Review

Cancer Research

Precision Oncology: Between Vaguely Right and Precisely Wrong

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Abstract

Precision Oncology seeks to identify and target the mutation that drives a tumor. Despite its straightforward rationale, concerns about its effectiveness are mounting. What is the biological explanation for the "imprecision?" First, Precision Oncology relies on indiscriminate sequencing of genomes in biopsies that barely represent the heterogeneous mix of tumor cells. Second, findings that defy the orthodoxy of oncogenic "driver mutations" are now accumulating: the ubiquitous presence of oncogenic mutations in silent premalignancies or the dynamic switching without mutations between various cell phenotypes that promote progression. Most troublesome is the observation that cancer cells that survive treatment still will have suffered cytotoxic stress and thereby enter a stem cell–like state, the seeds for recurrence. The benefit of "precision targeting" of mutations is inherently limited by this counterproductive effect. These findings confirm that there is no precise linear causal relationship between tumor genotype and phenotype, a reminder of logician Carveth Read's caution that being vaguely right may be preferable to being precisely wrong. An open-minded embrace of the latest inconvenient findings indicating nongenetic and "imprecise" phenotype dynamics of tumors as summarized in this review will be paramount if Precision Oncology is ultimately to lead to clinical benefits. *Cancer Res; 77(23); 6473–9. ©2017 AACR.*

Introduction

The application of "Precision Medicine" to the management of cancer, or "Precision Oncology," has quickly captured the imagination of scientists, clinicians, and the public because of its intuitively plausible scientific rationale: identification of the oncogenic mutation in a patient's cancer genome that drives the growth of her tumor, followed by treatment with target-selective drugs that block the phenotypic consequence of precisely that oncogenic mutation (1, 2). But critics have been quick to warn of an overly simplistic view, reminding us of the balance between warranted hope and runaway hype (3, 4). They point to the genetic heterogeneity of tumors, the diversity of pathways used by different tumors, and the inexorable evolution of drug resistance. These complications are readily comprehensible within the existing paradigm, but there is more outside this box of conventional thought that demands a more encompassing perspective.

Here, we go beyond the usual pushback against Precision Oncology and present emerging concepts of tumor dynamics that defy the orthodoxy in Precision Oncology. We summarize recent observations and insights that have been difficult to reconcile with the paradigm of oncogene-driven tumorigenesis and that will provide a biological basis to comprehend the immanent limitations of precision-guided treatment: (i) Identification of oncogenic mutations by tumor sequencing is obfuscated by intratumor genetic heterogeneity. (ii) Targeting of causative mutations is complicated by phenotypic plasticity of cells, which produces nongenetic heterogeneity and cell behaviors not predicted by a cell's genotype. Cancer is not a disease of DNA or the cell but of the tissue (3, 5, 6). Eradication of a tumor may fail because a tumor is a robust adaptive ecosystem of diverse, communicating cells that senses injuries, and repairs itself; this could explain why the very act of attempting to kill cancer cells may also plant the seed of recurrence (Fig. 1).

Current Public Perception and Cracks in the Paradigm

Clinical studies have failed to demonstrate that systematic cancer genome sequencing results in significant benefit to patients. One study showed that in only 5% of cases does genome sequencing result in "actionable" information (7), detection of a mutated driver oncogene for which there is a target-selective drug available and accessible (8). Often, well-established activating mutations are detected that cannot yet be pharmacologically blocked. For instance, one third of all human tumors are driven by mutations in *RAS* genes (9), yet development of drugs to block *KRAS* gene function has been challenging (10). One may ask: Given that Ras is so widely mutated and its intracellular pathways fan out to regulate thousands of genes (11), would targeting Ras indeed represent a "precision" intervention? Is precision the "right" strategy at all?

Despite limited clinical impact, Precision Oncology, glorified by a handful of highly publicized anecdotal success stories continues its ascendance in public awareness. The U.S. governments' new Cancer Moonshot Program launched in 2016 with great fanfare has Precision Oncology as one of its central pillars

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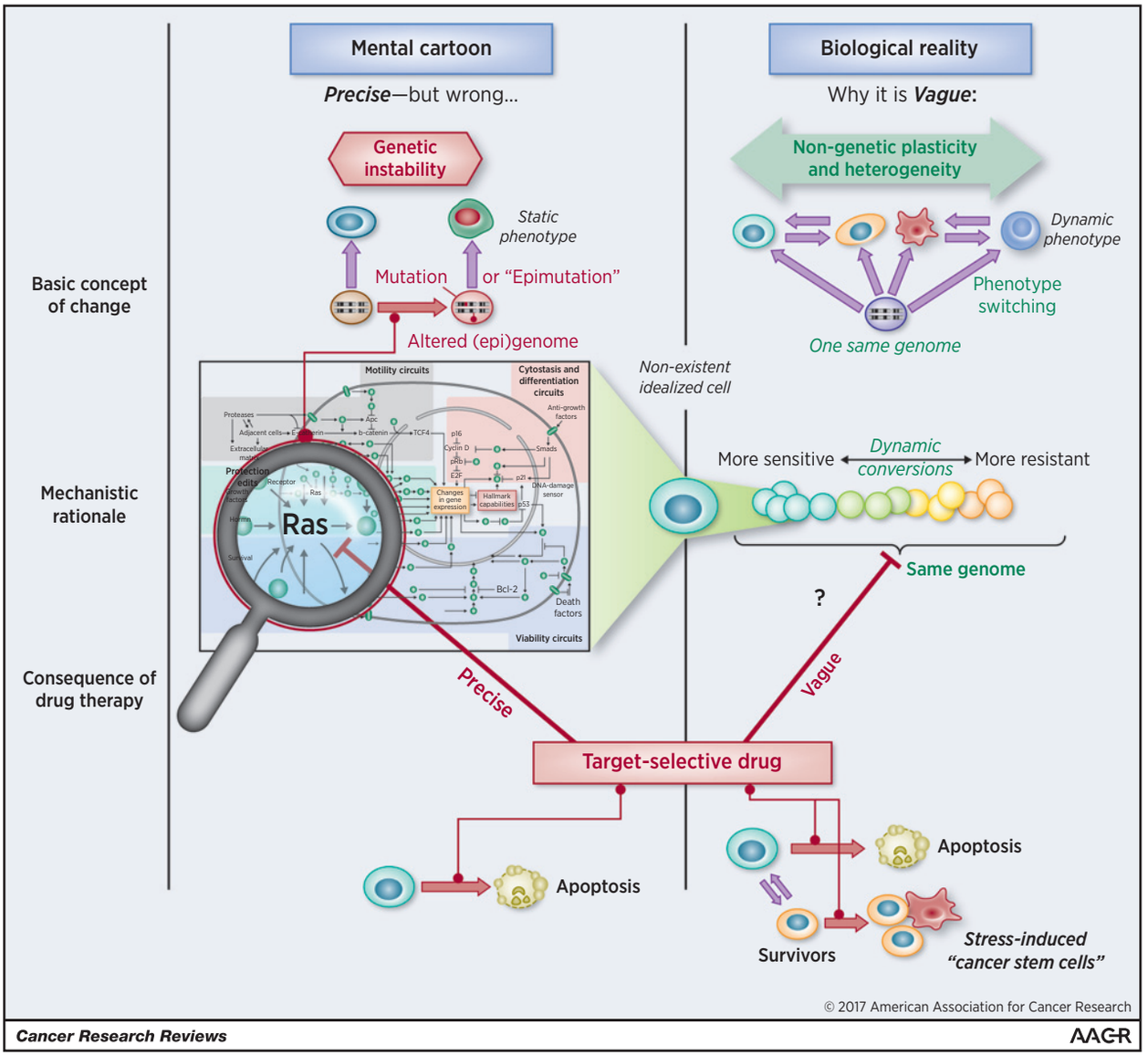

Figure 1.
Limitations of the precision approach. The belief in Precision Oncology relies on the assumption that tumor treatment is more effective if a target-specific therapy is selected to match the genetic or epigenetic alterations observed in an individual patient's tumor (e.g., shown here on the left in targeting altered Ras). However, in implementing such a precision approach, one encounters a number of biological realities that test the foundations of this mental model (right). Owing to heterogeneity, a single tumor displays intratumoral variation at the genetic and nongenetic level. Which specific alteration should then be targeted? Nongenetic heterogeneity is a critical consideration as cells respond to broad, environmental perturbations and drug treatments by converting to many other cell states, including stem-like, resistant cell phenotypes (Ras signaling inset image modified from ref. 42).

(12). Questions of cost effectiveness are drowned out by ubiquitous direct-to-consumer advertisements for Precision Oncology by cancer care centers (13). And the skepticism of some investigators regarding the very concept of "driver mutations" (14, 15) is overpowered by the gratifying logics of targeting a molecular root cause.

Ironically, the same genome sequencing technologies that have brought us Precision Oncology have uncovered a series of inconvenient facts that explain why Precision Oncology cannot be the universal solution. Deep sequencing with next-generation sequencing and single-cell omics technologies have revealed inconsistencies in the paradigm of "driver mutations" (16) and the somatic mutation theory of cancer, warranting a more critical stance toward Precision Oncology (15, 17).

Genetic Heterogeneity in Tumor Specimens

The notion of massive cell heterogeneity in tumors is now broadly acknowledged. But the minds of investigators still operate largely in the category of genetic as opposed to nongenetic

heterogeneity (18–20). Before clinical researchers recognized tumor cell heterogeneity as a challenge, cartoons of mutated oncogenic pathways (21) were used as a scheme of causality. These were mentally projected onto every cell of the tumor under the tacit assumption that the tumor is a homogenous mass of identical cells. This idea goes back to the textbook concept of clonal expansion of a dominant cell clone carrying the oncogenic mutation that "takes over" the entire tumor. Reality departs from such cartoonish pathway models in two ways: (i) Every individual cancer cell carries a distinct set of mutations, hardly surprising given the genomic instability of cancer cells; (ii) there is little evidence of a "purifying selective sweep" by the most malignant clone carrying "driver mutations" that would "homogenize" the tumor.

Although one finds signatures of clonal selection in some tumors (22–24), clonal diversity dominates. Genetic heterogeneity is now readily recognized by deep sequencing and genomic analysis of multiple biopsies from different regions of the same tumor (25). Analysis of regional diversity of genomes in colorectal and liver tumors (26, 27), with 100 million distinct coding region mutations found in a single tumor, illustrates this genetic diversity (26). A systematic analysis of the frequency distributions of mutations in bulk tumor genomes suggests that neutral evolution is the rule rather than exception (28). Single-cell studies confirm multiple, branching evolutionary trajectories proceeding concomitantly in leukemia populations (29).

With such genetic diversity within a single tumor, we must reexamine what exactly needs to be measured to identify "oncogenic mutations" in the diagnostic step of Precision Oncology. There is no single tumor genome to sequence. Genomic DNA extracted from a bulk tumor sample may capture the most dominant clone. The "other" tumor cells that do not carry the targeted "driver mutation" may yet survive and be the source of tumor recurrence.

In contrary to the regional genetic diversity uncovered by sequencing a tumor following surgery, temporal changes in genetic diversity have been less studied. Analyses of individual cell genomes in serial samples extracted at multiple time points indicate complex subclone dynamics that reflect independent evolutionary paths and copresence of distinct stages, far from the standard model of multistep cumulative acquisition of mutations (22, 29). Circulating tumor cells (30) or even tumor (ct) DNA (31) now afford a noninvasive window to the tumor, enabling longitudinal monitoring without repeated biopsies. But given the cellular heterogeneity of tumors, do these "liquid biopsies" offer representative samples of entire tumor cell populations? For example, ctDNA analysis of patients with colorectal cancer who had developed resistance to anti-EGFR therapy revealed up to 12 distinct subclones in one patient, each harboring a different mutation in *RAS* or *BRAF* genes (32).

Neither Mutations nor Epimutations Are Strictly the "Cause" of Cancer

Nowhere is the disconnect between nominal "oncogenic mutation" and cancer phenotype as lucidly manifested as in precancerous lesions that only rarely become cancer. With next-generation sequencing of somatic tissues, a large number of mutations that affect oncogenes have been detected in healthy skin cells. Potentially oncogenic mutations (in BRAF, TP56, PIKC3, etc.) are found in normal skin and at high frequency in nevi, the vast majority of which never develop into malignant melanoma (33–36). As in tumors, these mutations may even display signs of clonal selection and expansion. Similarly, a large fraction of epithelial cells in Barrett's esophagus also carry oncogenic mutations. But again, only <1% of Barrett's esophagus progresses to esophageal adenocarcinoma (37, 38). For a systematic review of driver mutations found in noncancer tissues, see Kato and colleagues' work (39). A logical interpretation is that so-called "driver mutations" are not actual omnipotent "drivers" but represent, in the simplest scheme, molecular lesions that are causatively necessary but not sufficient for pathogenesis. But the common notion that "additional hits" are needed may also be too simplistic.

If genomic mutations are not sufficient, one should also consider the logical complement that accumulation of specific mutations may not be necessary in the first place. Although absence of evidence is never evidence of absence, whole-genome sequencing of ependymoma has failed to identify recurrent somatic mutations (40), and several childhood cancers, notably leukemia and rhabdomyosarcoma, may be caused by just a single mutation (41). This is not as outlandish as one may think if one overcomes the tacit habit of reducing complex phenotypes to genotypes, a common epistemic practice in which each new "hallmark of cancer" (42), such as invasion or angiogenesis, is mapped to a plausible mutation. In reality, a single genetic alteration in a master regulatory gene may profoundly alter the developmental trajectory of cells on Waddington's canonical "epigenetic landscape" of cell development. In this (mathematically permissive) abstraction, every point on the landscape is a cell phenotype, and the major valleys into which cell naturally roll represent stable normal differentiated cell types (43). Mutations may push cells into "side-valleys" where further descent (=differentiation) is blocked, causing a maturation arrest that is a fundamental feature of all neoplasia (19). Once in the wrong developmental path, the derailing mutation may not be necessary anymore to maintain the malignant state. Such hysteresis in which activity of an oncogenic protein is required only in a transient developmental window (44) obviously defies the therapeutic targeting of "causal" mutations because dependency on the (tumor initiating) oncoprotein is lost.

A prosaic manifestation of "phenotype innovation without mutation" is the diversity of cell types in the metazoan body, each of which constitutes a distinct, stable, and inheritable cell phenotype that is not produced by genomic alterations. It is then only a small step to explain minimally mutated or even mutation-less cancer if one considers the old idea that cancer cells are pathologic (immature) cell types not meant to be implemented but once realized, are trapped in developmental dead ends (45).

When no plausible mutation is found, it is common practice to implicate "epimutations," that is, changes in DNA methylation or covalent histone modifications (ref. 46; such epigenetic alterations have little to do with Waddington's original "epigenetic landscape" despite the use of the same term; refs. 47, 48). These reversible changes of the "epigenetic state" of gene loci must be programmed by specific transcriptional regulation via recruitment of chromatin-modifying enzymes by site-specific transcription factors (49). Thus, chromatin changes reflect the altered gene expression patterns that

implement the pathologic cell phenotype and are the result rather than cause of the coordinated regulation of genes by the transcriptional network that integrates inputs from a variety of signaling pathways (48, 50). Therefore, when invoking "epigenetic" causation, one must consider that epigenetic modifications are fundamentally distinct from genetic alterations, belonging to a separate explanatory category: A chromatin modification is not a random event (the defining property of mutations) that occurs in a single cell and must be selected for to spread through the cell population. Instead, epigenetic modifications merely reflect the coordinated gene expression patterns, which in turn are controlled by regulatory signals in the tissue that act in parallel on large numbers of cells to modulate gene expression. These tissue-level signals have much more leverage than genetic mutations because they affect a large proportion of the cells in parallel. Perhaps, such events of abnormal signaling are the rate-limiting factors in progression of precancerous lesions, which may already carry oncogenic mutations. Then mutations would not be the "cause" of macroscopic (clinical) cancer.

This view applies to epigenetics in both senses and has fundamental epistemic and practical consequences: The development of clinical cancer in premalignant or dormant lesions may not be driven solely by "additional mutational hits." Nongenotoxic tissue-level stress, for example, chronic inflammation and toxic stress, alters the behavior of entire cell populations through signaling. Such nongenetic perturbations may be a much more potent trigger of growth in a dormant tumor and thus may be considered the "effective cause" of a clinical tumor. Such directed and concerted cell population level dynamics would explain rapid recurrence but defy the picture of clonal evolution that underlies Precision Oncology.

Paradoxical Responses to Targeted Therapy

A glaring illustration that cancer treatment is far more complex than can be captured by the Precision Oncology scheme is provided by a series of paradoxical effects: Attempts to block oncogenic pathways with precision drugs can instead stimulate progression (51, 52). Clinically, this is well documented in the case of melanoma treatment with BRAF inhibitors, a targeted therapy that has seen spectacular successes in the treatment of melanoma for a subset of patients (53, 54). Such paradoxical effects have also been reported in treatments with checkpoint inhibitors in immuno-oncology, the youngest child of Precision Ontology (55, 56).

Following the logics of target-selective therapy, benign nevi, which harbor BRAF mutations, should shrink when a melanoma patient is treated with a BRAF inhibitor. Some do but others actually grow under BRAF therapy (57, 58). BRAF inhibitors can trigger other (nonmelanoma) skin cancers, perhaps due to the compensatory activation of mitogenic MAPK when BRAF is inhibited (54, 59).

The rationale of Precision Oncology has therefore been expanded to targeting multiple pathways by combining treatment with BRAF and MEK inhibitors. Although combination treatment can substantially extend survival in melanoma patients, resistance and recurrence are observed in less than a year (60). The question is: how many backdoor pathways do tumors have available for escaping single-point targeted therapy, which becomes a whack-a-mole game due to the unforeseeably immense repertoire of evasion pathways. Where is then precision? Are we not better off acknowledging an inherent vagueness in intervening with complex processes and using broadly acting drugs, such as the class of chromatin modulators that target epigenetic enzymes? (61).

Expanding Precision Oncology to Embrace Complexity

The most salient manifestation of the limits of a "precision intervention" is the rapid and near inevitable development of therapy resistance and recurrence. Logically, tumor recurrence is at odds with Precision Oncology's targeting of the molecular root cause that drives the tumor, unless, as is done by default, recurrence is explained by a somatic evolution in the tumor: genetic mutations in the cancer cells' genome that confer resistance are selected for (62). Indeed, genetic mutations in the targeted pocket of kinases or those that activate alternative pathways can be routinely observed (63). However, in this gene-centric perspective, the convoluted and inevitable path to the phenotype of resistance is again reduced to a simple genetic cause, which takes effect thanks to a somatic equivalent of Darwinian evolution. But this rationale misses the complexity of the tumor, which provides many more ways for evading treatment. We summarize two central but forgotten principles (64):

Nongenetic plasticity

A growing number of reports may help explain why dynamic nongenetic heterogeneity of tumor cell states defies a "precise" genotype–phenotype causal relationship and allows them to adapt to treatment stress without mutations. First, any attempt to kill a cell by irradiation, chemotherapy, or targeted therapy will leave many cells alive for a variety of reasons, ranging from drug accessibility to cell-intrinsic susceptibility. In a 1 g tumor of approximately 10^9 cells even if (in the very optimistic case) treatment kills 99% of cells, still 10^7 cells would, independent of resistance mutations, survive (18). A neglected reason for such uneven killing efficiency is the enormous nongenetic cell-to-cell variability due to the physics of molecular fluctuations: the abundance of any given protein, including the drug target, varies up to 1,000-fold between cells within the same genetic clone (18). This untold heterogeneity of cell states results in a wide statistical spread of susceptibility: some cells will always receive substantially smaller effective (relative) dose and thus are more likely to survive.

Second, a more vexatious biological fact is that the surviving cells are not static bystanders. In response to cytotoxic stress, a robust protective response is activated through induction of specific biological pathways, long before Darwinian selection of randomly occurring genetically encoded behaviors. This induced response to treatment stress is manifested in the acquisition of a resilient stem-like state by surviving cancer cells (51, 52) after their near-death experience, as is ubiquitously seen following chemotherapy or irradiation (reviewed in ref. 65). Similarly, chemotherapy promotes immune evasion by stimulating expression of the immune checkpoint protein PD-1 (55, 56).

Third, yet more troublesome is the cytokine storm released by cells stressed by therapy and the "danger signals" emitted by dying cells, which promote the transition to the stem-like state

in other cells (66, 67). This explains the old observation that coinjection of radiation-generated tumor cell debris along with tumor cells drastically stimulates tumor growth in xenograft models (68). This response of surviving tumor cells to dying cells constitutes a non-cell–autonomous, tumor–tissue level damage response that stimulates inflammation and suppresses antitumor immunity (69). Therapy-induced cell stemness and tissue inflammation suggest a primordial wound-healing and regenerative response of the tissue that if nonphysiologically activated and unopposed, might sustain the tumor. This picture of perpetuating self-healing was articulated by the 19th century pathologist Virchow and recently revived by Dvorak (70): Tumors are wounds that do not heal.

Thus, if mutations are not the sufficient cause of progression under therapy, genetic alterations combined with the sea of nongenetic regenerative signals stimulated by tissue destruction may reinforce each other to produce a pathologic healing response that escapes tissue homeostasis. Therefore, any treatment (targeted or not) that involves killing tumor cells but, as is nearly universally the case, leaves some cancer cells alive and stressed, is a double-edged sword. Recurrence is not driven solely by the passive Darwinian scheme of "Survival of the fittest" (cell). Instead, tumors may follow Nietzsche's principle (71): "What does not destroy me, strengthens me." Conquering this active dynamics, of which we have currently only a vague notion, will require more sophisticated, multipronged precision than that of current Precision Oncology.

Outlook: "Precision" Must Not Mean "Narrow"

What can we do to elevate the bar for "precision" and embrace all the aforementioned complexities? First and foremost, we need to learn more about the interplay between genetic and nongenetic heterogeneity of tumor cells and their dynamics, the plasticity of individual cells and of cell populations. The very technology that gave rise to Precision Oncology now affords high-throughput single-cell analysis to study these features. Advanced methods for single-cell resolution molecular profiling, such as RNA-seq, CyTOF, and MIBI (72), open a new window to the molecular signatures of individual tumor cells and to single-cell resolution of cell population structures (73, 74).

But only if interpreted within the appropriate theoretical framework of tumors as highly evolved complex adaptive systems (64) that are stochastic and nonlinear, and by considering that any intervention in such self-sustaining reactive systems can backfire (75), can these new analytic tools guide us beyond the confines of linear–mechanistic schemes of current Precision Oncology.

The sentiment of "precision" in current Precision Oncology depended on a narrow view that ignores the grander scheme of complex dynamics. The associated naïve mental picture of the molecular network of tumor cells working like a clockwork makes us, to recall Carveth Read, "precisely wrong," when we need to first be "vaguely right," to see beyond the trees and acknowledge the forest, and understand how the latter as a whole relates to the finer details of its parts. With new technologies for studying genetic and nongenetic cell population heterogeneity, tumor cell plasticity, and intercell communication, we are only at the beginning of achieving a new "precision" at the more encompassing level of systems behaviors to better understand the inherent resilience of tumors and to learn how to conquer it.

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References

1. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015;372:793–5.
2. Tsimberidou AM, Eggermont AM, Schilsky RL. Precision cancer medicine: the future is now, only better. Am Soc Clin Oncol Educ Book 2014:61–9. doi:10.14694/EdBook_AM.2014.34.61.
3. Joyner MJ, Paneth N. Seven questions for personalized medicine. JAMA 2015;314:999–1000.
4. Tannock IF, Hickman JA. Limits to personalized cancer medicine. N Engl J Med 2016;375:1289–94.
5. Sonnenschein C, Soto AM. The death of the cancer cell. Cancer Res 2011;71:4334–7.
6. Lambert G, Estevez-Salmeron L, Oh S, Liao D, Emerson BM, Tlsty TD, et al. An analogy between the evolution of drug resistance in bacterial communities and malignant tissues. Nat Rev Cancer 2011; 11:375–82.
7. Beltran H, Eng K, Mosquera JM, Sigaras A, Romanel A, Rennert H, et al. Whole-exome sequencing of metastatic cancer and biomarkers of treatment response. JAMA Oncol 2015;1:466–74.
8. West HJ. No solid evidence, only hollow argument for universal tumor sequencing: show me the data. JAMA Oncol 2016;2:717–8.
9. Kranenburg O. The kras oncogene: past, present, and future. Biochim Biophys Acta 2005;1756:81–2.
10. Ostrem JM, Shokat KM. Direct small-molecule inhibitors of kras: from structural insights to mechanism-based design. Nat Rev Drug Discov 2016;15:771–85.
11. Zuber J, Tchernitsa OI, Hinzmann B, Schmitz AC, Grips M, Hellriegel M, et al. A genome-wide survey of ras transformation targets. Nat Genet 2000;24:144–52.
12. Office of the Press Secretary TWH. Fact sheet: At cancer moonshot summit, vice president biden announces new actions to accelerate progress toward ending cancer as we know it. 2016. Available from: https://www.whitehouse.gov/the-press-office/2016/06/28/fact-sheet-cancer-moonshot-summit-vice-president-biden-announces-new%3E.
13. Kontos EZ, Viswanath K. Cancer-related direct-to-consumer advertising: a critical review. Nat Rev Cancer 2011;11:142–50.
14. Soto AM, Sonnenschein C. The somatic mutation theory of cancer: growing problems with the paradigm? Bioessays 2004;26:1097–107.
15. Satgé D. Analysis of somatic mutations in cancer tissues challenges the somatic mutation theory of cancer. eLS 2013. doi: 10.1002/9780470015902.a0024465.
16. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–58.
17. Soto AM, Sonnenschein C. Emergentism as a default: cancer as a problem of tissue organization. J Biosci 2005;30:103–18.

18. Brock A, Chang H, Huang S. Non-genetic heterogeneity -a mutation-independent driving force for the somatic evolution of tumours. Nat Rev Genet 2009;10:336–42.
19. Huang S. Genetic and non-genetic instability in tumor progression: Link between the fitness landscape and the epigenetic landscape of cancer cells. Cancer Metastasis Rev 2013;32:423–48.
20. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 2012;12:323–34.
21. Hahn WC, Weinberg RA. A subway map of cancer pathways. Nat Rev Cancer 2002. Available from: http://www.nature.com/nrc/posters/subpathways/index.html.
22. Navin N, Krasnitz A, Rodgers L, Cook K, Meth J, Kendall J, et al. Inferring tumor progression from genomic heterogeneity. Genome Res 2010;20:68–80.
23. Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, et al. Evolution of human bcr-abl1 lymphoblastic leukaemia-initiating cells. Nature 2011;469:362–7.
24. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 2010;467:1114–7.
25. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 2014;46:225–33.
26. Ling S, Hu Z, Yang Z, Yang F, Li Y, Lin P, et al. Extremely high genetic diversity in a single tumor points to prevalence of non-darwinian cell evolution. Proc Natl Acad Sci U S A 2015;112:E6496–505.
27. Sottoriva A, Spiteri I, Piccirillo SG, Touloumis A, Collins VP, Marioni JC, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci U S A 2013;110:4009–14.
28. Williams MJ, Werner B, Barnes CP, Graham TA, Sottoriva A. Identification of neutral tumor evolution across cancer types. Nat Genet 2016;48:238–44.
29. Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature 2011;469:356–61.
30. Plaks V, Koopman CD, Werb Z. Cancer. Circulating tumor cells. Science 2013;341:1186–8.
31. Ma M, Zhu H, Zhang C, Sun X, Gao X, Chen G. "Liquid biopsy"-ctdna detection with great potential and challenges. Ann Transl Med 2015;3:235.
32. Siravegna G, Mussolin B, Buscarino M, Corti G, Cassingena A, Crisafulli G, et al. Clonal evolution and resistance to egfr blockade in the blood of colorectal cancer patients. Nat Med 2015;21:795–801.
33. Hafner C, Lopez-Knowles E, Luis NM, Toll A, Baselga E, Fernandez-Casado A, et al. Oncogenic pik3ca mutations occur in epidermal nevi and seborrheic keratoses with a characteristic mutation pattern. Proc Natl Acad Sci U S A 2007;104:13450–4.
34. Ling G, Persson A, Berne B, Uhlen M, Lundeberg J, Ponten F. Persistent p53 mutations in single cells from normal human skin. Am J Pathol 2001;159:1247–53.
35. Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 2015;348:880–6.
36. Tschandl P, Berghoff AS, Preusser M, Burgstaller-Muehlbacher S, Pehamberger H, Okamoto I, et al. Nras and braf mutations in melanoma-associated nevi and uninvolved nevi. PLoS One 2013;8:e69639.
37. Weaver JM, Ross-Innes CS, Shannon N, Lynch AG, Forshew T, Barbera M, et al. Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis. Nat Genet 2014;46:837–43.
38. Kapoor H, Agrawal DK, Mittal SK. Barrett's esophagus: Recent insights into pathogenesis and cellular ontogeny. Transl Res 2015;166:28–40.
39. Kato S, Lippman SM, Flaherty KT, Kurzrock R. The conundrum of genetic "drivers" in benign conditions. J Natl Cancer Inst 2016;108:pii:djw036.
40. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stutz AM, et al. Epigenomic alterations define lethal cimp-positive ependymomas of infancy. Nature 2014;506:445–50.
41. Greaves M. When one mutation is all it takes. Cancer Cell 2015;27:433–4.
42. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
43. Waddington CH. Canalization of development and the inheritance of acquired characters. Nature 1942;150:563–5.
44. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol 1997;139:1861–72.
45. Kauffman S. Differentiation of malignant to benign cells. J Theor Biol 1971;31:429–51.
46. Peltomaki P. Mutations and epimutations in the origin of cancer. Exp Cell Res 2012;318:299–310.
47. Pisco A, Fouquier d'Herouel A, Huang S. `Epigenetics': Many meanings - one common concept. 2016. Available from: http://biorxiv.org/content/early/2016/05/12/053009.
48. Ptashne M. `On the use of the word epigenetic'. Curr Biol 2007;17:R233–6.
49. Goode DK, Obier N, Vijayabaskar MS, Lie ALM, Lilly AJ, Hannah R, et al. Dynamic gene regulatory networks drive hematopoietic specification and differentiation. Dev Cell 2016;36:572–87.
50. Huang S. The molecular and mathematical basis of waddington's epigenetic landscape: a framework for post-darwinian biology. Bioessays 2012;34:149–55.
51. Seguin L, Kato S, Franovic A, Camargo MF, Lesperance J, Elliott KC, et al. An integrin beta(3)-kras-ralb complex drives tumour stemness and resistance to egfr inhibition. Nat Cell Biol 2014;16:457–68.
52. Pisco AO, Brock A, Zhou J, Moor A, Mojtahedi M, Jackson D, et al. Non-darwinian dynamics in therapy-induced cancer drug resistance. Nat Commun 2013;4:2467.
53. Sanchez-Laorden B, Viros A, Girotti MR, Pedersen M, Saturno G, Zambon A, et al. Braf inhibitors induce metastasis in ras mutant or inhibitor-resistant melanoma cells by reactivating mek and erk signaling. Sci Signal 2014;7:ra30.
54. Robert C, Arnault JP, Mateus C. Raf inhibition and induction of cutaneous squamous cell carcinoma. Curr Opin Oncol 2011;23:177–82.
55. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after immunotherapy: analysis of genomic alterations associated with accelerated growth rate. Clin Cancer Res 2017;23:4242–50.
56. Champiat S, Dercle L, Ammari S, Massard C, Hollebecque A, Postel-Vinay S, et al. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-pd-1/pd-l1. Clin Cancer Res 2017;23:1920–8.
57. Anforth RM, Carlos GR, Scolyer RA, Chou S, Fernandez-Penas P. Eruptive naevi in a patient treated with lgx818 for braf mutant metastatic melanoma. Melanoma Res 2015;25:91–4.
58. Haenssle HA, Kraus SL, Brehmer F, Kretschmer L, Volker B, Asper H, et al. Dynamic changes in nevi of a patient with melanoma treated with vemurafenib: Importance of sequential dermoscopy. Arch Dermatol 2012;148:1183–5.
59. Poulikakos PI, Zhang C, Bollag G, Shokat KM, Rosen N. Raf inhibitors transactivate raf dimers and erk signalling in cells with wild-type braf. Nature 2010;464:427–30.
60. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined braf and mek inhibition in melanoma with braf v600 mutations. N Engl J Med 2012;367:1694–703.
61. Schmoch T, Gal Z, Mock A, Mossemann J, Lahrmann B, Grabe N, et al. Combined treatment of atra with epigenetic drugs increases aggressiveness of glioma xenografts. Anticancer Res 2016;36:1489–96.
62. Diaz LA Jr, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted egfr blockade in colorectal cancers. Nature 2012;486:537–40.
63. Rueff J, Rodrigues AS. Cancer drug resistance: a brief overview from a genetic viewpoint. Methods Mol Biol 2016;1395:1–18.
64. Huang S. Tumor progression: chance and necessity in darwinian and lamarckian somatic (mutationless) evolution. Prog Biophys Mol Biol 2012;110:69–86.
65. Pisco AO, Huang S. Non-genetic cancer cell plasticity and therapy-induced stemness in tumour relapse: `What does not kill me strengthens me'. Br J Cancer 2015;112:1725–32.
66. Kornbluth RS. The immunological potential of apoptotic debris produced by tumor cells and during hiv infection. Immunol Lett 1994;43:125–32.

67. Kuraishy A, Karin M, Grivennikov SI. Tumor promotion via injury- and death-induced inflammation. Immunity 2011;35:467–77.
68. Revesz L. Effect of tumour cells killed by x-rays upon the growth of admixed viable cells. Nature 1956;178:1391–2.
69. Hangai S, Ao T, Kimura Y, Matsuki K, Kawamura T, Negishi H, et al. Pge2 induced in and released by dying cells functions as an inhibitory damp. Proc Natl Acad Sci U S A 2016;113:3844–9.
70. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986; 315:1650–9.
71. Nietzsche F. Twilight of the idols. Oxford, United Kingdom: Oxford University Press; 1998.
72. Marr C, Zhou JX, Huang S. Single-cell gene expression profiling and cell state dynamics: collecting data, correlating data points and connecting the dots. Curr Opin Biotechnol 2016;39:207–14.
73. Muller S, Liu SJ, Di Lullo E, Malatesta M, Pollen AA, Nowakowski TJ, et al. Single-cell sequencing maps gene expression to mutational phylogenies in pdgf- and egf-driven gliomas. Mol Syst Biol 2016; 12:889.
74. Tirosh I, Izar B, Prakadan SM, Wadsworth MH II, Treacy D, Trombetta JJ, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell rna-seq. Science 2016;352:189–96.
75. Huang S. The war on cancer: lessons from the war on terror. Front Oncol 2014;4:293.