

Genomic Sequencing for Cancer Diagnosis and Therapy

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Abstract

For a decade, the technologies behind DNA sequencing have improved rapidly in cost reduction and speed. Sequencing in large populations of cancer patients is leading to dramatic advances in our understanding of the cancer genome. The wide variety of cancer types sequenced and analyzed using these technologies has revealed many novel fundamental genetic mechanisms driving cancer initiation, progression, and maintenance. We have deepened our understanding of the signaling pathways, demonstrating disruption in epigenetic regulation and destabilization of the splicing machinery. The molecular mechanisms of resistance to targeted therapies are being elucidated for the first time. The translation of genome-scale variation into clinically actionable information is still in its infancy; nevertheless, insights from sequencing studies have led to the discovery of a variety of novel diagnostic biomarkers and therapeutic targets. Here, we review recent advances in cancer genomics and discuss what the new findings have taught us about cancer biology and, more importantly, how these new findings guide more effective diagnostic and treatment strategies.

Somatic mutations: mutations that occur in the tumor cell but not in the germ cell of the body

INTRODUCTION

In 2008, 7.6 million people died of cancer—13% of all deaths worldwide. The number of cancer deaths globally is projected to increase 51% from 2008 to 2030 (11.5 million deaths), influenced in part by an aging global population (<http://www.who.int/en/>). According to the 2013 statistics from the World Health Organization (<http://www.who.int/research/en/>), cancer has replaced heart disease as the primary cause of death in many developed countries including the United States, Canada, Japan, and Australia, and it remains the leading cause of death worldwide in developing countries. This fact is driving increased research and public health efforts to fight against cancer both nationally and internationally.

Today cancer is recognized as a disease of the genome; however, recognition of that fact was slow in coming. In spite of the publication of the central dogma of molecular biology, which elucidated the flow of biological information from DNA to RNA to protein, in 1958 (1), not until 1973 did the first concrete hints at the genetic underpinnings of cancer appear. In that year, Dr. Janet Rowley, studying chronic myeloid leukemia, proposed a link between a specific chromosomal translocation, the Philadelphia chromosome, and a particular cancer type (2). Almost 10 years later in 1982, Reddy and colleagues found a single point mutation changing guanosine to thymidine

in *HRAS* associated with bladder cancer (3). This seminal discovery provided evidence that cancer can result from the alteration of a normal cell's genetic material and inaugurated an era of vigorous research aimed at enumerating the list of normal genes that when mutated or aberrantly expressed could promote the development of human cancer.

Cancer researchers have continued to accumulate knowledge on the basic mechanisms of the disease, and by 2004, an inventory of the genes associated with cancer included 291 entries—approximately 1% of the coding sequence (4). The most common form of variation in the 2004 inventory, at the dawn of the genomic era, was translocation leading to the production of oncogenic fusion proteins.

The completion of the human reference genome (5) ushered in the “genomics era” of cancer research, wherein it became possible, at least in principle, to determine the DNA sequence of any tumor and identify the somatic mutations present through comparison to the reference sequence. In practice, it required the development and refinement of next-generation sequencing (NGS) instruments (see sidebar, Sequencing) together with an understanding of principles of normal genetic variation (6), both of which developed rapidly between the finished reference genome in 2003 and the completion of the HapMap project (HapMap Phase I, II, and III) to catalog the majority of common normal human variation in 2009. (See also the supplemental section Practical Aspects of DNA Sequencing Applied to Cancer Research. Follow the **Supplemental Material** link from the Annual Reviews home page at <http://www.annualreviews.org>.)

SEQUENCING

Whole-exome sequencing: Genomic DNA is enriched using oligonucleotide probes complementary to portions of the genome encoding proteins. Sequencing only the coding portion of the genome focuses the effort to 1–2% of the genome and is more cost-effective, requiring the generation of only 6–9 billion bases of sequence.

Whole-genome sequencing: Unbiased sequencing of the whole genome. In the context of cancer genome sequencing, this requires the generation of 90–180 billion bases from the tumor and 90 billion bases from the patient's normal DNA.

REPertoire OF SOMATIC MUTATIONS IN CANCER

The knowledge of mutations accumulated in the 40 years between the discovery of the Philadelphia chromosome and the completion of the human reference genome indicates that mutations occur over vast scales (**Figure 1**). From point mutations changing a single base to

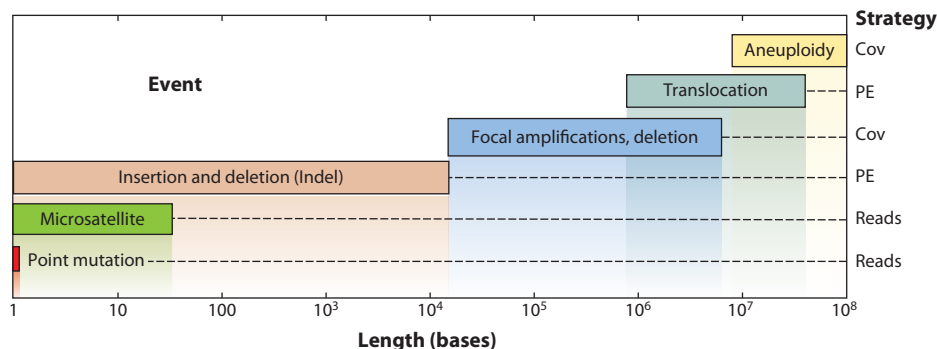


Figure 1

Size scale of genetic variation in tumor genomes. Cov, 0.5 to 1 × coverage by unique reads: Method compares coverage in tumor with that in normal and detects significant deviation. PE, paired-end reads: Detection of clusters of discordantly mapping reads pairs in tumor is compared with normal. Reads, individual sequencing reads: Detection of disruption in read's alignment is shown.

large-scale chromosomal rearrangements, the cancer genome can be reconstructed on size scales over six orders of magnitude. Large-scale DNA sequencing is the only technology that can reconstruct all the changes that occur in a given tumor over all scales (7).

LARGE-SCALE CANCER GENOME PROJECTS

New NGS technologies have enabled a systematic cataloging of cancer genomes through national and international genome projects. The Cancer Genome Atlas (TCGA) (<http://cancergenome.nih.gov/>), the International Cancer Genome Consortium (ICGC) (8), the Cancer Genome Project (<http://www.sanger.ac.uk/genetics/CGP/>), Therapeutically Applicable Research to Generate Effective Treatments (<http://target.cancer.gov/>), and other privately funded large-scale projects (9) seek to systematically catalog all the mutations in a wide variety of adult and pediatric cancers (10).

TCGA has collected a total of 7,589 patients from 25 tumor types/subtypes and has completed whole-exome sequencing for 6,602 cases and RNA-seq for 6,225 cases (<https://tcga-data.nci.nih.gov/tcga/>); ICGC currently has collected 7,774 patients from 17 tumor types (<http://icgc.org/>) and has mutation data for 5,689 cases

and gene expression data for 7,042 cases (Supplemental Figure 2; follow the link from the Annual Reviews home page at <http://www.annualreviews.org>). These projects make their data available to the wider cancer research community, providing substantial resources for cancer research. These worldwide collaborative efforts have already had a profound impact on our understanding of cancer biology over the past 5 years in large patient populations. These studies afford unparalleled statistical power to discover the majority of somatically mutated genes contributing to tumor phenotypes. That is, the results of these studies comprise a reliable “genetic parts list” for cancer. The task at hand, for clinical and basic researchers, is to devise strategies to exploit the parts list to effect improved diagnosis, prognosis, and therapy, thus improving cancer patient care.

ADVANCES IN TUMOR BIOLOGY

Tumor Mutation Frequencies: By Disease

The variation in somatic mutation frequency is a function of the number of somatic cell divisions prior to initiation of the tumor, the exposure to environmental mutagens—notably UV radiation and tobacco leaf by-products—and in some cancers, altered fidelity of the

[Supplemental Material](#)

TCGA: The Cancer Genome Atlas

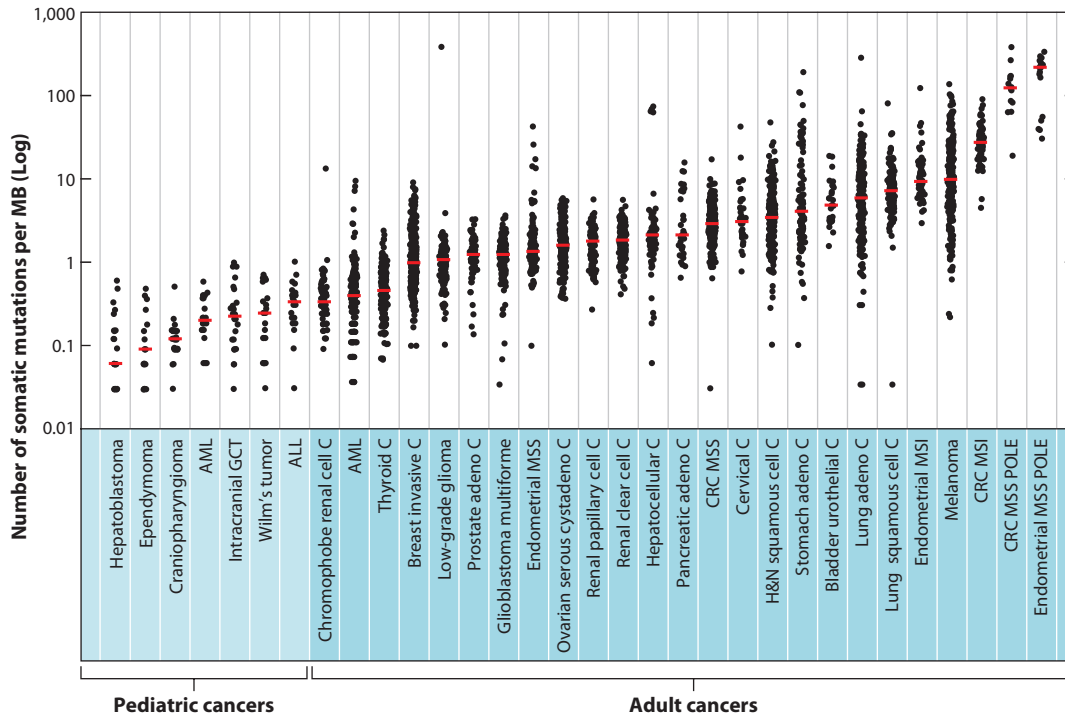


Figure 2

Frequencies of point mutations in different cancers. Each black dot represents a single patient's tumor mutation frequency as determined through whole-exome sequencing. The pediatric cancers are shaded in light blue and adult cancers are shaded in dark blue. All data are from primary tumors prior to the initiation of therapy. Only non-silent mutations (missense, nonsense, frameshift, and splice site) are tallied. Cancers are arranged left to right in order of increasing median mutation frequency. Red horizontal lines within each cluster of points indicate median value of the mutation frequency of each tumor type. Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; C, carcinoma; GCT, germ cell tumor; CRC, colorectal carcinoma; MSI, microsatellite instability; H&N, head and neck; MSS, microsatellite stable; POLE, patients with somatic mutation in the nuclease (proofreading) domain of the *POLE* gene. The outlier in the low-grade glioma patient with >100 mutations per Mb is also *POLE* mutated. The sequencing data for all the pediatric tumors, CRC, and hepatocellular carcinoma were generated at the Human Genome Sequencing Center at Baylor College of Medicine. The sequencing data for all other adult tumors were from the TCGA Genome Data Analysis Center (<https://confluence.broadinstitute.org/display/GDAC/Home>). Pediatric AML, ALL, and Wilm's tumor data were obtained from the TARGET project (<http://www.targetproject.net/>). Reproduced from Reference 65 with permission.

tumor DNA replication system. The variation in nucleotide substitution frequency among patients with the same type of tumor is approximately one order of magnitude, whereas it is more than three orders of magnitude across different human tumors (see **Figure 2**). At the low end of the range are pediatric cancers, followed by adult chromophobe renal cell carcinoma, adult leukemia and other adult solid tumors. Most pediatric tumors have approximately 0.1 to 0.2 mutations per megabase pairs (Mb) of DNA sequence. Some individuals

surprisingly have none! The absence of any observable driving genetic alterations has been referred to recently as the “dark matter” (11) of the cancer genome. Although the mechanisms of tumorigenesis in these patients are not currently understood, it is assumed that developmental transcriptional programs are disrupted by errors in epigenetic programming (12, 13). Integrated analysis of genome-wide epigenetic profiles coupled with transcriptional profiles may shed light on this important issue. Most adult solid tumors harbor between 1 and 2

mutations per Mb, tenfold more than pediatric cancer. The difference observed between pediatric and adult mutation frequencies is consistent with accumulation of mutations over the longer adult lifetimes in affected tissues (14). Adult tumors, with mutation frequencies above 2 per Mb, result from exposure of the target tissue to environmental mutagens such as cigarette smoke for lung cancers or UV radiation for melanoma. Adult tumors with coding mutation frequencies of 10 per Mb or greater are often deficient in mismatch repair, either through mutation or epigenetic silencing of the mismatch repair genes, in particular *MLH1*, a key gene for sensing errors in DNA replication (15). Exceptional tumors with coding mutation frequencies of 100 per Mb or greater are mutated in the exonuclease domain of *POLE*, which is one of two DNA-replicative enzymes of the cells (16).

These patterns of mutation may have important implications for clinical management. At the low-frequency end of the mutation range, DNA sequencing may less often lead to the discovery of actionable mutations. Instead, clinicians may seek insights into genes driving the disease from gene expression data. Mutation discovery for adult cancers is becoming increasingly important in subclassifying disease for prognosis and treatment (18). Cancers with >1 mutation per Mb are most likely to be mutation-driven, and thus DNA sequencing will be a highly effective diagnostic tool. At the high end of the mutation frequency range, i.e., >10 mutations per Mb, some cancers, paradoxically, have a better prognosis and therefore require less aggressive therapy than their lower-rate counterparts (16). Although the reason for the improved prognosis is presently not understood, two mechanisms have been suggested (17). One is that the high mutation rate leads to an enhanced immune response by virtue of the generation of a wide variety of novel epitopes from mutated proteins. The other possibility is that, at elevated mutation rates, functions essential to cell survival are impaired, causing cells to die at such a high rate that robust tumor growth is not possible.

Tumor Mutation Frequencies: By Gene

A pattern has been established on the basis of the first completed exome screening in colorectal and breast cancers (19) and repeated in every cancer since: One to three genes are mutated in more than 20% of the tumors. A shoulder on the distribution may display several more genes mutated in 10–20% of the tumors. Then, infrequently mutated genes form a long tail to an ever-decreasing mutation frequency (**Figure 3**).

The lower-frequency genes have great importance for understanding tumor biology. They may exhibit redundancy of mutation in a given signaling pathway in a single patient, which contributes to our understanding of how the pathway is utilized by the cancer cell. Less frequent drivers have often revealed entirely new pathways or processes. In addition, by potentially having one or more downstream genes that are already mutated, these genes point to added complexity in the application of therapies targeted to a specific gene in a pathway. Redundant mutation in key pathways is an increasingly common feature of cancer genomes, as revealed by comprehensive exome or genome sequencing. The well-known EGFR inhibitors erlotinib (Tarceva) and gefitinib (Iressa) fail in the context of *KRAS* mutation in nonsmall-cell lung cancers (20).

Genetic Heterogeneity of Tumors

Two types of genetic heterogeneity have been recognized in tumorigenesis: interpatient heterogeneity and intratumoral heterogeneity. The former has been observed by every oncologist: No two cancer patients have exactly the same clinical course, with or without therapy, which has been a central principle of personalized medicine (11). Although patients with a given tumor type may share a few mutations, at the genome-wide level every patient has a different collection of somatic mutations.

Over the past three years, researchers have begun to dissect the intratumoral genetic

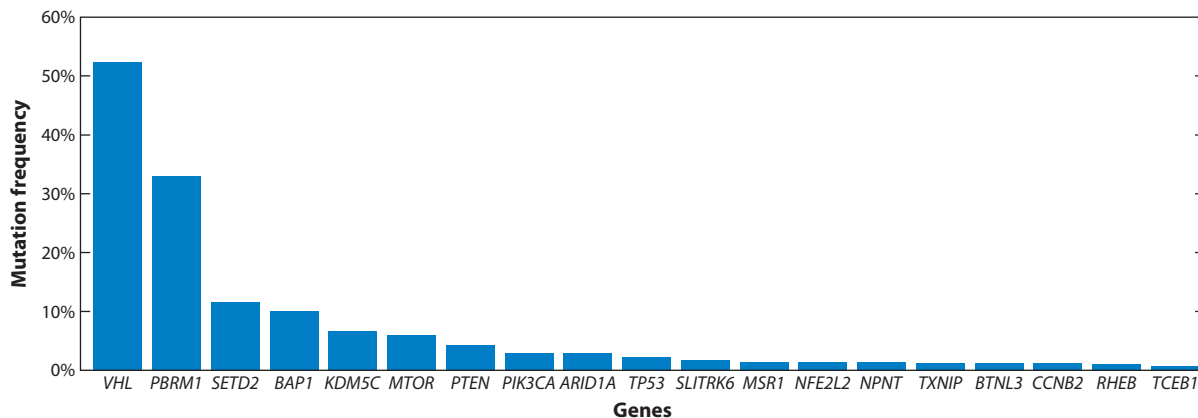


Figure 3

The distribution of mutation frequencies of significantly mutated genes (SMGs) in clear cell renal cell carcinoma ($n = 417$) (78). Only those genes with a false discovery rate (q) less than 0.1 are shown.

heterogeneity by NGS in both primary and metastatic cancers, including childhood acute lymphoblastic leukemia (21) and secondary acute myeloid leukemia (22), adult pancreatic cancers (23, 24), breast cancers (25–27), renal clear cell carcinomas (28), and metastatic medulloblastoma (29). The intratumoral heterogeneity was also demonstrated at the single-cell level using single-nucleus or single-cell sequencing (27, 30, 31). Intratumoral heterogeneity has now been noted as a common feature in both solid tumors and hematological malignancies.

Studies of intratumoral heterogeneity shed light on the mechanisms underlying acquired drug resistance to targeted therapy observed for multiple cancers. Colorectal tumors that are wildtype for *KRAS* are often sensitive to anti-EGFR antibodies, but they almost always develop resistance within several months of initiating therapy owing to the emergence of *KRAS* mutations in tumors. The general consensus is that the *KRAS* mutations were likely present in a small subpopulation of cells within the tumor prior to the initiation of therapy (32). Mathematical modeling showed that it takes only 5 to 6 months for a resistant subclone to expand and regrow into a tumor that is resistant to the anti-EGFR antibody (32). The genetic heterogeneity observed within most tumors is one of

the main obstacles to the discovery of predictive biomarkers and the development of antitumor molecular therapeutics (33).

NOVEL DISCOVERIES FROM SEQUENCING

Cancer genome studies have greatly expanded our knowledge of the genes and pathways driving cancer progression. In many cases, genes involved in one cancer are also involved in other cancers, thereby revealing the action of well-understood pathways in a new context. For instance, *NF1*, first discovered in neurofibromatosis and also involved in pediatric myeloid neoplasms, was identified as a significantly mutated gene in glioblastoma multiforme (34). The importance of identifying well-understood cancer genes in the context of a new cancer type cannot be underestimated. Sometimes such genes are already targets of therapies, and thus their employment against other cancers in clinical trials is straightforward (35). In terms of clinical management, these findings have led to the general proposition that cancers may be more logically grouped according to a particular collection of driver genes than according to organ site (35). Completely new avenues of research have also been opened as a result of the power of the whole-exome approach

Table 1 New cancer genes discovered by NGS^a

Pathway		Examples of new cancer genes discovered by NGS	Cancer types	
Genome stability		<i>POLE</i>	Colorectal and endometrial cancers	
		<i>TERT</i> promoter mutations	Melanomas, bladder, hepatocellular carcinomas, and many other cancer types	
RNA splicing machinery		<i>SF3B1</i>	Myelodysplasia, chronic lymphocytic leukemia	
		<i>SF3A1, U2AF1,U2AF65, ZRSR2, SRSF2, PRPF40B, SF1</i>	Myelodysplasia	
Metabolism		<i>IDH1, IDH2</i>	Glioblastoma, oligodendrogliomas, astrocytomas, AML, and many other cancer types	
		<i>NT5C2</i>	Acute lymphoblastic leukemia	
Epigenetic regulation	DNA methylation	<i>DNMT3A</i>	AML, myelodysplastic syndrome	
	DNA hydroxymethylation	<i>TET2</i>	AML, myelodysplastic syndrome	
	Histone methyltransferase	<i>EZH2</i>	Large B cell lymphomas	
	Histone demethylase	<i>KDM6A</i>	Multiple myeloma, renal cell carcinoma, AML and other cancers	
		<i>KDM5A, KDM5C</i>	AML, renal clear cell carcinoma	
	Histone deubiquitinase	<i>BAP1</i>	Mesothelioma, uveal melanoma, renal clear cell carcinoma	
	SWI/SNF chromatin complex	<i>PBRM1, SMARCA1, SMARCA4</i>	Renal clear cell carcinoma	
		<i>SMARCA4</i>	Melanomas	
		<i>ARID1A</i>	Ovarian clear cell carcinomas	
		<i>ARID1B, ARID2</i>	Melanoma, hepatocellular, and pancreatic cancers	
	Chromatin compaction	<i>CHD1</i>	Prostate cancer	
		<i>CHD2</i>	Chronic lymphocytic leukemia	
		<i>CHD4</i>	Endometrial cancers	
	Transcription factors		<i>SOX2</i>	Esophageal cancer
			<i>SOX9, CDX2</i>	Colorectal cancer
<i>GATA3</i>			Breast cancer	
<i>NKX2-1</i>			Lung adenocarcinoma	
<i>FOXA1, MED12</i>			Prostate cancer	
Ubiquitination		<i>SPOP</i>	Prostate and endometrial cancers	
		<i>FBXW7</i>	Endometrial, head/neck, bladder, gastrointestinal cancers	
		<i>WWP1</i>	Liver cancer	
RAC/PAK-signaling		<i>RAC1, RAC2, CDC42</i>	Melanoma	
		<i>ELMO1, DOCK2</i>	Esophageal cancer	
Tumor suppressor		<i>PTPN12</i>	Breast cancer	
		<i>PPP6C</i>	Melanoma	

(Continued)

Table 1 (Continued)

Pathway	Examples of new cancer genes discovered by NGS	Cancer types
Notch signaling	<i>NOTCH2</i> , <i>NOTCH3</i>	Head and neck squamous cell carcinoma
SLIT/ROBO signaling	<i>ROBO1</i> , <i>ROBO2</i> , <i>SLIT2</i>	Pancreatic cancer
Semaphorin signaling	<i>SEMA3A</i> , <i>SEMA3E</i> , <i>SEMA5A</i>	Pancreatic cancer
NF-KB signaling	<i>MYD88</i>	Diffuse large B cell lymphoma
RNA abundance	<i>DIS3</i>	Multiple myeloma
Translation regulation	<i>FAM46C</i>	Multiple myeloma
Protein homeostasis	<i>XBPI</i>	Multiple myeloma
Cell cycle	<i>CCNE1</i>	Uterine serous carcinoma

^aAbbreviations: AML, acute myeloid leukemia; NGS, next-generation sequencing.

manifest in the discovery of genes previously not associated with cancer (**Table 1**).

Genome Stability

Whole-exome sequencing recently identified a new subgroup (10%) of endometrial cancer with an unusually high mutation frequency (more than 100 mutations per Mb) (**Figure 2**). Patients in this ultramutated group harbor a somatic mutation in the exonuclease domain of *POLE* (16). *POLE*, one of two DNA replicative enzymes, is responsible for the synthesis of the leading strand during DNA replication. *POLE* also has proofreading capability through its exonuclease domain (36), which is critical for high-fidelity copying of the DNA template during the S-phase of the cell division cycle. Eighty percent of the mutations reported in *POLE* are clustered in two codons: Pro286 to Arg286 and Val411 to Leu411. Somatic *POLE* mutations were also found in similar ultramutated colorectal cancers. With high penetrance, germline variants at codons 424 and 478 of *POLE* may also be associated with susceptibility to colorectal cancer (37). This class of patients is clinically important because they have an improved progression-free survival, suggesting that *POLE* could be used as a favorable prognostic marker in these cancers.

In normal functioning, the telomeric ends of chromosomes gradually shorten over the life

of the cell, leading to senescence or cell death. Cancer cells overcome this block to replication by overexpressing telomerase encoded by *TERT*. Excess *TERT* maintains long telomeres and generates the limitless replicative potential of cancer cells. Though the great majority of cancer cells increase expression of *TERT*, the mechanism by which this is accomplished was not understood (38). Two recent studies reported somatic mutations in the telomerase *TERT* promoter region in 70% of melanoma patients as well as human cell lines derived from metastatic melanomas (39, 40). *TERT* encodes the catalytic reverse transcriptase subunit of telomerase, the ribonucleoprotein complex that maintains telomere length. The majority of *TERT* mutations occur at two positions in the *TERT* promoter, and both positions generate a de novo consensus binding motif for the *ETS* transcription factors. Functional studies demonstrate that the mutations could upregulate *TERT* transcription (39, 40). To extend these findings to other cancer types, Huang and colleagues assessed the prevalence of *TERT* promoter mutations in a large number of cancer cell lines derived from diverse tumor types and validated the *TERT* promoter mutations in 10 other cancer types (40). Killela and colleagues surveyed 1,230 tumors of 60 different types and validated *TERT* promoter mutations in 16 tumor types: Eight tumor types had a high frequency (16–83%) of

TERT mutations, whereas 18 other tumor types had a low frequency of *TERT* mutations (<15%) (41). The widespread *TERT* promoter mutations suggest that these mutations may function as driver events that contribute to oncogenesis through *TERT* dysregulation in a variety of human cancers, consistent with earlier experiments showing the requirements for *TERT* in transformation of primary human fibroblast and epithelial cells in culture (42).

Metabolic Genes

Both IDH1 and IDH2 convert isocitrate to α -ketoglutarate (α -KG), a cofactor for α -KG dioxygenases including TET family DNA demethylases, KDM-family histone demethylases, and many other proteins (43). The isocitrate dehydrogenases 1 (*IDH1*) mutations were surprising additions to the list of cancer drivers emerging from glioblastoma multiforme sequencing studies (44). Following these findings, *IDH1* and *IDH2* mutations were reported in multiple human cancer types and are now recognized as the most frequently mutated metabolic genes in human cancer (43). Mutated *IDH1/2* lose their normal activity to produce α -KG and gain a new activity producing D-2-hydroxyglutarate (D-2-HG), a structural analogue of α -KG but potent inhibitor of α -KG-dependent enzymes, the methyltransferases involved in DNA and chromatin methylation. These inhibitors therefore result in aberrant epigenetic modification as well as the potential for deregulation of many other cellular pathways.

Chromatin Remodeling

The most impressive advances resulting from large-scale genetic studies have provided insights into the role of chromatin remodeling in tumorigenesis. The discovery of *PBRM1* produced the first member of the large SWI/SNF chromatin-remodeling complex shown to be mutated to high levels (i.e., greater than 20% of patients) in any cancer; it is found in 41% of clear cell renal

carcinomas (45). SWI/SNF is a multisubunit chromatin-remodeling complex that uses the energy of ATP hydrolysis to reposition nucleosomes, thereby modulating gene expression. Researchers have uncovered mutations in one or more SWI/SNF components including *ARID1A*, *ARID1B*, *ARID2*, and *SMARCA4*, usually at low frequency. SWI/SNF mutations are widespread across diverse human cancers including renal, ovarian, hepatocellular, and gastric, among others (46), with a combined mutation frequency approaching that of *TP53*, the single most commonly mutated gene across all cancers.

RNA Splicing Machinery

Mutations of various components involved in RNA splicing, including *U2AF1*, *ZRSR2*, *SRSF2*, and *SF3B1*, were first discovered in myelodysplastic syndrome (47). Most mutations affect genes involved in 3' splice site recognition during pre-mRNA processing and induce abnormal RNA splicing and compromised hematopoiesis. In a large series analysis of 582 cases with various myeloid neoplasms, frequent mutations of this splicing pathway (45–85%), specifically in patients showing features of myelodysplasia, were observed (47). Subsequent studies in myeloid neoplasms and other cancer types validated these findings and found mutations in additional splicing components (48–50). *U2AF1* is a U2 auxiliary factor protein that recognizes the AG splice acceptor dinucleotide at the 3' end of the intron. *U2AF1* was recently reported in lung adenocarcinoma at a similar frequency as seen in acute myeloid leukemia (51). Functional testing of the commonly recurrent mutation sites in *U2AF1* demonstrated that they promote enhanced splicing and exon skipping in reporter assays in vitro (49). These findings implicate abnormalities of messenger RNA splicing in the pathogenesis of myelodysplastic syndrome and other human cancers. Such potential abnormalities may be among the most perplexing contributors to the biology of cancer, first, because it is not clear why splicing defects would

preferentially affect myeloid neoplasms and, second, because it remains unexplained why splicing impairment, which could potentially impact the expression of every gene in the cell, would specifically contribute to cancer.

CLINICAL IMPLICATIONS OF NEXT-GENERATION SEQUENCING

NGS has expanded beyond research applications to deliver clinically actionable information that can effectively guide therapeutic decision making. Researchers are now seeking to translate the immense volume of genomic data to clinic applications (35). From a clinical perspective, NGS has the potential to fill major gaps in molecular markers for diagnosis and prognosis and in the management and treatment of many human cancers. Characterization of patients' tumor mutation profiles may soon be required to make informed choices regarding therapy, given that the effectiveness of agents targeted to specific genes or pathways may be modulated by particular mutations. DNA sequence data could provide clinicians with a comprehensive view of the genetic alterations at the DNA and RNA levels, help clinicians identify specific characteristics in each patient, and discover new actionable biomarkers, thereby paving the way toward personalized medicine.

Circulating Cell-Free Tumor DNA

Recently, sequencing of circulating cell-free tumor DNA (ctDNA) has emerged as a promising and noninvasive tool for quantifying tumor burdens and monitoring treatment responses. ctDNA is present in healthy subjects at an average concentration of 30 ng/ml of blood (range 0–100) (52), representing an average of 5,000 (range 0–15,000) genome equivalents per milliliter of blood. Two main biological mechanisms have been proposed for the release of ctDNA in the blood: (a) apoptosis/necrosis or (b) release of intact cells in the bloodstream and their subsequent lysis (53). The ctDNA carries

information regarding tumor mutations and tumor burden; thus, it has potentially transformative applications in cancer management.

Studies since 1998 have shown the feasibility of using ctDNA as a tool for cancer diagnosis and prognosis. With traditional technologies, different types of DNA alterations including point mutations, DNA methylation, microsatellite instabilities, and chromosomal alterations have been reported in ctDNA of a large variety of cancer types including colorectal, pancreas, lung, bladder, head and neck, and liver (54–59). However, most of these studies targeted a single gene or a small subset of genes with known alterations.

With the power of NGS technologies, researchers are able to identify genome-wide *de novo* tumor-derived alterations in ctDNA. Last year, a study performed whole-genome sequencing of ctDNA samples of colorectal and breast cancer patients and demonstrated that whole-genome analyses of ctDNA could be used as a tool for detecting structural alterations specific to patients (60). Using deep sequencing of a single gene by NGS, researchers demonstrated the capability of detecting low-frequency mutations (as low as 2%) in ctDNA with great sensitivity and specificity (61). In 2013, Dawson and colleagues compared the sensitivity of measuring ctDNA, cancer antigen 15–3, and circulating tumor cells to monitor tumor burden in patients with metastatic breast cancer using whole-genome or targeted sequencing (62). Their data showed that the sensitivity of ctDNA was superior to that of other circulating biomarkers and that ctDNA had a greater dynamic range that correlated with changes in tumor burden. In more than half of the patients, ctDNA provided the earliest measure of treatment response. Recently, Murtaza and colleagues performed whole-exome sequencing of serially collected ctDNA samples from patients with advanced breast, ovarian, and lung cancers (63). Their data confirmed the genome-wide representation of the tumor genome in plasma and identified increased representation of mutant alleles associated with the emergence of therapy resistance

including mutations in *PIK3CA* and *RB1*. Two other studies performed in colorectal cancers suggested that ctDNA-based noninvasive monitoring of patients undergoing treatment with anti-EGFR therapies for the emergence of KRAS mutant subpopulation could allow for the early initiation of combination therapies that may delay or prevent disease progression (32, 64).

Sequencing of ctDNA has the potential to detect cancers in a noninvasive and unbiased manner even without prior knowledge of disease. This approach, representing a “liquid biopsy” alternative, could complement current invasive biopsy approaches, and for some applications such as serial sampling during the course of treatment, it could potentially replace invasive biopsies as a means to assess genetic characteristics and track genomic evolution of cancers. In addition, ctDNA measurements within clinical trials may serve as important biomarkers for real-time monitoring of the efficacy of systemic therapies in cancer patients, thereby accelerating drug development and the ability to define subpopulations of patients with the highest treatment benefit.


Therapeutic Opportunities

The number of drugs in oncology either with FDA approval (**Supplemental Figure 3**; follow the **Supplemental Material** link from the Annual Reviews home page at <http://www.annualreviews.org>) or under development is still limited but has increased rapidly over the past several years (65), suggesting that the impact of discovery fostered by the genomic era is beginning to make its mark. There is no doubt that NGS studies have led to the discovery of a variety of novel putative biomarkers as well as known actionable biomarkers in new cancer types (35). The question now is, How do we translate the immense sequencing data into effective therapies? Potential applications for putative cancer biomarkers include predictive, prognostic, and pharmacogenomic biomarkers that will provide

decision-making support for answering questions about who should be given treatment and which therapy should be chosen.

Studies of the therapeutic application of inhibitors that specifically target KIT mutations provide an illustrative example. Oncogenic KIT mutations are common in testicular seminomas (66, 67), gastrointestinal stromal tumors (GISTs) (68), and melanomas (69). Accordingly, they are becoming a crucial diagnostic marker for such tumors. KIT mutation leads to ligand-independent kinase activation (70). So far, eight tyrosine kinase inhibitors (TKIs) targeting KIT have been approved by the US Food and Drug Administration (<http://www.fda.gov/Drugs/default.htm>), and many more are in clinical trials. Several of these have been approved or tested in GIST and melanoma patients.

Correlation between the location of KIT mutations and the efficacy of TKIs is now established (68, 70–76) and could guide the more effective application of TKIs in KIT-mutated new cancer types. For GISTs, the majority of KIT mutations affect the juxtamembrane domain of the protein encoded by exon 11 of the gene such as V560F (**Figure 4**). KIT exon-11 mutations are often sensitive to imatinib mesylate, which is the first-line treatment for advanced GIST (74). Unfortunately, the majority of patients eventually develop resistance to this drug: Approximately 14% of patients are initially insensitive to imatinib, and ~50% of patients acquire secondary gene mutations in the KIT TK domains (70). Certain imatinib-resistant KIT TK-domain mutations respond to sunitinib and sorafenib (70, 73, 76–78). However, the D816 mutation in exon 17 that leads to constitutive activation of KIT by altering the structure of the juxtamembrane domain and destabilizing the A-loop inactive conformation (70) is resistant to most of the available TKIs; fortunately, however, it responds well to midostaurin (71). The KIT story provides an applicable model of how detailed mutational analysis can influence decision making for effective cancer therapy.

 **Supplemental Material**

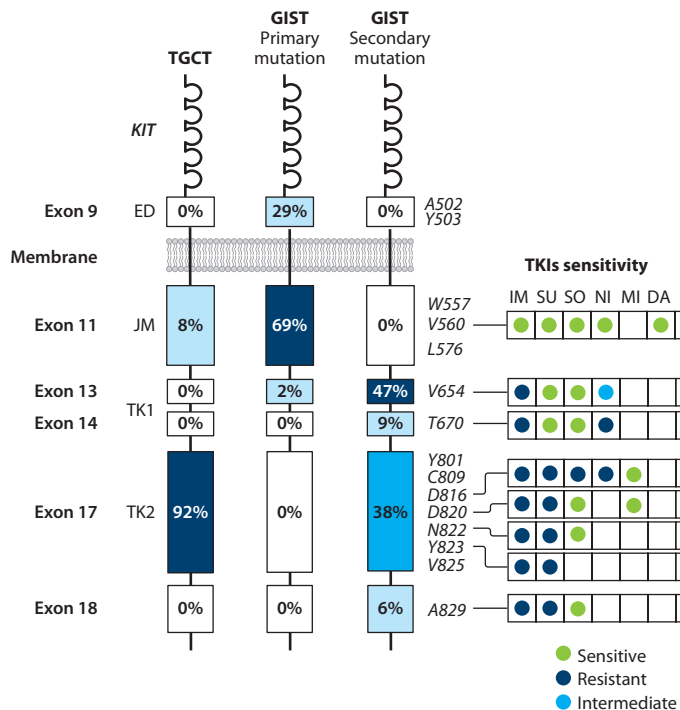


Figure 4

Functional differences in receptor tyrosine kinase (RTK) mutations in different cancers. The *KIT* oncogene plays a role in testicular germ cell tumors (TGCTs) as well as gastrointestinal stromal tumors (GISTs). This schematic of the mutational profile of the *KIT* oncogene compares TGCTs and GISTs. The known drug (tyrosine kinase inhibitors, TKIs) sensitivities for different mutations are shown to the right. Somatic mutation frequencies are mapped to different functional domains, with denser shading corresponding to higher mutation frequencies. Note most TGCT patients harbored a *KIT* mutation in the TK2 domain, whereas the primary pretreated GIST patients usually had *KIT* mutations in the JM domain. In RTK, different tyrosine kinase domains target different pathways; therefore, these data suggest different functional roles of *KIT* in the two diseases. Of immediate clinical import for treatment of *KIT*-driven tumors is the observation that different TKIs target different mutations; thus, molecular characterization of *KIT* is required for proper treatment of these two diseases. Interestingly, after targeted therapy, GIST patients usually develop secondary mutations in tyrosine kinase domains TK1 and to a lesser extent TK2. Abbreviations: ED, extracellular domain; JM, juxtamembrane domain; TK1 (tyrosine kinase I), ATP binding domain; TK2, (tyrosine kinase II), activation loop; IM, imatinib; SU, sunitinib; SO, sorafenib; NI, nilotinib; MI, midostaurin; DA, dasatinib.

SUMMARY AND FUTURE DIRECTIONS

Taken together, these findings reveal how the use of advanced NGS technologies has enabled researchers to obtain an in-depth understanding of cancer biology and identify

numerous diagnostic, prognostic, and actionable biomarkers for cancer management. Today, we know that cancer is far more complicated than imagined in 1971. Although we have made dramatic inroads in fighting cancer, the war on cancer is still being waged.

ctDNA as a Diagnostic Biomarker

ctDNA has emerged as a promising noninvasive tool for cancer diagnosis, monitoring of tumor progression, and treatment response. To make it a routine diagnostic tool, much more work remains. First, it is essential to understand the relationship between levels of ctDNA and the state of the tumor. Further experimentation into the dynamics of release and clearance of ctDNA in the plasma, the timing of its occurrence, and quantification with regard to cancer progression are needed. Second, different cancer types should be systematically compared to determine whether some cancers are more prone than others to release ctDNA. Third, the characterization of the proteins associated with ctDNA may become a source of very interesting and characteristic molecular signature for cells that release DNA.

Targeting Core Signaling Pathways

Although tumorigenesis in certain cancers appears to be driven by a single cancer driver gene, most human cancers have genetic alterations from a large number of cancer genes. Across all cancers, genomic studies have identified hundreds of cancer driver genes, and the number continues to grow. Developing agents that target specific mutated genes is a great challenge and principally impractical because most genes of interest are difficult to target. Currently, all the known driver genes can be classified into one or more of 12 core signaling pathways (11). Thus, developing agents that broadly target downstream mediators, regulators, or key nodal points of the dysregulated pathways is preferable. A representative example is the recent advances in the development of inhibitors of the KRAS pathway. So far, no approved targeted

therapies are available for patients with KRAS mutations and developing direct inhibitors of KRAS has proved to be challenging. So the focus has turned to targeting the immediate downstream signaling targets of KRAS, such as MEK. A recent preclinical study of the MEK inhibitor selumetinib indicated great efficacy against KRAS-mutated nonsmall-cell lung cancer cells (79), suggesting MEK inhibitors may also be promising agents for KRAS-mutated cancer patients.

Combination Therapy

Following the initiation of targeted therapies, some cancer cells find a way to generate drug-resistant mutations to survive, expand quickly, and eventually lead to recurrence. Resistance usually involves secondary mutations within the targeted protein or compensatory changes within the targeted pathway that bypass the drug-mediated inhibition. For example, imatinib-treated GISTs are likely to generate a secondary mutation in exon 17 of KIT and

become resistant to imatinib (70). Anti-EGFR-treated colorectal cancers tend to develop resistant KRAS mutations (32, 64). These scenarios prompted researchers to consider combining therapies: Colorectal cancers may be sensitive to treatment combined with an MEK inhibitor (79), and GISTs can be treated more effectively by combining imatinib with another TKI that responds to KIT exon 17 mutations (71, 73, 75). More recently, researchers have proposed new treatment options that combine targeted therapy and immunotherapy in cancer treatment (80). Targeted approaches inhibit essential molecular pathways that are required for tumor growth and survival; by contrast, immunotherapy aims to stimulate a host immune response that effectuates long-lived tumor destruction. Analysis of the strengths and weaknesses of targeted therapy and immunotherapy suggests that the two approaches may have complementary roles in cancer treatment and that combined therapy could prove synergistic and lead to more effective treatments (80).

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LITERATURE CITED

1. Crick FH. 1958. On protein synthesis. *Symp. Soc. Exp. Biol.* 12:138–63
2. Rowley JD. 1973. Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 243:290–93
3. Reddy EP, Reynolds RK, Santos E, Barbacid M. 1982. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* 300:149–52
4. Futreal PA, Coin L, Marshall M, et al. 2004. A census of human cancer genes. *Nat. Rev. Cancer* 4:177–83
5. Int. Hum. Genome Seq. Consort. 2004. Finishing the euchromatic sequence of the human genome. *Nature* 431:931–45
6. Gonzaga-Jauregui C, Lupski JR, Gibbs RA. 2012. Human genome sequencing in health and disease. *Annu. Rev. Med.* 63:35–61

7. Meyerson M, Gabriel S, Getz G. 2010. Advances in understanding cancer genomes through second-generation sequencing. *Nat. Rev. Genet.* 11:685–96
8. Hudson TJ, Anderson W, Artez A, et al. 2010. International network of cancer genome projects. *Nature* 464:993–98
9. Downing JR, Wilson RK, Zhang J, et al. 2012. The Pediatric Cancer Genome Project. *Nat. Genet.* 44:619–22
10. Garraway LA, Lander ES. 2013. Lessons from the cancer genome. *Cell* 153:17–37
11. Vogelstein B, Papadopoulos N, Velculescu VE, et al. 2013. Cancer genome landscapes. *Science* 339:1546–58
12. Gonzalez-Perez A, Jene-Sanz A, Lopez-Bigas N. 2013. The mutational landscape of chromatin regulatory factors across 4623 tumor samples. *Genome Biol.* 14:r106
13. Jaffe JD, Wang Y, Chan HM, et al. 2013. Global chromatin profiling reveals NSD2 mutations in pediatric acute lymphoblastic leukemia. *Nat. Genet.* 45:1386–91
14. Welch JS, Ley TJ, Link DC, et al. 2012. The origin and evolution of mutations in acute myeloid leukemia. *Cell* 150:264–78
15. Prolla TA, Pang Q, Alani E, et al. 1994. MLH1, PMS1, and MSH2 interactions during the initiation of DNA mismatch repair in yeast. *Science* 265:1091–93
16. Cancer Genome Atlas Res. Netw. 2013. Integrated genomic characterization of endometrial carcinoma. *Nature* 497:67–73
17. Drescher KM, Sharma P, Lynch HT. 2010. Current hypotheses on how microsatellite instability leads to enhanced survival of Lynch syndrome patients. *Clin. Dev. Immunol.* 2010:170432
18. Eroles P, Bosch A, Perez-Fidalgo JA, et al. 2012. Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. *Cancer Treat. Rev.* 38:698–707
19. Wood LD, Parsons DW, Jones S, et al. 2007. The genomic landscapes of human breast and colorectal cancers. *Science* 318:1108–13
20. Pao W, Wang TY, Riely GJ, et al. 2005. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med.* 2:e17
21. Anderson K, Lutz C, van Delft FW, et al. 2011. Genetic variegation of clonal architecture and propagating cells in leukaemia. *Nature* 469:356–61
22. Walter MJ, Shen D, Ding L, et al. 2012. Clonal architecture of secondary acute myeloid leukemia. *N. Engl. J. Med.* 366:1090–98
23. Campbell PJ, Yachida S, Mudie LJ, et al. 2010. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* 467:1109–13
24. Yachida S, Jones S, Bozic I, et al. 2010. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 467:1114–17
25. Geyer FC, Weigelt B, Natrajan R, et al. 2010. Molecular analysis reveals a genetic basis for the phenotypic diversity of metaplastic breast carcinomas. *J. Pathol.* 220:562–73
26. Navin N, Krasnitz A, Rodgers L, et al. 2010. Inferring tumor progression from genomic heterogeneity. *Genome Res.* 20:68–80
27. Navin N, Kendall J, Troge J, et al. 2011. Tumour evolution inferred by single-cell sequencing. *Nature* 472:90–4
28. Gerlinger M, Rowan AJ, Horswell S, et al. 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366:883–92
29. Wu X, Northcott PA, Dubuc A, et al. 2012. Clonal selection drives genetic divergence of metastatic medulloblastoma. *Nature* 482:529–33
30. Hou Y, Song L, Zhu P, et al. 2012. Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. *Cell* 148:873–85
31. Xu X, Hou Y, Yin X, et al. 2012. Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. *Cell* 148:886–95
32. Diaz LA Jr, Williams RT, Wu J, et al. 2012. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 486:537–40
33. Yap TA, Gerlinger M, Futreal PA, et al. 2012. Intratumor heterogeneity: seeing the wood for the trees. *Sci. Transl. Med.* 4:127ps10

34. Cancer Genome Atlas Res. Netw. 2008. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455:1061–68
35. Simon R, Roychowdhury S. 2013. Implementing personalized cancer genomics in clinical trials. *Nat. Rev. Drug Discov.* 12:358–69
36. Pursell ZF, Isoz I, Lundstrom EB, et al. 2007. Yeast DNA polymerase epsilon participates in leading-strand DNA replication. *Science* 317:127–30
37. Palles C, Cazier JB, Howarth KM, et al. 2013. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat. Genet.* 45:136–44
38. Cerni C. 2000. Telomeres, telomerase, and *myc*. An update. *Mutat. Res.* 462:31–47
39. Horn S, Figl A, Rachakonda PS, et al. 2013. TERT promoter mutations in familial and sporadic melanoma. *Science* 339:959–61
40. Huang FW, Hodis E, Xu MJ, et al. 2013. Highly recurrent TERT promoter mutations in human melanoma. *Science* 339:957–59
41. Killela PJ, Reitman ZJ, Jiao Y, et al. 2013. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc. Natl. Acad. Sci. USA* 110:6021–26
42. Hahn WC, Weinberg RA. 2002. Rules for making human tumor cells. *N. Engl. J. Med.* 347:1593–603
43. Yang H, Ye D, Guan KL, et al. 2012. IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clin. Cancer Res.* 18:5562–71
44. Parsons DW, Jones S, Zhang X, et al. 2008. An integrated genomic analysis of human glioblastoma multiforme. *Science* 321:1807–12
45. Varela I, Tarpey P, Raine K, et al. 2011. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene *PBRM1* in renal carcinoma. *Nature* 469:539–42
46. Shain AH, Pollack JR. 2013. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS One* 8:e55119
47. Yoshida K, Sanada M, Shiraishi Y, et al. 2011. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 478:64–69
48. Papaemmanuil E, Cazzola M, Boulton J, et al. 2011. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N. Engl. J. Med.* 365:1384–95
49. Graubert TA, Shen D, Ding L, et al. 2012. Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. *Nat. Genet.* 44:53–57
50. Quesada V, Conde L, Villamor N, et al. 2012. Exome sequencing identifies recurrent mutations of the splicing factor *SF3B1* gene in chronic lymphocytic leukemia. *Nat. Genet.* 44:47–52
51. Imielinski M, Berger AH, Hammerman PS, et al. 2012. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 150:1107–20
52. Anker P, Stroun M. 2000. Circulating DNA in plasma or serum. *Medicina* 60:699–702
53. Gormally E, Caboux E, Vineis P, et al. 2007. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. *Mutat. Res.* 635:105–17
54. Esteller M, Sanchez-Cespedes M, Rosell R, et al. 1999. Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res.* 59:67–70
55. Kirk GD, Lesi OA, Mendy M, et al. 2005. 249^{ser} TP53 mutation in plasma DNA, hepatitis B viral infection, and risk of hepatocellular carcinoma. *Oncogene* 24:5858–67
56. Mulcahy HE, Lyautey J, Lederrey C, et al. 1998. A prospective study of K-ras mutations in the plasma of pancreatic cancer patients. *Clin. Cancer Res.* 4:271–75
57. Salbe C, Trevisiol C, Ferruzzi E, et al. 2000. Molecular detection of codon 12 K-ras mutations in circulating DNA from serum of colorectal cancer patients. *Int. J. Biol. Markers* 15:300–7
58. Utting M, Werner W, Dahse R, et al. 2002. Microsatellite analysis of free tumor DNA in urine, serum, and plasma of patients: a minimally invasive method for the detection of bladder cancer. *Clin. Cancer Res.* 8:35–40
59. Chang HW, Chan A, Kwong DL, et al. 2003. Detection of hypermethylated *RIZ1* gene in primary tumor, mouth, and throat rinsing fluid, nasopharyngeal swab, and peripheral blood of nasopharyngeal carcinoma patient. *Clin. Cancer Res.* 9:1033–38
60. Leary RJ, Sausen M, Kinde I, et al. 2012. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. *Sci. Transl. Med.* 4:162ra54

61. Forshew T, Murtaza M, Parkinson C, et al. 2012. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci. Transl. Med.* 4:136ra68
62. Dawson SJ, Tsui DW, Murtaza M, et al. 2013. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N. Engl. J. Med.* 368:1199–209
63. Murtaza M, Dawson SJ, Tsui DW, et al. 2013. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497:108–12
64. Misale S, Yaeger R, Hobor S, et al. 2012. Emergence of *KRAS* mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 486:532–36
65. Wheeler DA, Wang L. 2013. From human genome to cancer genome: the first decade. *Genome Res.* 23:1054–62
66. Kemmer K, Corless CL, Fletcher JA, et al. 2004. *KIT* mutations are common in testicular seminomas. *Am. J. Pathol.* 164:305–13
67. Coffey J, Linger R, Pugh J, et al. 2008. Somatic *KIT* mutations occur predominantly in seminoma germ cell tumors and are not predictive of bilateral disease: report of 220 tumors and review of literature. *Genes Chromosom. Cancer* 47:34–42
68. Corless CL, Barnett CM, Heinrich MC. 2011. Gastrointestinal stromal tumours: origin and molecular oncology. *Nat. Rev. Cancer* 11:865–78
69. Garrido MC, Bastian BC. 2010. *KIT* as a therapeutic target in melanoma. *J. Invest. Dermatol.* 130:20–27
70. Gajiwala KS, Wu JC, Christensen J, et al. 2009. *KIT* kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. *Proc. Natl. Acad. Sci. USA* 106:1542–47
71. Growney JD, Clark JJ, Adelsperger J, et al. 2005. Activation mutations of human c-*KIT* resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. *Blood* 106:721–24
72. Woodman SE, Trent JC, Stemke-Hale K, et al. 2009. Activity of dasatinib against L576P *KIT* mutant melanoma: molecular, cellular, and clinical correlates. *Mol. Cancer Ther.* 8:2079–85
73. Heinrich MC, Marino-Enriquez A, Presnell A, et al. 2012. Sorafenib inhibits many kinase mutations associated with drug-resistant gastrointestinal stromal tumors. *Mol. Cancer Ther.* 11:1770–80
74. Heinrich MC, Corless CL, Demetri GD, et al. 2003. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J. Clin. Oncol.* 21:4342–49
75. Guo T, Agaram NP, Wong GC, et al. 2007. Sorafenib inhibits the imatinib-resistant KITT670I gate-keeper mutation in gastrointestinal stromal tumor. *Clin. Cancer Res.* 13:4874–81
76. Minor DR, Kashani-Sabet M, Garrido M, et al. 2012. Sunitinib therapy for melanoma patients with *KIT* mutations. *Clin. Cancer Res.* 18:1457–63
77. Heinrich MC, Maki RG, Corless CL, et al. 2008. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J. Clin. Oncol.* 26:5352–59
78. Wang WL, Conley A, Reynoso D, et al. 2011. Mechanisms of resistance to imatinib and sunitinib in gastrointestinal stromal tumor. *Cancer Chemother. Pharmacol.* 67(Suppl. 1):S15–24
79. Janne PA, Shaw AT, Pereira JR, et al. 2013. Selumetinib plus docetaxel for *KRAS*-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol.* 14:38–47
80. Vanneman M, Dranoff G. 2012. Combining immunotherapy and targeted therapies in cancer treatment. *Nat. Rev. Cancer* 12:237–51