

Cancer Research Meets Evolutionary Biology

John W. Pepper, C. Scott Findlay, Rees Kassen, Sabrina L. Spencer, and Carlo C. Maley

Abstract

There is increasing evidence that Darwin's theory of evolution by natural selection provides insights into the etiology and treatment of cancer. On a microscopic scale, neoplastic cells meet the conditions for evolution by Darwinian selection: cell reproduction with heritable variability that affects cell survival and replication. This suggests that, like other areas of biological and biomedical research, Darwinian theory can provide a general framework for understanding many aspects of cancer, including problems of great clinical importance. With the availability of raw molecular data increasing rapidly, this theory may provide guidance in translating data into understanding and progress. Several conceptual and analytical tools from evolutionary biology can be applied to cancer biology. Two clinical problems may benefit most from the application of Darwinian theory: neoplastic progression and acquired therapeutic resistance. The Darwinian theory of cancer has especially profound implications for drug development, both in terms of explaining past difficulties, and pointing the way toward new approaches. Because cancer involves complex evolutionary processes, research should incorporate both tractable (simplified) experimental systems, and also longitudinal observational studies of the evolutionary dynamics of cancer in laboratory animals and in human patients. Cancer biology will require new tools to control the evolution of neoplastic cells.

The fact that mortality rates in many cancers have fallen only slowly since the declaration of the "war on cancer" in 1971 [1] suggests that novel approaches to both therapy and prevention are required. Two such efforts were launched this year. In February, the National Cancer Institute sponsored a meeting titled, "Integrating and Leveraging the Physical Sciences to Open a New Frontier in Oncology", based on the premise that, "Cancer research needs new ideas, deep innovation, and new and unprecedented transdisciplinary teams of scientists...". Independently, a working group of cancer biologists, evolutionary biologists, systems and computational biologists, and physicians convened in May at the Santa Fe Institute (SFI) with similar goals [2]. In both cases, consensus emerged that somatic (within-body) cellular selection and evolution is the fundamental process by which neoplasms arise, acquire malignancy, and evade therapeutic interventions [3-6]. This hypothesis was introduced in the 1970's [7, 8], and has since garnered enough empirical support to rise to the level of a scientific theory, which, "explains various large and independent classes of facts" [9, p. 107].

The emerging interest in somatic evolution in cancer biology is timely. New technologies are providing extensive data on the molecular and cellular mechanisms of cancer, data that can be used to test Darwinian theories of cancer progression and therapeutic response by employing both a well-developed body of theory and an armamentarium of analytical tools from evolutionary biology. Here we review some of these tools, and how they can be applied to basic problems in cancer biology. Although the role of somatic evolution in cancer is rarely disputed, it has seldom been integrated

into biomedical research. We begin by discussing how tools from evolutionary theory may be applied to cancer biology. We examine two key clinical challenges as opportunities for the integration of evolutionary biology into cancer biology: predicting progression to malignancy and preventing acquired therapeutic resistance. We also consider the implications of the somatic evolution theory of cancer for drug development. Finally, we discuss how research into cancer stem cells may be integrated into the evolutionary theory of cancer.

Importing tools from evolutionary biology into cancer biology

The mathematical theory of Darwinian dynamics provides tools for understanding and predicting responses to somatic selection, including clonal adaptation, diversification and extinction [10]. This framework has been used to develop explanatory models of cancer initiation, promotion, and progression [11, 12]. Consideration of cellular evolutionary dynamics can also guide development of novel therapeutic strategies [13].

Techniques originally developed to reconstruct the evolutionary history of species have since been applied to tracing the somatic lineages of normal and cancer cells within an individual [14, 15]. In organismal biology, such phylogenetic reconstruction permits the comparative analysis of adaptation through methods such as independent contrasts [16]. Similar comparative analysis of cancer cell phylogenies could reveal which molecular changes occurring during neoplastic progression facilitate the development of invasive, metastatic and resistant cell phenotypes.

Two further developments in evolutionary theory may also be applicable. The first results from renewed interest in the evolutionary genetics of adaptation [17] which suggests that just a few mutations are typically involved in conferring the bulk of adaptive evolution in mutation-limited populations such as cancer cells. This theory may be particularly relevant in predicting the progression of cancers following treatment failure, as it suggests that compensatory evolution (the recovery of fitness losses due to the acquisition of costly resistance mutations) may occur extremely fast. There is even evidence that many resistance mutations may not be costly in the first place [18], a phenomenon that is also seen in bacteria [19].

A second applicable advance in evolutionary biology is the development of theory for “multilevel selection”, or natural selection at multiple levels of biological organization [20, 21]. Somatic selection occurs solely at the level of the cell. However, for understanding human defenses against and vulnerability to cancer, the history of selection among individuals due to cancer is also important [22, 23]. It is also useful to consider how these two levels of selection have interacted and shaped each other. For example, individual selection has apparently shaped patterns of ongoing somatic cell differentiation that suppress somatic selection [24]. Efforts at cancer prevention may benefit from the study of the mechanisms that evolution has discovered for suppressing cancer in various organisms [22].

Conceptual contributions to cancer biology from organismal biology have derived from the consideration of ecological (e.g. competition, predation) as well as evolutionary processes [3]. One novel application is the use of engineered bacteria as competitors and predators of cancer cells in the hypoxic environments they create inside tumors, where other agents lose effectiveness [25]. Another possibility involves the use of oncolytic viruses as predators [26], and speaks to the nature of the therapeutic agents themselves. As pointed out by Levin and Bull [27] in the context of treatment of bacterial diseases, phage have one outstanding advantage over conventional antibiotics: a phage population

can itself evolve to overcome bacterial resistance whereas antibiotics – and both conventional and targeted cancer chemotherapies - are evolutionarily inert; once drug resistance evolves, the therapy fails. By contrast, oncolytic viruses are, at least in principle, capable of evolving rapidly. The potential therefore exists for using selection to evolve oncolytic viruses with desirable attributes such as attenuated (or enhanced) virulence or increased tumor cell tropism. Of course, this evolvability is a double-edged sword, for it also opens up the possibility of genetic adaptation of the therapeutic to non-target tissues, i.e. evolution of a pathogenic virus. Early results suggest, however, that at least for some candidate oncolytic viruses (e.g. poliovirus employed to treat glioblastoma multiforme), evolution of pathogenicity does not occur [28]. Moreover, in at least one model system, persistent infection by oncolytic reovirus dramatically impedes tumor development, and although infected cells subsequently cleared of reovirus are tumorigenic, they have not acquired resistance to the virus [29].

New tools for understanding cancer biology will be adopted only to the extent that they translate into clear directions for clinical advances. The two clinical challenges where we seem likely to benefit most directly from adopting the perspective of somatic evolution are predicting progression to malignancy and preventing acquired therapeutic resistance.

Neoplastic progression

Not all pre-malignant neoplasms progress to cancer. It is therefore important to identify risk factors for progression as early as possible because, in many cancers, early detection and intervention improve survival [30]. Moreover, the risks, hardship and expense of intervention can be minimized by recognizing when intervention is unnecessary. Predictors of progression to cancer that are independent of particular genes or tumor types – and hence, may be generic indicators of cancer risk – include genetic instability [31, 32] and genetic diversity [33, 34], as well as signatures of ongoing somatic evolution such as clonal expansion [33, 35]. Monitoring these attributes of cell populations may allow us to tailor interventions to the current level of risk [31].

Because neoplastic progression is a process of somatic evolution, reducing evolutionary rates should decrease cancer incidence. Evolutionary theory suggests that this could be accomplished by reducing the mutation rate, reducing the effective population size of cells, increasing the generation time of the self-renewing cells (e.g. through cytostatic agents or agents capable of inducing cell-cycle arrest or senescence), or reducing the relative fitness of carcinogenic mutations. Unfortunately, we currently lack tools to measure those attributes of neoplasms, let alone manipulate them. One possibility is to reduce the mutation rate via therapeutic reduction in mutagen exposure. For example, non-steroidal anti-inflammatory drugs, such as aspirin, are associated with as much as a 5-fold reduction of risk of progression in Barrett's esophagus [36, 37]. This may be due to a reduction in mutagens in the form of oxygen radicals produced during inflammation. Suppressing inflammation may also remove proliferative signals normally involved in wound healing, and so may prolong the cell cycle of neoplastic cells and reduce the number of multiplying tissue stem cells, thereby reducing evolutionary rates.

Acquired drug resistance

Acquired drug resistance is a major problem in the treatment of most cancers [38, 39]. In the clinic, patients often respond to the initial application of a therapy but are prone to relapse, at which point repeating the same therapy is rarely effective. The situation is

even more dire for patients presenting with metastatic cancers, where initial response to therapy is undermined by subsequent disease progression. In both instances, it is clear that therapeutic sensitivity of the tumor has declined over the course of treatment.

Decades ago, Nowell postulated that the emergence of drug resistance in cancer was driven by somatic evolution [7], an hypothesis for which there is now substantial empirical support. For example, early work found methotrexate (a common chemotherapy employed for many different cancers) resistance due to amplification (extra copies) of its target gene, dihydrofolate reductase (DHFR), in clinical samples after methotrexate therapy [40-43]. Similarly, 5-fluorouracil – another common chemotherapy – selects for amplification in its target gene, thymidylate synthase (TYMS), causing acquired therapeutic resistance [44]. Some of the most compelling evidence comes from chronic myeloid leukemia, where longitudinal blood samples have revealed the acquisition of a series of mutations in the gene BCR-ABL, inducing resistance to sequential ABL kinase inhibitor therapies (imatinib and dasatinib) [18]. Similarly, gefitinib selects for mutations in its target gene, epidermal growth factor receptor (EGFR) [45]. However, amplification of a downstream gene, MET, can also induce acquired resistance to gefitinib [46]. Anti-androgen therapies in prostate cancer select for mutations that cause hypersensitivity in the androgen receptor (AR) [47] as well as amplification of that gene [48]. Consistent with the idea that therapies impose new selective pressures, a recent, genetic comparison of pre-therapy and relapse samples in acute lymphoblastic leukemia found that the clone detected at relapse was often present as a minority clone prior to therapy [49].

If acquired therapeutic resistance reflects largely a Darwinian dynamic, then the key will be to design therapeutic interventions that both reduce tumor burden and delay or prevent the evolution of therapeutic resistance. Here, several possibilities arise. If resistance to different drugs is conferred by different mutations, then the likelihood of a patient having cancer cells with the multiple mutations required for resistance to combination therapy should be smaller than the likelihood of having the mutation required for single agent resistance. Hence, combination therapy should result in improved response rates relative to single agent therapy, and reduce the likelihood of relapse. In a meta-analysis of single drugs vs. adding additional drugs, in metastatic breast cancer, combination therapies increased response, reduced relapse, but had increased toxicity and only reduced overall mortality by modest amount [50]. Similarly, a meta-analysis of single vs. double or triple drug therapies in non-small cell lung cancer found that double drug therapies increased response rate, increased 1-year survival moderately, and increased median survival time, but also increased toxicity [51]. However, triple drug therapies increased response rates, but did not significantly improve 1-year survival or median survival time relative to single drug therapies [51]. Thus, in combination therapy with cytotoxins, there appears to be a trade-off between toxicity and blocking therapeutic resistance.

Unlike multidrug cocktails used in the treatment of other diseases (e.g. HAART therapy for HIV AIDS), combination therapies have not transformed cancer into a chronic disease. The reasons for this failure are unknown, but may be due to single mutations that up-regulate efflux pumps causing multi-drug resistance [52]. This notwithstanding, there is an urgent need for more research into the evolution of chemotherapeutic resistance and the design of multidrug therapies that act synergistically to reduce the likelihood of relapse, and thereby increase overall survival.

The Darwinian perspective suggests that interventions that ameliorate progression or virulence without directly killing neoplastic cells would delay the emergence of resistance. Tamoxifen, and second generation selective estrogen receptor modulators (SERMs) inhibit the proliferative stimulation of breast cancer cells, generated by estrogen, by blocking the estrogen receptor and hence, are cytostatic rather than cytotoxic. Yet they have proven effective in breast cancer therapy [53] and show improved toxicity profiles compared to standard cytotoxins. The mechanism by which breast cancer tumors shrink under SERM therapy is not fully understood [54] but may involve both autophagy [55] and apoptosis [56]. The fact that they reduce cancer cell proliferation should also slow the rate at which novel resistance mutations arise.

Recent computational models suggest that hypothetical benign cell boosters, which increase the fitness of either benign neoplastic clones or normal cells, may help to drive the more dysplastic clones extinct and thereby delay cancer progression. Since the drug would act to increase fitness, natural selection should lead to increased sensitivity rather than resistance [13]. Proton pump inhibitors (PPIs) may be acting as a benign cell booster in Barrett's esophagus. If PPIs are being used to suppress gastric acid reflux when the Barrett's epithelium is wounded, normal squamous epithelium grows to heal the wound instead of neoplastic Barrett's epithelium [57]. A similar strategy might be employed to boost the fitness of chemosensitive cells, so that they out-compete innately resistant cells before chemotherapy is initiated [13].

Implications of somatic evolution for drug development

The somatic evolution theory of neoplastic progression and acquired therapeutic resistance has several important implications for drug development. The first is that high tumor cell toxicity does not invariably imply effective treatment. Usually in cancer therapy the clinical objective is to reduce the size of the tumor as quickly as possible to achieve immediate clinical benefit. This objective in part underlies the twin concepts of a Maximum Tolerable Dose (MTD) and the Therapeutic Index: the idea is to design killing agents for which maximum cancer cell mortality is achieved at a dose considerably lower than the dose at which the therapy is toxic to the patient. However, if heritable variation in susceptibility to the killing agent exists in the tumor cell population, high mortality implies that only cells with very high resistance escape killing. The result is a large difference in the average value of the trait (resistance) in those cells that are killed, compared to those that survive, i.e. a large selection differential. A basic principle of quantitative genetics holds that the rate of evolution of a trait is proportional to the selective differential [58] such that, all else being equal, therapies causing high cell mortality will increase the rate of evolution of resistance compared to those inducing lower mortality.

The second point follows from the first. As a consequence of selection caused by therapeutic interventions, the short-term therapeutic response may bear little relationship to the likelihood of effective longer-term treatment. The relationship between short- and long-term therapeutic responses depends on the extent of heritable phenotypic variation in cytotoxicity susceptibility: if no such variability exists, there is no selection and dramatic reductions in tumor burden can, at least in principle, be achieved without significant evolutionary response. If such variation exists at the time therapy is initiated, or arises soon thereafter, then dramatic initial reductions imply strong selection, with a resulting dramatic rebound effect. Thus while there are undoubtedly short-term clinical

benefits associated with rapid and large reductions in pathogen populations, the longer-term cost may well be an accelerated rate of resistance evolution.

These considerations suggest that a key strategy for the design of effective cancer therapeutics is to develop systematic methods for identifying drug targets for which heritable variation in resistance is minimal [59]. Cancer cells thrive by altering their micro-environment to make it more hospitable. The number of neoplastic micro-environments, and the variation among them, are expected to be much smaller than the number of neoplastic cells, and the variation among them. By targeting the cancer cell products that alter the micro-environment, it is possible to halt or reverse tumor growth without using cytotoxins to directly kill cancer cells. This should, as noted above, be substantially less prone to evolved resistance. Anti-angiogenic drugs are well-established therapeutics that have been less prone to acquired resistance than cytotoxic drugs [60]. It has been proposed that this advantage results from the fact that anti-angiogenics target the microenvironment of tumor cells, rather than directly killing individual cancer cells [59].

Cancer Stem Cells

Although we advocate for somatic evolution as the central organizing theory of cancer biology, other ideas have also been suggested as candidates for this role. Most prominent among these is the idea of cancer stem cells. Recently, cell surface markers have been identified that are associated with the capacity for neoplastic cells to engraft and propagate a neoplasm through serial xenografts into immune compromised mice [61, 62]. These results have led to the revival of the “cancer stem cell” hypothesis which posits that only a small proportion of neoplastic cells are capable of self-renewal and propagation.

Relapse can only occur if some self-renewing cells survive therapy. Do patients relapse because cancer stem cells are inherently resistant to therapy [63, 64], or because therapy selected for a resistant genetic or epigenetic variants in the cancer stem cell pool? There is experimental evidence to support both alternatives. The observation of clones with resistance mutations and amplifications after therapy suggests that in those cases, therapy resulted in positive Darwinian selection on the cancer stem cells [18, 40-48]. There is also experimental evidence that cancer stem cells may have up-regulated efflux pumps (ATP-binding cassette transporters) that protect the cancer stem cells from cytotoxins [63]. They also may have active DNA repair and suppressed apoptosis [63, 64]. If cancer stem cells are relatively quiescent, then they should also be more resistant to chemotherapies that target S-phase compared to highly proliferative cancer cells [63]. It is likely that both selection for resistance mutations and a stem-like cell phenotype contribute to the refractory nature of the disease.

Perhaps inevitably, the cancer stem cell hypothesis remains controversial [9, 65]. Certainly from an evolutionary perspective, the presence of a large majority of cancer cells with a putative limited potential to proliferate is a surprise [66]. Cancer stem cells, that expend a portion of their reproductive potential on progeny with limited proliferative capacity, should be at a competitive disadvantage relative to cancer stem cells that always divide symmetrically to produce more cancer stem cells. If the cancer stem cells are indeed rare [67], one possibility is that there has not been enough time in most neoplasms to select for an increased frequency of cancer stem cells, an hypothesis that could be tested by serial passage of neoplastic cells through immune compromised mice. Indeed, McBride [66] hypothesizes that the putative non-stem cells are really self-renewing cells

that have acquired a transient migratory, non-proliferative phenotype [66]. Another possibility is that cancer non-stem cells may be altering the microenvironment of the cancer stem cells so as to increase the fitness of the stem cells. In this way, selection may be acting on the ensemble of cells.

The relative importance of stem versus non-stem components in acquired resistance is still largely unknown. Experiments that involve engrafting mouse cancer cells into syngenic mice suggest that self-renewing cells may not be rare [67], and a recent study found a genetic lesion present in most of the breast cancer non-stem cell component that was absent in the (putative) stem cell compartment, suggesting that the non-stem cells comprised an independent, self-renewing clone [68].

The cancer stem cell hypothesis is not a mutually exclusive alternative to the somatic evolution theory of acquired therapeutic resistance [69, 70]. Moreover, as currently articulated, the cancer stem cell hypothesis does not appear to offer an alternative explanation for neoplastic progression, clonal expansions or tumor cell phylogenies. In fact, the question of whether the entire neoplasm or a minority of neoplastic cells is capable of self-renewal is, at least in part, a question merely of the effective population size of the evolving cells in a neoplasm. But irrespective of whether the stem or non-stem cell component is responsible for neoplasm self-renewal, there is broad agreement that therapeutic targeting of the self-renewing cells is crucial for effective disease management [71].

Conclusions

The somatic evolutionary theory of cancer progression and acquired therapeutic resistance has profound implications for cancer therapy. It is, therefore, crucial that further efforts be devoted to testing predictions of the theory, especially in the clinical setting. For example, an understanding of the selective effects of therapeutic interventions requires that the genetic and epigenetic structure of tumors be evaluated before, during and after therapy. It is no accident that rapid progress in understanding therapeutic resistance has been made in hematopoietic malignancies where post-therapy samples are easily acquired with minimally invasive procedures [18]. In solid tumors, clinicians are understandably reluctant to initiate further invasive procedures to biopsy a neoplasm after the extended trauma of cancer therapy has failed. Yet, longitudinal sampling of neoplasms, during both progression and therapeutic response, will be critical to our understanding of cancer progression and the acquisition of resistance. This can be done in hematopoietic neoplasms and some solid tumors like Barrett's esophagus and superficial bladder cancer where the standard of care is serial biopsy surveillance. In other neoplasms, monitoring somatic evolution will depend on developing assays of cells shed from the tissues in blood, urine, feces or sputum samples.

Longitudinal evaluation will also be important in cell culture or animal model studies. One underexploited experimental design in animal models is serial biopsies of a neoplasm as it develops and changes in response to therapy. In contrast, most animal experiments in cancer biology rely on sacrificing the animal to take a tissue sample and consequently do not generate longitudinal data. Similarly, the long-term evolution of human cancer cells could be tested by serially passaging the cells through immune compromised mice, as is done in the routine maintenance of some cell lines.

Most of evolutionary biology has been focused on describing evolution. In order to prevent or cure cancer, we will need to develop new methods to control or manipulate the evolutionary process. It is our hope that cancer biology may help to drive new

evolutionary biology research into the methods and theory for controlling evolution. This is already a topic of intense interest in infectious disease evolution [59, 72-75].

As in other complex diseases, model systems are useful for controlled experimentation, but often suffer from the problem of limited extrapolation to clinical studies. Yet the possibility arises of supplementing controlled experiments in more tractable model systems with observational studies of somatic evolution in human neoplasms. Direct observational studies of human neoplasms have provided insights into how somatic evolution leads to cancer outcomes [32, 34] and to therapeutic resistance [18, 40-49, 76, 77]. Longitudinal sampling of some tissues is either non-invasive or already routine (e.g., certain biopsies, pap smears, urine, blood, feces, etc.). Detailed analysis of such samples will transform the clinic into an environment for basic research on pre-malignant neoplasms that provides critical information for the elaboration and testing of hypotheses for human carcinogenesis.

Acknowledgments

This paper reflects the contributions of all participants in the SFI working group, including Stephanie Forrest, Rafe Furst, Henry H.Q. Heng, Brian J. Reid, and Thomas L. Vincent. We are grateful to Chris Wood and the Santa Fe Institute for hosting and supporting this meeting. CCM is supported by NIH P01 CA91955, NIH R03 CA137811, NIH P30 CA010815, a Bioinformatics Research Starter grant from the PhRMA Foundation, and the Landon AACR Innovator Award for Cancer Prevention. CSF and RK are supported by the Natural Sciences and Engineering Research Council of Canada.

References

1. Espey, D.K., et al., Annual report to the nation on the status of cancer, 1975-2004, featuring cancer in American Indians and Alaska Natives. *Cancer*, 2007. 110(10): p. 2119-52.
2. Integrating Evolutionary Biology into Cancer Biology. Santa Fe Institute, Santa Fe, NM, May 18-23, 2008.
http://www.santafe.edu/events/workshops/index.php/Integrating_Evolutionary_Theory_into_Cancer_Biology
3. Merlo, L.M., et al., Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer*, 2006. 6(12): p. 924-35.
4. Frank, S.A. and M.A. Nowak, Problems of somatic mutation and cancer. *Bioessays*, 2004. 26(3): p. 291-9.
5. Crespi, B. and K. Summers, Evolutionary Biology of Cancer. *Trends in Ecology and Evolution*, 2005. 20(10): p. 545-552.
6. Maley, C.C. and B.J. Reid, Natural selection in neoplastic progression of Barrett's esophagus. *Semin Cancer Biol*, 2005. 15: p. 474-483.
7. Nowell, P.C., The clonal evolution of tumor cell populations. *Science*, 1976. 194(4260): p. 23-8.
8. Cairns, J., Mutation Selection and the Natural History of Cancer. *Nature*, 1975. 255: p. 197-200.

9. Polyak, K., Breast cancer stem cells: a case of mistaken identity? *Stem Cell Rev.*, 2007. 3(2): p. 107-9.
10. Vincent, T.L. and J.S. Brown, *Evolutionary Game Theory*. 2005, Cambridge: Cambridge University Press.
11. Vincent, T.L. and R.A. Gatenby 2008. An evolutionary model for initiation, promotion, and progression in carcinogenesis. *International Journal of Oncology* 31(4): 729-737.
12. Michor, F., Y. Iwasa, and M.A. Nowak, Dynamics of cancer progression. *Nat. Rev. Cancer*, 2004. 4(3): p. 197-205.
13. Maley, C.C., B.J. Reid, and S. Forrest, Cancer prevention strategies that address the evolutionary dynamics of neoplastic cells: simulating benign cell boosters and selection for chemosensitivity. *Cancer Epidemiol Biomarkers Prev*, 2004. 13(8): p. 1375-84.
14. Frumkin, D., et al., Cell lineage analysis of a mouse tumor. *Cancer Res.*, 2008. 68(14): p. 5924-31.
15. Shibata, D., Stem cells as common ancestors in a colorectal cancer ancestral tree. *Curr. Opin. Gastroenterol.*, 2008. 24(1): p. 59-63.
16. Felsenstein, J., Phylogenies and the comparative method. *Am. Nat.*, 1985. 125: p. 1-15.
17. Orr, H.A., The genetic theory of adaptation: a brief history. *Nat. Rev. Genet*, 2005. 6(2): p. 119-27.
18. Shah, N.P., et al., Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J. Clin Invest*, 2007. 117(9): p. 2562-9.
19. Kassen, R. and T. Bataillon, Distribution of fitness effects among beneficial mutations before selection in experimental populations of bacteria. *Nat Genet*, 2006. 38(4): p. 484-8.
20. Keller, L.K., *Levels of Selection in Evolution*. 1999, Princeton, NJ: Princeton University Press.
21. Okasha, S., *Evolution and the Levels of Selection*. 2006, Oxford: Clarendon Press.
22. Greaves, M., Darwinian medicine: a case for cancer. *Nat Rev Cancer*, 2007. 7(3): p. 213-21.
23. Leroi, A.M., V. Koufopanou, and A. Burt, Cancer selection. *Nat. Rev. Cancer*, 2003. 3(3): p. 226-31.
24. Pepper, J.W., K. Sprouffske, and C.C. Maley, Animal Cell Differentiation Patterns Suppress Somatic Evolution. *PLoS Comput Biol*, 2007. 3(12): p. e250.
25. Dang, L.H., et al., Combination Bacteriolytic therapy for the Treatment of Experimental Tumors. *Proceedings of the National Academy of Science, USA*, 2001. 98(26): p. 15155-15160.
26. Davis, J.J. and B. Fang, Oncolytic virotherapy for cancer treatment: challenges and solutions. *J Gene Med*, 2005. 7(11): p. 1380-9.
27. Levin, B.R. and J.J. Bull, Population and evolutionary dynamics of phage therapy. *Nat Rev Microbiol*, 2004. 2(2): p. 166-73.

28. Dobrikova, E.Y., et al., Recombinant oncolytic poliovirus eliminates glioma in vivo without genetic adaptation to a pathogenic phenotype. *Mol Ther*, 2008. 16(11): p. 1865-72.
29. Alain, T., et al., The oncolytic effect in vivo of reovirus on tumour cells that have survived reovirus cell killing in vitro. *Br J Cancer*, 2006. 95(8): p. 1020-7.
30. Etzioni, R., et al., The Case for Early Detection. *Nature Reviews Cancer*, 2003. 3(April): p. 1-10.
31. Galipeau, P.C., et al., NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med*, 2007. 4(2): p. e67.
32. Maley, C.C., et al., The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. *Cancer Res*, 2004. 64(20): p. 7629-33.
33. Heng, H.H., Cancer genome sequencing: the challenges ahead. *Bioessays*, 2007. 29(8): p. 783-94.
34. Maley, C.C., et al., Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nat Genet*, 2006. 38(4): p. 468-73.
35. Maley, C.C., et al., Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res*, 2004. 64(10): p. 3414-27.
36. Vaughan, T.L., et al., Non-steroidal anti-inflammatory drugs and risk of neoplastic progression in Barrett's oesophagus: a prospective study. *Lancet Oncol*, 2005. 6(12): p. 945-52.
37. Corley, D.A., et al., Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. *Gastroenterology*, 2003. 124(1): p. 47-56.
38. Moscow, J., C.S. Morrow, and C.H. Cowan, Drug Resistance and its Clinical Circumvention, in *Cancer Medicine*, D.W. Kufe, J.F. Holland, and E. Frei, Editors. 2003, B. C. Decker: Hamilton, Ont.
39. O'Connor, R., et al., Drug resistance in cancer - searching for mechanisms, markers and therapeutic agents. *Expert Opin Drug Metab Toxicol*, 2007. 3(6): p. 805-17.
40. Horns, R.C., Jr., W.J. Dower, and R.T. Schimke, Gene amplification in a leukemic patient treated with methotrexate. *J Clin Oncol*, 1984. 2(1): p. 2-7.
41. Carman, M.D., et al., Resistance to methotrexate due to gene amplification in a patient with acute leukemia. *J Clin Oncol*, 1984. 2(1): p. 16-20.
42. Curt, G.A., et al., Unstable methotrexate resistance in human small-cell carcinoma associated with double minute chromosomes. *N Engl J Med*, 1983. 308(4): p. 199-202.
43. Trent, J.M., et al., Cytologic evidence for gene amplification in methotrexate-resistant cells obtained from a patient with ovarian adenocarcinoma. *J Clin Oncol*, 1984. 2(1): p. 8-15.
44. Wang, T.L., et al., Digital karyotyping identifies thymidylate synthase amplification as a mechanism of resistance to 5-fluorouracil in metastatic colorectal cancer patients. *Proc Natl Acad Sci USA*, 2004. 101(9): p. 3089-94.
45. Kobayashi, S., et al., EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*, 2005. 352(8): p. 786-92.
46. Engelman, J.A., et al., MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*, 2007. 316(5827): p. 1039-43.

47. Taplin, M.E., et al., Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. *Cancer Res*, 1999. 59(11): p. 2511-5.
48. Visakorpi, T., et al., In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet*, 1995. 9(4): p. 401-6.
49. Mullighan, C.G., et al., Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science*, 2008. 322(5906): p. 1377-80.
50. Carrick, S., et al., Single agent versus combination chemotherapy for metastatic breast cancer. *Cochrane Database Syst Rev*, 2005(2): p. CD003372.
51. Delbaldo, C., et al., Benefits of adding a drug to a single-agent or a 2-agent chemotherapy regimen in advanced non-small-cell lung cancer: a meta-analysis. *JAMA*, 2004. 292(4): p. 470-84.
52. Gottesman, M.M., T. Fojo, and S.E. Bates, Multidrug Resistance in Cancer: Role of ATP-dependent Transporters. *Nature Reviews Cancer*, 2002. 2(1): p. 48-58.
53. Robertson, J.F., Selective oestrogen receptor modulators/new antioestrogens: a clinical perspective. *Cancer Treat Rev*, 2004. 30(8): p. 695-706.
54. Dowsett, M., et al., Effect of raloxifene on breast cancer cell Ki67 and apoptosis: a double-blind, placebo-controlled, randomized clinical trial in postmenopausal patients. *Cancer Epidemiol Biomarkers Prev*, 2001. 10(9): p. 961-6.
55. Bursch, W., et al., Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. *Carcinogenesis*, 1996. 17(8): p. 1595-607.
56. Mandlekar, S. and A.N. Kong, Mechanisms of tamoxifen-induced apoptosis. *Apoptosis*, 2001. 6(6): p. 469-77.
57. Paulson, T.G., et al., Neosquamous epithelium does not typically arise from Barrett's epithelium. *Clin Cancer Res*, 2006. 12(6): p. 1701-6.
58. Falconer, D.S. and T.F.C. Mackay, *Introduction to Quantitative Genetics*. 4th ed. 1996, Harlow, Essex, UK: Longmans Green. 480.
59. Pepper, J.W., Defeating Pathogen Drug Resistance: Guidance from Evolutionary Theory. *Evolution*, 2008. 62(12): 3185-3191.
60. Boehm, T., et al., Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. *Nature*, 1997. 390(6658): p. 404-7.
61. Hope, K.J., L. Jin, and J.E. Dick, Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. *Nat Immunol*, 2004. 5(7): p. 738-43.
62. Cho, R.W. and M.F. Clarke, Recent advances in cancer stem cells. *Curr Opin Genet Dev*, 2008. 18(1): p. 48-53.
63. Dean, M., T. Fojo, and S. Bates, Tumour stem cells and drug resistance. *Nat Rev Cancer*, 2005. 5(4): p. 275-84.
64. Costello, R.T., et al., Human acute myeloid leukemia CD34+/CD38- progenitor cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis, reduced immunogenicity, and impaired dendritic cell transformation capacities. *Cancer Res*, 2000. 60(16): p. 4403-11.

65. Hill, R.P., Identifying cancer stem cells in solid tumors: case not proven. *Cancer Res*, 2006. 66(4): p. 1891-5; discussion 1890.
66. McBride, S.M., Natural selection's challenge to the cancer stem cell hypothesis. *Med Hypotheses*, 2008. 71(3): p. 471-2.
67. Adams, J.M. and A. Strasser, Is tumor growth sustained by rare cancer stem cells or dominant clones? *Cancer Res*, 2008. 68(11): p. 4018-21.
68. Shipitsin, M., et al., Molecular definition of breast tumor heterogeneity. *Cancer Cell*, 2007. 11(3): p. 259-73.
69. Campbell, L.L. and K. Polyak, Breast tumor heterogeneity: cancer stem cells or clonal evolution? *Cell Cycle*, 2007. 6(19): p. 2332-8.
70. Visvader, J.E. and G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat. Rev. Cancer*, 2008. 8(10): p. 755-68.
71. Wang, J.C., Evaluating Therapeutic Efficacy against Cancer Stem Cells: New Challenges Posed by a New Paradigm. *Cell Stem Cell*, 2007. 1(5): p. 497-501.
72. Levin, B.R., M. Lipsitch, and S. Bonhoeffer, Population biology, evolution, and infectious disease: convergence and synthesis. *Science*, 1999. 283(5403): p. 806-9.
73. Stearns, S.C. and J.C. Koella, eds. *Evolution in Health and Disease*. 2nd ed. 2008, Oxford University Press: Oxford.
74. Ewald, P.W., Using Evolution as a Tool for Controlling Infectious Diseases, in *Evolutionary Medicine*, W.R. Trevathan, E.O. Smith, and J.J. McKenna, Editors. 1999, Oxford University Press: Oxford. p. 245-270.
75. Rowe-Magnus, D.A. and D. Mazel, The Evolution of Antibiotic Resistance, in *Evolution of Microbial Pathogens*, H.S. Seifert and V.J. Dirita, Editors. 2006, ASM Press: Washington, D.C. p. 221-241.
76. Roche-Lestienne, C. and C. Preudhomme, Mutations in the ABL kinase domain pre-exist the onset of imatinib treatment. *Semin Hematol*, 2003. 40(2 Suppl 2): p. 80-2.
77. Gorre, M.E., et al., Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*, 2001. 293(5531): p. 876-80.