diversity during breast cancer evolution. *Clin. Cancer Res.* **14**, 370–378 (2008).
9. Gatenby, R. A. & Gillies, R. J. A microenvironmental model of carcinogenesis. *Nature Rev. Cancer* **8**, 56–61 (2008).
10. Gatenby, R. A. & Gillies, R. J. Why do cancers have high aerobic glycolysis? *Nature Rev. Cancer* **4**, 891–899 (2004).
11. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. *Cancer Res.* **51**, 3075–3079 (1991).
12. Cahill, D. P., Kinzler, K. W., Vogelstein, B. & Lengauer, C. Genetic instability and darwinian selection in tumours. *Trends Cell Biol.* **9**, M57–M60 (1999).
13. Garber, J. E. & Offit, K. Hereditary cancer predisposition syndromes. *J. Clin. Oncol.* **23**, 276–292 (2005).
14. Chung, C. C. & Chanock, S. J. Current status of genome-wide association studies in cancer. *Hum. Genet.* **130**, 59–78 (2011).
15. Negrini, S., Gorgoulis, V. G. & Halazonetis, T. D. Genomic instability--an evolving hallmark of cancer. *Nature Rev. Mol. Cell Biol.* **11**, 220–228 (2010).
16. Podsypanina, K. *et al.* Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. *Proc. Natl Acad. Sci. USA* **96**, 1563–1568 (1999).
17. Michiels, F. M. *et al.* Development of medullary thyroid carcinoma in transgenic mice expressing the RET protooncogene altered by a multiple endocrine neoplasia type 2A mutation. *Proc. Natl Acad. Sci. USA* **94**, 3330–3335 (1997).
18. Ferrara, N. *et al.* Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* **380**, 439–442 (1996).
19. Zhang, Q. *et al.* Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* **333**, 1764–1767 (2011).
20. Davies, P. C. & Lineweaver, C. H. Cancer tumors as Metazoa 1.0: tapping genes of ancient ancestors. *Phys. Biol.* **8**, 015001 (2011).
21. Goncharova, E. I., Nadas, A. & Rossman, T. G. Serum deprivation, but not inhibition of growth per se, induces a hypermutable state in Chinese hamster G12 cells. *Cancer Res.* **56**, 752–756 (1996).
22. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
23. Grivennikov, S. I., Greten, F. R. & Karin, M. Immunity, inflammation, and cancer. *Cell* **140**, 883–899 (2010).
24. Aggarwal, B. B., Vijayalekshmi, R. V. & Sung, B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. *Clin. Cancer Res.* **15**, 425–430 (2009).
25. Papp-Szabo, E., Josephy, P. D. & Coomber, B. L. Microenvironmental influences on mutagenesis in mammary epithelial cells. *Int. J. Cancer* **116**, 679–685 (2005).
26. Reynolds, T. Y., Rockwell, S. & Glazer, P. M. Genetic instability induced by the tumor microenvironment. *Cancer Res.* **56**, 5754–5757 (1996).
27. Wilkinson, D., Sandhu, J. K., Breneman, J. W., Tucker, J. D. & Birnboim, H. C. Hprt mutants in a transplantable murine tumour arise more frequently *in vivo* than *in vitro*. *Br. J. Cancer* **72**, 1234–1240 (1995).
28. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. & Howley, P. M. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* **63**, 1129–1136 (1990).
29. Brechot, C., Pourcel, C., Louise, A., Rain, B. & Tiollais, P. Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature* **286**, 533–535 (1980).
30. Anand, P. *et al.* Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* **25**, 2097–2116 (2008).
31. Minamoto, T., Mai, M. & Ronai, Z. Environmental factors as regulators and effectors of multistep carcinogenesis. *Carcinogenesis* **20**, 519–527 (1999).
32. DeMarini, D. M. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. *Mutat. Res.* **567**, 447–474 (2004).
33. Ikehata, H. & Ono, T. The mechanisms of UV mutagenesis. *J. Radiat. Res.* **52**, 115–125 (2011).
34. Chitneni, S. K., Palmer, G. M., Zalutsky, M. R. & Dewhirst, M. W. Molecular imaging of hypoxia. *J. Nucl. Med.* **52**, 165–168 (2011).
35. Wykoff, C. C. *et al.* Expression of the hypoxia-inducible and tumor-associated carbonic anhydrases in ductal carcinoma *in situ* of the breast. *Am. J. Pathol.* **158**, 1011–1019 (2001).
36. Gillies, R. J. & Gatenby, R. A. Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? *J. Bioenerg. Biomembr.* **39**, 251–257 (2007).
37. Bristow, R. G. & Hill, R. P. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nature Rev. Cancer* **8**, 180–192 (2008).
38. Klein, T. J. & Glazer, P. M. The tumor microenvironment and DNA repair. *Semin. Radiat. Oncol.* **20**, 282–287 (2010).
39. Lu, Y., Chu, A., Turker, M. S. & Glazer, P. M. Hypoxia-induced epigenetic regulation and silencing of the BRCA1 promoter. *Mol. Cell. Biol.* **31**, 3339–3350 (2011).
40. Mihaylova, V. T. *et al.* Decreased expression of the DNA mismatch repair gene Mlh1 under hypoxic stress in mammalian cells. *Mol. Cell. Biol.* **23**, 3265–3273 (2003).
41. Hammond, E. M., Dorie, M. J. & Giaccia, A. J. ATR/ATM targets are phosphorylated by ATR in response to hypoxia and ATM in response to reoxygenation. *J. Biol. Chem.* **278**, 12207–12213 (2003).
42. Graeber, T. G. *et al.* Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* **379**, 88–91 (1996).
43. Shi, Q. *et al.* Constitutive and inducible interleukin 8 expression by hypoxia and acidosis renders human pancreatic cancer cells more tumorigenic and metastatic. *Clin. Cancer Res.* **5**, 3711–3721 (1999).
44. Bindra, R. S. & Glazer, P. M. Genetic instability and the tumor microenvironment: towards the concept of microenvironment-induced mutagenesis. *Mutat. Res.* **569**, 75–85 (2005).
45. Young, S. D., Marshall, R. S. & Hill, R. P. Hypoxia induces DNA overreplication and enhances metastatic potential of murine tumor cells. *Proc. Natl Acad. Sci. USA* **85**, 9533–9537 (1988).
46. Rice, G. C., Hoy, C. & Schimke, R. T. Transient hypoxia enhances the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells. *Proc. Natl Acad. Sci. USA* **83**, 5978–5982 (1986).
47. Otsuka, J. The large-scale evolution by generating new genes from gene duplication; similarity and difference between monoploid and diploid organisms. *J. Theor. Biol.* **278**, 120–126 (2011).
48. van Sluis, R. *et al. In vivo* imaging of extracellular pH using 1H MRSI. *Magn. Reson. Med.* **41**, 743–750 (1999).
49. Gillies, R. J., Liu, Z. & Bhujwalla, Z. 31P-MRS measurements of extracellular pH of tumors using 3-aminopropylphosphonate. *Am. J. Physiol.* **267**, C195–203 (1994).
50. Morita, T., Nagaki, T., Fukuda, I. & Okumura, K. Clastogenicity of low pH to various cultured mammalian cells. *Mutat. Res.* **268**, 297–305 (1992).
51. Zhang, H. Y., Hormi-Carver, K., Zhang, X., Spechler, S. J. & Souza, R. F. In benign Barrett's epithelial cells, acid exposure generates reactive oxygen species that cause DNA double-strand breaks. *Cancer Res.* **69**, 9083–9089 (2009).
52. Xiao, H., Li, T. K., Yang, J. M. & Liu, L. F. Acidic pH induces topoisomerase II-mediated DNA damage. *Proc. Natl Acad. Sci. USA* **100**, 5205–5210 (2003).
53. Hashim, A. I., Zhang, X., Wojtkowiak, J. W., Martinez, G. V. & Gillies, R. J. Imaging pH and metastasis. *NMR Biomed.* **24**, 582–591 (2011).
54. Webb, B. A., Chimenti, M., Jacobson, M. P. & Barber, D. L. Dysregulated pH: a perfect storm for cancer progression. *Nature Rev. Cancer* **11**, 671–677 (2011).
55. Radu, C. G., Nijagal, A., McLaughlin, J., Wang, L. & Witte, O. N. Differential proton sensitivity of related G protein-coupled receptors T cell death-associated gene 8 and G2A expressed in immune cells. *Proc. Natl Acad. Sci. USA* **102**, 1632–1637 (2005).
56. Leffler, A., Monter, B. & Koltzenburg, M. The role of the capsaicin receptor TRPV1 and acid-sensing ion channels (ASICS) in proton sensitivity of subpopulations of primary nociceptive neurons in rats and mice. *Neuroscience* **139**, 699–709 (2006).
57. Dong, X. *et al.* Expression of acid-sensing ion channels in intestinal epithelial cells and their role in the regulation of duodenal mucosal bicarbonate secretion. *Acta Physiol. (Oxf.)* **201**, 97–107 (2011).
58. Yun, J. *et al.* Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. *Science* **325**, 1555–1559 (2009).

59. Rose, C. J. *et al.* Quantifying spatial heterogeneity in dynamic contrast-enhanced MRI parameter maps. *Magn. Reson. Med.* **62**, 488–499 (2009).
60. Kawata, Y. *et al.* Quantitative classification based on CT histogram analysis of non-small cell lung cancer: Correlation with histopathological characteristics and recurrence-free survival. *Med. Phys.* **39**, 988 (2012).
61. Drew, P. J. *et al.* Dynamic contrast enhanced magnetic resonance imaging of the breast is superior to triple assessment for the pre-operative detection of multifocal breast cancer. *Ann. Surg. Oncol.* **6**, 599–603 (1999).
62. Knopp, M. V., Giesel, F. L., Marcos, H., von Tengg-Kobligk, H. & Choyke, P. Dynamic contrast-enhanced magnetic resonance imaging in oncology. *Top. Magn. Reson. Imag.* **12**, 301–308 (2001).
63. Lamer, S. *et al.* Radiologic assessment of intranodal vascularity in head and neck squamous cell carcinoma. Correlation with histologic vascular density. *Invest. Radiol* **31**, 673–679 (1996).
64. Venkatasubramanian, R., Arenas, R. B., Henson, M. A. & Forbes, N. S. Mechanistic modelling of dynamic MRI data predicts that tumour heterogeneity decreases therapeutic response. *Br. J. Cancer* **103**, 486–497 (2010).
65. Helmlinger, G., Yuan, F., Dellian, M. & Jain, R. K. Interstitial pH and pO2 gradients in solid tumors *in vivo*: high-resolution measurements reveal a lack of correlation. *Nature Med.* **3**, 177–182 (1997).
66. Kimura, H. *et al.* Fluctuations in red cell flux in tumor microvessels can lead to transient hypoxia and reoxygenation in tumor parenchyma. *Cancer Res.* **56**, 5522–5528 (1996).
67. Costouros, N. G. *et al.* Microarray gene expression analysis of murine tumor heterogeneity defined by dynamic contrast-enhanced MRI. *Mol. Imag.* **1**, 301–308 (2002).
68. Guccione, S. *et al.* Functional genomics guided with MR imaging: mouse tumor model study. *Radiology* **228**, 560–568 (2003).
69. Hobbs, S. K. *et al.* Magnetic resonance image-guided proteomics of human glioblastoma multiforme. *J. Magn. Reson. Imag.* **18**, 530–536 (2003).
70. Winge, O. Zytologische untersuchungen uber die natur maligner tumoren. II. Teerkarzinome bei mausen. *Z. Zellforsch. Mikrosk. Anat.* **10**, 683–735 (1930).
71. Sandberg, A. A. & Hossfeld, D. K. Chromosomal abnormalities in human neoplasia. *Annu. Rev. Med.* **21**, 379–408 (1970).
72. Bakhoum, S. F., Danilova, O. V., Kaur, P., Levy, N. B. & Compton, D. A. Chromosomal instability substantiates poor prognosis in patients with diffuse large B-cell lymphoma. *Clin. Cancer Res.* **17**, 7704–7711 (2011).
73. Loeb, L. A. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nature Rev. Cancer* **11**, 450–457 (2011).
74. Yachida, S. *et al.* Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* **467**, 1114–1117 (2010).
75. Araujo, R. P., Liotta, L. A. & Petricoin, E. F. Proteins, drug targets and the mechanisms they control: the simple truth about complex networks. *Nature Rev. Drug Discov.* **6**, 871–880 (2007).
76. Lage, H. An overview of cancer multidrug resistance: a still unsolved problem. *Cell. Mol. Life Sci.* **65**, 3145–3167 (2008).
77. Marty, M. *et al.* Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J. Clin. Oncol.* **23**, 4265–4274 (2005).
78. Mahtani, R. L. & Vogel, C. L. Pleomorphic lobular carcinoma of the breast: four long-term responders to trastuzumab--coincidence or hint? *J. Clin. Oncol.* **26**, 5823–5824 (2008).
79. Untch, M. *et al.* Pathologic complete response after neoadjuvant chemotherapy plus trastuzumab predicts favorable survival in human epidermal growth factor receptor 2-overexpressing breast cancer: results from the TECHNO trial of the AGO and GBG study groups. *J. Clin. Oncol.* **29**, 3351–3357 (2011).
80. Druker, B. J. *et al.* Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* **355**, 2408–2417 (2006).
81. Engelman, J. A. & Settleman, J. Acquired resistance to tyrosine kinase inhibitors during cancer therapy. *Curr. Opin. Genet. Dev.* **18**, 73–79 (2008).
82. Aktipis, C. A., Kwan, V. S., Johnson, K. A., Neuberg, S. L. & Maley, C. C. Overlooking evolution: a systematic analysis of cancer relapse and therapeutic resistance research. *PLoS ONE* **6**, e26100 (2011).
83. O'Hare, T., Corbin, A. S. & Druker, B. J. Targeted CML therapy: controlling drug resistance, seeking cure. *Curr. Opin. Genet. Dev.* **16**, 92–99 (2006).
84. Blackwell, K. L. *et al.* Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J. Clin. Oncol.* **28**, 1124–1130 (2010).
85. Massarweh, S. & Schiff, R. Resistance to endocrine therapy in breast cancer: exploiting estrogen receptor/growth factor signaling crosstalk. *Endocr. Relat. Cancer* **13** Suppl. 1, 15–24 (2006).
86. Baselga, J. *et al.* Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N. Engl. J. Med.* **366**, 520–529 (2012).
87. Komarova, N. L. & Wodarz, D. Drug resistance in cancer: principles of emergence and prevention. *Proc. Natl Acad. Sci. USA* **102**, 9714–9719 (2005).
88. Chmielecki, J. *et al.* Optimization of dosing for EGFR-mutant non-small cell lung cancer with evolutionary cancer modeling. *Sci Transl Med* **3**, 90ra59 (2011).
89. Gatenby, R. A., Brown, J. & Vincent, T. Lessons from applied ecology: cancer control using an evolutionary double bind. *Cancer Res.* **69**, 7499–7502 (2009).
90. Gatenby, R. A., Silva, A. S., Gillies, R. J. & Frieden, B. R. Adaptive therapy. *Cancer Res.* **69**, 4894–4903 (2009).
91. Mirski, S. E., Gerlach, J. H. & Cole, S. P. Multidrug resistance in a human small cell lung cancer cell line selected in adriamycin. *Cancer Res.* **47**, 2594–2598 (1987).
92. Abrahamsson, P. A. Potential benefits of intermittent androgen suppression therapy in the treatment of prostate cancer: a systematic review of the literature. *Eur. Urol.* **57**, 49–59 (2010).
93. Beex, L. *et al.* Continuous versus intermittent tamoxifen versus intermittent/alternated tamoxifen and medroxyprogesterone acetate as first line endocrine treatment in advanced breast cancer: an EORTC phase III study (10863). *Eur. J. Cancer* **42**, 3178–3185 (2006).
94. Farber, S. & Diamond, L. K. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. *N. Engl. J. Med.* **238**, 787–793 (1948).
95. De Bock, K., Mazzone, M. & Carmeliet, P. Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? *Nature Rev. Clin. Oncol.* **8**, 393–404 (2011).
96. Iwamoto, F. M. *et al.* Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology* **73**, 1200–1206 (2009).
97. Jain, R. K. Lessons from multidisciplinary translational trials on anti-angiogenic therapy of cancer. *Nature Rev. Cancer* **8**, 309–316 (2008).
98. Goel, S. *et al.* Normalization of the vasculature for treatment of cancer and other diseases. *Physiol. Rev.* **91**, 1071–1121 (2011).
99. Chan, D. A. *et al.* Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. *Sci Transl Med* **3**, 94ra70 (2011).
100. Ganapathy-Kanniappan, S. *et al.* 3-bromopyruvate: a new targeted antiglycolytic agent and a promise for cancer therapy. *Curr. Pharm. Biotechnol.* **11**, 510–517 (2010).
101. Michelakis, E. D., Webster, L. & Mackey, J. R. Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. *Br. J. Cancer* **99**, 989–994 (2008).
102. Le, A. *et al.* Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc. Natl Acad. Sci. USA* **107**, 2037–2042 (2010).
103. Gatenby, R. A., Gawlinski, E. T., Gmitro, A. F., Kaylor, B. & Gillies, R. J. Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Res.* **66**, 5216–5223 (2006).
104. Ibrahim Hashim, A. *et al.* Reduction of metastasis using a non-volatile buffer. *Clin. Exp. Metastasis* **28**, 841–849 (2011).
105. Hashim, A. I. *et al.* Sytemic buffers inhibit carcinogenesis in TRAMP mice. *J. Urology* (2012). (in press Jan, < /pub > 2012).
106. Ibrahim Hashim, A. *et al.* Reduction of metastasis using a non-volatile buffer. *Clin Exp Metastasis* (2011).
107. Wojtkowiak, J. W., Verduzco, D., Schramm, K. J. & Gillies, R. J. Drug resistance and cellular adaptation to tumor acidic pH microenvironment. *Mol. Pharm.* **8**, 2032–2038 (2011).
108. Ghobrial, I. M., Witzig, T. E. & Adjei, A. A. Targeting apoptosis pathways in cancer therapy. *CA Cancer J. Clin.* **55**, 178–194 (2005).
109. Ludwig, H., Khayat, D., Giaccone, G. & Facon, T. Proteasome inhibition and its clinical prospects in the treatment of hematologic and solid malignancies. *Cancer* **104**, 1794–1807 (2005).
110. Madhok, B. M., Yeluri, S., Perry, S. L., Hughes, T. A. & Jayne, D. G. Dichloroacetate induces apoptosis and cell-cycle arrest in colorectal cancer cells. *Br. J. Cancer* **102**, 1746–1752 (2010).
111. Holoch, P. A. & Griffith, T. S. TNF-related apoptosis-inducing ligand (TRAIL): a new path to anti-cancer therapies. *Eur. J. Pharmacol.* **625**, 63–72 (2009).
112. Smolewski, P. Recent developments in targeting the mammalian target of rapamycin (mTOR) kinase pathway. *Anticancer Drugs* **17**, 487–494 (2006).
113. Agulnik, M. New developments in mammalian target of rapamycin inhibitors for the treatment of sarcoma. *Cancer* **118**, 1486–1497 (2012).
114. Houghton, P. J. Everolimus. *Clin. Cancer Res.* **16**, 1368–1372 (2010).
115. Pouget, J. P. *et al.* Clinical radioimmunotherapy--the role of radiobiology. *Nature Rev. Clin. Oncol.* **8**, 720–734 (2011).
116. Richman, C. M. *et al.* High-dose radioimmunotherapy combined with fixed, low-dose paclitaxel in metastatic prostate and breast cancer by using a MUC-1 monoclonal antibody, m170, linked to indium-111/yttrium-90 via a cathepsin cleavable linker with cyclosporine to prevent human anti-mouse antibody. *Clin. Cancer Res.* **11**, 5920–5927 (2005).
117. Behr, T. M. *et al.* High-linear energy transfer (LET) alpha versus low-LET beta emitters in radioimmunotherapy of solid tumors: therapeutic efficacy and dose-limiting toxicity of 213Bi- versus 90Y-labeled CO17-1A Fab' fragments in a human colonic cancer model. *Cancer Res.* **59**, 2635–2643 (1999).
118. Chatal, J. F., Davodeau, F., Cherel, M. & Barbet, J. Different ways to improve the clinical effectiveness of radioimmunotherapy in solid tumors. *J. Cancer Res. Ther.* **5** Suppl. 1, S36–40 (2009).
119. Wilson, W. R. & Hay, M. P. Targeting hypoxia in cancer therapy. *Nature Rev. Cancer* **11**, 393–410 (2011).
120. Duan, J. X. *et al.* Potent and highly selective hypoxia-activated achiral phosphoramidate mustards as anticancer drugs. *J. Med. Chem.* **51**, 2412–2420 (2008).
121. Meng, F. *et al.* Molecular and cellular pharmacology of the hypoxia-activated prodrug TH-302. *Mol. Cancer Ther.* **6**, 6 (2011).
122. Sun, J. D. *et al.* Selective tumor hypoxia targeting by hypoxia-activated prodrug TH-302 inhibits tumor growth in preclinical models of cancer. *Clin. Cancer Res.* **18**, 758–770 (2011).
123. Tian, L. & Bae, Y. H. Cancer nanomedicines targeting tumor extracellular pH. *Colloids Surf B Biointerfaces* DOI colsurfb.2011.10.039 (2011).
124. Kaminskas, L. M. *et al.* Doxorubicin-conjugated PEGylated dendrimers show similar tumoricidal activity but lower systemic toxicity when compared to PEGylated liposome and solution formulations in mouse and rat tumor models. *Mol. Pharm.* **9**, 422–432 (2012).
125. Gatenby, R. A. & Vincent, T. L. Application of quantitative models from population biology and evolutionary game theory to tumor therapeutic strategies. *Mol. Cancer Ther.* **2**, 919–927 (2003).
126. Casanueva, M. O., Burga, A. & Lehner, B. Fitness trade-offs and environmentally induced mutation buffering in isogenic C. elegans. *Science* **335**, 82–85 (2012).
127. Jacob, F. Evolution and Tinkering. *Science* **196**, 1161–1166 (1977).

Acknowledgements

The authors would like to thank Z. Bhujwalla, K. Brindle, J. Brown, J. Evelhoch, A. Giuliano, P. Glazer, C. Hart, L. Loeb, T. Sellers, B. Vogelstein and J. Zhang for their insightful and helpful comments. Supported by a grant from the McDonnell Foundation and US NIH grants U54 CA143970 ("Physical Sciences in Oncology") and R01 CA077575 ("Causes and Consequences of Acid pH in Tumors") to R.A.G. and R.J.G.

Competing interests statement

The authors declare competing financial interests. See Web version for details.

FURTHER INFORMATION

Robert J. Gillies's homepage: http://imaging.moffitt.org/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF