Leading Edge
Review
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The Genetic Basis for Cancer Treatment Decisions

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Personalized cancer medicine is based on increased knowledge of the cancer mutation repertoire and availability of agents that target altered genes or pathways. Given advances in cancer genetics, technology, and therapeutics development, the timing is right to develop a clinical trial and research framework to move future clinical decisions from heuristic to evidence-based decisions. Although the challenges of integrating genomic testing into cancer treatment decision making are wide-ranging and complex, there is a scientific and ethical imperative to realize the benefits of personalized cancer medicine, given the overwhelming burden of cancer and the unprecedented opportunities for advancements in outcomes for patients.

Introduction

Numerous models have been proposed to explain the complex nature of cancer at molecular, cellular, and pathological levels (Hanahan and Weinberg, 2000, 2011). One model that explains cancer initiation, progression, dissemination, treatment response, and emergence of drug resistance is based on the progressive accumulation of mutations throughout the history of a tumor and its downstream colonies (Figure 1). Though incomplete, the somatic mutation model does incorporate one of the most consistent hallmarks of cancer: DNA mutations are found in all cancers. In addition, specific mutations have been linked to one or more forms of cancers, and mutant gene products have been associated with biological characteristics of cancer.

The spectrum of cancer mutations is diverse in terms of type, number, and functional consequences. Examples include single base changes that alter protein activity, amplifications, and deletions that modify the abundance of a gene and its products and alternative splicing or translocations that can create novel proteins. Mutations are abundant in cancer cells, numbering between thousands and hundreds of thousands per tumor (Stratton, 2011; Wong et al., 2011). However, most mutations in cancer cells do not appear to play a role in cancer progression; rather, they are indicative of the high mutation rate resulting from carcinogens and DNA instability (Pleasance et al., 2010a, 2010b). Such mutations have been called "passengers." A minority of cancer mutations are thought to be "drivers," defined as mutations involved in the development or progression of a tumor. A subset of these drivers and their component cellular pathways may be "actionable," i.e., have significant diagnostic, prognostic, or therapeutic implications in subsets of cancer patients and for specific therapies. A subset of mutations may also be druggable, i.e., targets for therapeutic development. Given current knowledge on gene function, classifying mutations into drivers and passengers—actionable and/or druggable—is difficult. It is still too early to deduce how many mutations are active at any given stage of a tumor. Moreover, the constant accumulation of mutations, with or without exogenous selective pressures of therapy, implies that tumors evolve to encompass many subpopulations that have distinct differences in mutation load within and between patients (Figure 1). Although there is great diversity in the types and numbers of mutations in human cancer, our ability to annotate and to assess functional and clinical consequence has expanded remarkably.

DNA sequencing technologies now allow whole-genome, exome, and transcriptome sequencing at rates that are dramatically faster and cheaper than traditional Sanger-based methods. Quantifying and cataloguing mutations, transcriptomes, and methylomes for many forms of cancer are underway in dozens of countries through coordinated projects of the International Cancer Genome Consortium (ICGC) (Hudson et al., 2010). Already, partial cancer genome data sets are available for several thousands of tumors with protein-altering mutations affecting more than 7,500 genes inventoried to date (ICGC Dataset Version 6; http://www.icgc.org). The availability of these large cancer data sets in the public domain will foster significant follow-up research by academia and industry and will lead to the validation of many new driver mutations, drug targets, and clinically useful biomarkers.

A subset of mutations are being branded as "actionable" by clinicians, based on evidence from clinical studies that the presence or absence of gene mutations in tumor (and occasionally

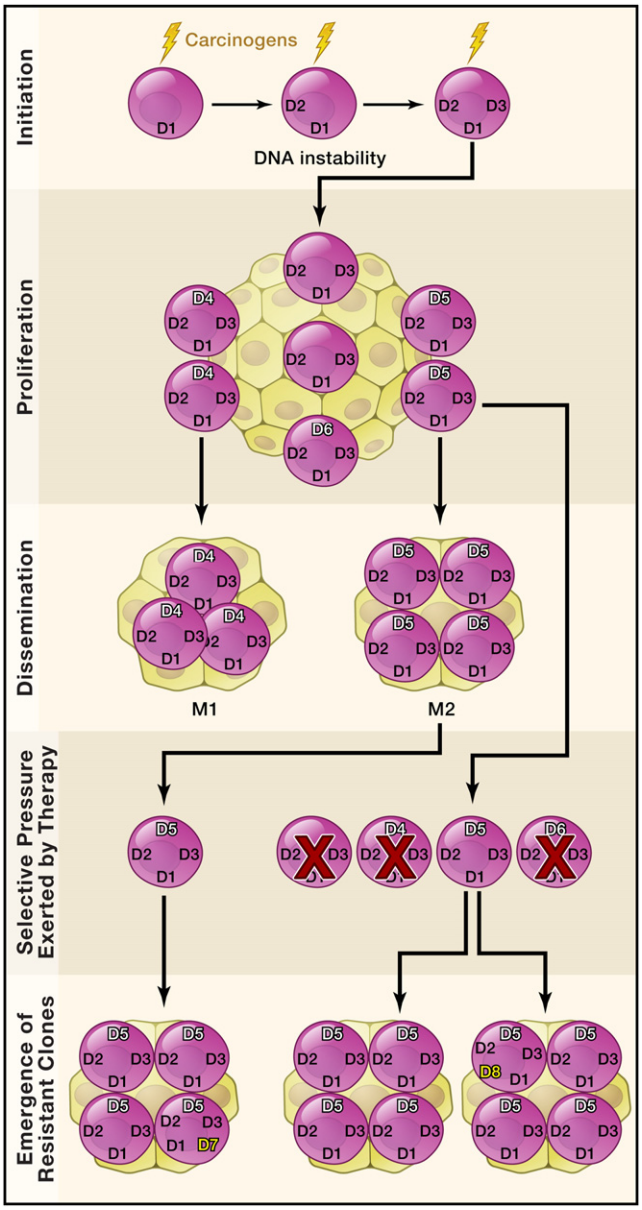

Figure 1. Accumulation of Driver Mutations in the History of a Tumor
Exposure to carcinogens, failure of DNA repair, and progressive genetic instability lead to accumulation of mutations that drive cancer development, growth, and metastases. Subclones with new mutations may become dominant within metastases or within persistent or recurrent cancer deposits through selective pressures exerted by cytotoxic or targeted chemotherapies.

germline) DNA can be used to inform clinical management (Table 1). Some examples include *KRAS* mutations that correlate with resistance to epidermal growth factor receptor (EGFR) antibodies in colorectal cancer and *BCR-ABL* fusion gene products that are pathognomonic of chronic myelogenous leukemia and can be inhibited by agents such as imatinib. The list of potential actionable mutations that may impact on treatment recommendations for predictive or prognostic reasons, or those with known prognostic or diagnostic implications, is growing.

Notwithstanding the historical links between certain actionable mutations and specific cancer histologies, further exploration has revealed that specific mutations are often observed across a range of tumor histologies, albeit at different frequencies. Figure 2 highlights many genes, including some with known actionable mutations, which are altered in several common cancers. One testable hypothesis is that mutations act as "drivers" in most if not all tumors where they are observed. Moreover, if a mutation is predictive of a drug response in one form of tumor (for example, BRAF V600E and vemurafenib for melanoma; Chapman et al., 2011), then there may be some likelihood that the same drug could affect tumors from other origins with the same mutation (for example, BRAF V600E and ovarian cancer; Sieben et al., 2004). It is clear, however, that this hypothesis requires formal testing, as experience to date suggests that the presence of a specific genetic abnormality may not confer the same sensitivity to an agent across all cancers, as exemplified by trastuzumab, which has been shown to benefit patients with HER2-amplified breast and gastric cancer, but not those with ovarian or endometrial cancer (Bang et al., 2010; Bookman et al., 2003; Fleming et al., 2010).

If, in fact, the functional consequence of a specific mutation is similar across different cancers, the clinical implications are unavoidable. Rather than approaching each patient's tumor investigation with an organ-based list of mutation tests, one could systematically perform a global search for all such "actionable" mutations in any type of cancer and test targeted therapeutics in patients with the specific mutation(s) regardless of cancer histology. One of the key reasons to test this approach in the clinical setting now is the need to elucidate further whether many of the targeted anticancer therapies that are approved for specific cancer types may benefit patients with other forms of cancer that share similar genetic profiles and biologic features.

Framework Requirements for Evaluating the Genetic Basis for Cancer Treatment

It is important to recognize early that obtaining convincing evidence to guide future clinical decisions for matching therapies to mutations affecting unique patients with different tumor types will require large numbers of patients in meticulously conducted clinical trials. The goal of these initial trials will be to determine which mutation profiles correlate with sensitivity or resistance to specific therapies and whether the mutation profile and treatment outcome is consistent among different cancer histologies. As opposed to a classical randomized controlled trial in which a novel therapeutic strategy is assessed against current standard practice, a genomics-based clinical trial offers the potential for many different therapeutic options to be selected on the basis of genomic profiling. Each subgroup of patients harboring a specific mutation and receiving a targeted therapy (or assigned to a control group) will represent a minority of patients recruited. To achieve power to determine whether outcome is improved in subgroups, genomics-based clinical trials require both large sample sizes and large treatment effects within the mutation-defined subgroups. For example, a 1,000 patient genomic profiling trial could recruit 100 subjects harboring mutations in a target gene at 10% frequency, allowing a two-armed nested

Table 1. Selected Genetic Markers and Their Application in Cancer Treatment

Genetic Marker	Application	Drug
BCR-ABL	Ph+ CML; Ph+ ALL	Imatinib, dasatinib, nilotinib
BCR-ABL/T315I	Resistance to anti-BCR-ABL agents	Imatinib, dasatinib, nilotinib
BRAF V600E	Metastatic melanoma	Vemurafenib
BRCA1/2	Metastatic ovarian cancer and breast cancer with BRCA 1/2 mutations	Olaparib, veliparib, iniparib
c-Kit	Kit (CD117)-positive malignant GIST	Imatinib
EGFR	Locally advanced, unresectable, or metastatic NSCLC	Erlotinib, gefitinib
EGFR T790M	Resistance to EGFR tyrosine kinase inhibitors in advanced NSCLC	Erlotinib, gefitinib
EML4-ALK	ALK kinase inhibitor for metastatic NSCLC with this fusion gene	Crizotinib
HER2 amplification	HER2-positive breast cancer or metastatic gastric or gastroesophageal junction adenocarcinoma	Trastuzumab
KRAS	Resistance to EGFR antibodies in metastatic colorectal cancer	Cetuximab, panitumumab
PML/RAR	Acute promyelocytic leukemia	ATRA, arsenic trioxide
TPMT	Deficiency is associated with increased risk of myelotoxicity	Mercaptopurine, azathioprine
UGT1A1	Homozygosity for UGT1A1*28 is associated with risk of toxicity	Irinotecan
DPD	Deficiency is associated with risk of severe toxicity	5-Fluorouracil

ATRA, all trans retinoic acid; Ph+, Philadelphia-positive chromosome; DPD, dihydropyrimine dehydrogenase; EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase; HER2, human epidermal growth receptor 2; GIST, gastrointestinal stromal tumors; ALL, acute lymphocytic leukemia; NSCLC, non-small cell lung cancer; TPMT, thiopurine S-methyltransferase.

phase II study comparing a new therapy in selected cases and controls. The same genomics trials could support several nested phase II studies testing different agents in patients with different mutations. However, the frequency of mutated cancer genes is often less than 10%, and there will be a need to know the influence of the tissue of origin on outcome. Thus, the sample size requirement for genomics trials may be larger by at least one order of magnitude if there is interest in assessing treatment effect across and within patients with different cancer histologies that share the same mutations. Although the number of patients profiled may be greater, the numbers of patients per treatment arm may be smaller, as the magnitude of treatment effect should be greater to justify this complex approach. To achieve large patient numbers, genomics trials need to recruit patients at multiple centers and, ultimately, leverage several large multi-institutional trial networks. It is also likely that, in the future, the scientific community will want to synthesize data from multiple studies performed across the globe. This will be enabled not only by instituting appropriate data sharing policies (see below), but also by using similar standard operating procedures (SOPs) for sample collection, processing, analyses, mutation calling, and data collection and management as much as possible. The net outcome of the approach will be a new system of cancer classification that will include genomic factors that make a difference in patient prognosis and treatment.

Efficient workflows are required that incorporate all steps: initial invitations to participate, consent, sample collection, genomic analyses, validation of actionable mutations, expert deliberations, reporting to clinicians, intervention(s) including access to appropriate therapies, and follow-up (Figure 3). The addition of complex genetic or genomic testing and interpretation to clinical trials imposes some time-consuming activities that could delay the start of therapies. This is particularly true at this time, as most high-throughput genomic technologies require weeks and/or months for data generation and analysis. One way to minimize the potential impact on genomic testing to create a delay in treatment initiation is to sequence patient tumors early in the management of their disease—for example, at the time when metastatic disease is diagnosed and patients begin their first-line standard of care regimen—as the genomic information will inform subsequent choices of therapies. The caveat is, of course, that, over time and with each treatment, new mutations can be acquired. An alternative to early genomic testing is to sequence at the time of progression when a change in therapy is considered. To achieve a turnaround time of less than 3 weeks (a threshold suggested by clinical trials leaders), the choice of sequencing technologies and streamlining of data analysis steps are important.

Patient Recruitment and Informed Consent

Participation in genomics trials requires that prospective patients be informed of genomic testing and the potential of future therapies based on genomics results. Whereas the latter can be administered after genomics results are known and the informed consent document can be customized according to the specific intervention being considered, all participants need to be aware that extensive genomic information will be generated and that, in addition to generating data regarding "actionable" mutations that may modify treatment decisions, there may be "incidental findings," such as germline mutations associated with risk to other diseases (i.e., long QT syndrome). Furthermore, germline mutation data could also provide risk information relevant to family members (i.e., mutations in breast cancer type 1 susceptibility [*BRCA1*] or cystic fibrosis genes). The issue of returning such data to research participants and patients is currently a controversial topic in bioethics (McGuire et al., 2008). There is a clear need for experts and stakeholders

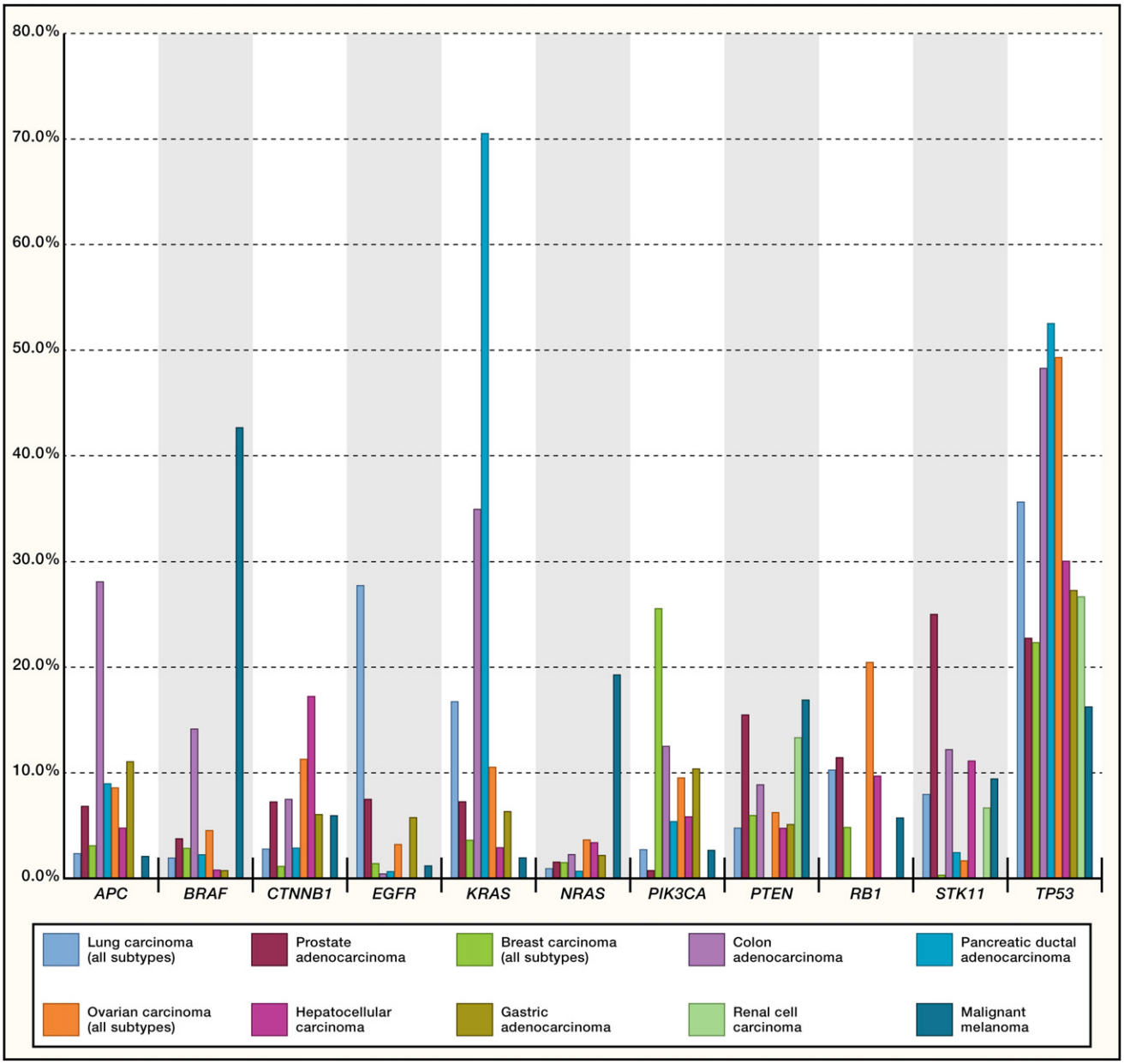

Figure 2. Mutation Frequencies in Common Cancers

Selected mutations are those found on Snapshop and OncoCarta panels. The mutation data were obtained from the Sanger Institute Catalogue of Somatic Mutations in Cancer website at http://www.sanger.ac.uk/cosmic, COSMIC v54 Release (Forbes et al., 2011).

to develop a framework that addresses ethical and legal obligations to inform subjects and family members. In addition, this framework should consider the preferences and concerns of research subjects and family members to receive information on germline variants of risk of cancer and other diseases.

In addition to the "risks" of identifying germline or somatic mutations that may affect patients or family members, patients should be aware of the extent that data will be shared and of the risks and potential consequences of breach of confidentiality. To support data sharing across participating networks and ideally across the scientific community (described later), the consent process should notify participants that data will be made accessible to national and international researchers under robust mechanisms to protect confidentiality of participants (Toronto International Data Release Workshop Authors et al., 2009).

Tissue Requirements: Quantity, Quality, Processing, and Timing

Any framework for clinical decision making on the basis of somatic genomic alterations requires timely access to tumor

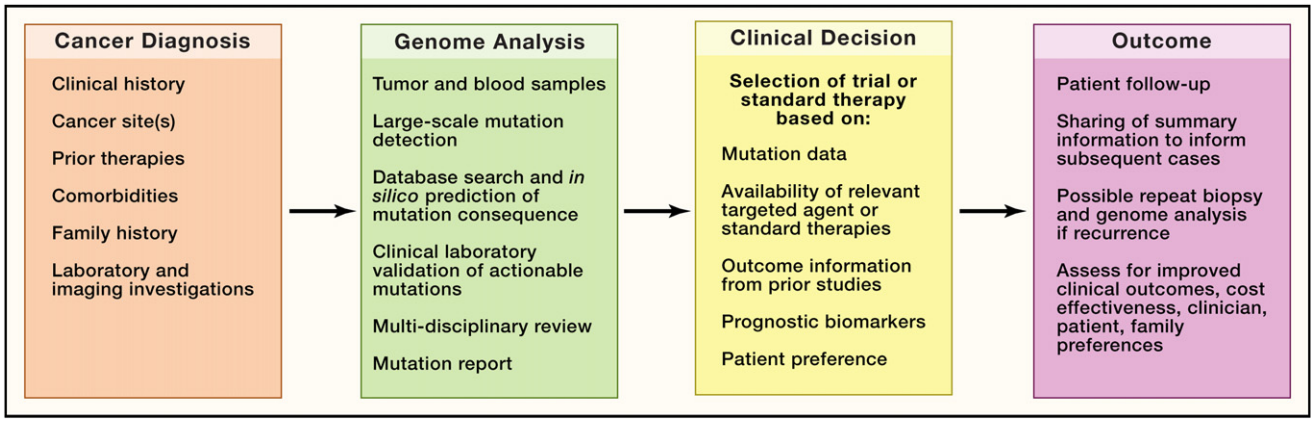

Figure 3. The Genetic Basis for Cancer Treatment

The key steps for the application and evaluation of clinical genomics for cancer treatment include the following. The recruitment of patients and acquisition of relevant clinical information. Sample collection and analyses for cancer genes. Interpretation of results of genomic analysis based on known functional and clinical significance of mutation. Provision of information to clinicians and patients for management. Clinical trials of novel treatments offered to cancer patients who are unlikely to benefit from standard of care and thus have a relatively poor prognosis and/or are more likely to benefit from a novel therapy due to the presence of tumor genetic abnormalities that predict sensitivity, lack of resistance, or toxicity to a treatment. Assessment of outcomes and sharing of results across cancer networks to accelerate clinical cancer genomic knowledge.

tissue for high-throughput molecular profiling that is readily available, of sufficient quality and quantity for successful analysis, and obtained at a time that the generated mutation profile remains relevant for the potential available treatments (Dias-Santagata et al., 2010; MacConaill et al., 2009). Most cancer patients have archival tissue available for molecular profiling from either their primary tumor and/or metastasis obtained from diagnostic biopsy or surgical excision. Local regulations dictate the minimum time period that hospital pathology departments must retain archival tumor tissue for the benefit of the medical care of the patient. In North America, most hospitals collect and archive tumor specimens as formalin-fixed, paraffin-embedded (FFPE) blocks to optimize histological assessment. Although collected on all patients, the diagnostic samples may or may not be representative of the mutations that subsequently arise in metastases or as a consequence of treatment. In addition, DNA and RNA preservation in FFPE tissue is challenging, as formalin fixation causes crosslinking and degradation into smaller fragments (Wang et al., 2009). Snap freezing tumor tissues in liquid nitrogen is the optimal method of nucleic acid preservation; however, this is not routinely performed outside of select European cancer centers. FFPE does provide preservation of histological features that allows for pathological review of hematoxylin and eosin stained slides to assess tumor cellularity and to mark the regions of tumor for macrodissection to isolate regions of nonnecrotic tumor from surrounding stroma.

Quantity and quality of tumor DNA are key sample considerations. Unfortunately, key parameters of tumor cellularity and optimal quantity of DNA remain unknown. Some authors suggest > 70% tumor cellularity with < 10% necrotic tumor tissue as guidelines (MacConaill et al., 2009), although less stringent thresholds may be employed if there is more tumor tissue available for macrodissection and DNA extraction or a more limited panel of gene mutation will be assayed. The minimum tumor tissue requirement and optimal method of DNA extraction remain unknown. As few as four 5 micron sections to isolate 15 ng or less of genomic DNA has been described as the requirement for successful sequencing using a customized multiplex colorectal cancer mutation (Colocarta) panel derived from the Oncocarta v1.0 platform (Sequenom, San Diego, CA) (Fumagalli et al., 2010). Increased genomic coverage requires greater quantities of tumor DNA. The Oncocarta v1.0 and OncoMap panels, which include, respectively, 238 mutations in 19 oncogenes and ~400 mutations in 33 oncogenes and tumor suppressors, recommend at least 500 ng of DNA (MacConaill et al., 2009; Thomas et al., 2007). There will always be a direct relationship between the extent of genetic testing and the quantity of DNA required; however, methods to allow expanded and deeper genomic sequencing, likely to be the mainstay technologies in the future for clinical laboratory testing, using small quantities of DNA from tumor, circulating tumor cells, or DNA would greatly enhance the successful evaluation of genomic testing in cancer management.

An unresolved issue is whether archival tissue from the primary tumor or a fresh biopsy of a metastatic lesion should be profiled for treatment selection for patients with advanced refractory disease. It is well recognized that cancers are genomically unstable and new mutations arise during the process of metastasis to distant sites, and/or treatment-resistant clones emerge over time (Campbell et al., 2010; Jones et al., 2010; Lee et al., 2010; Shah et al., 2009). Although metastatic tumor biopsies are increasingly acceptable to patients and their physicians if they may inform treatment decision making (Agulnik et al., 2007), it is not feasible in the current clinical practice environment to perform a metastatic tumor biopsy at the time of treatment resistance in all patients with advanced cancer, and at each point, a new treatment may be considered. Clonal evolution may differ across metastatic sites within an individual patient (Yachida et al., 2010), suggesting that genomic profiling of

biopsy material from a single metastatic lesion may not be sufficient to completely capture the genomic diversity of advanced solid cancers.

Nevertheless, available data suggest that individual mutations may be highly concordant between primary and metastatic sites and that mutations identified in primary tumors predict benefit to certain drugs in patients with metastatic disease. For instance, concordance of *KRAS* mutations in colorectal primary cancers and metastases was 96% in two published series (Knijn et al., 2011; Santini et al., 2008). In non-small cell lung cancer, one report of a small cohort of 25 cases demonstrated concordance rates for *EGFR* and *KRAS* mutations of 76% (Kalikaki et al., 2008). Furthermore, the effectiveness of currently available targeted treatments for advanced cancer patients such as gefitinib or erlotinib for *EGFR*-mutated lung cancer or trastuzumab for *HER2*-amplified breast cancer has largely been demonstrated from trials that have identified genetic mutations in archived diagnostic samples rather than new biopsies from metastatic lesions (Mok et al., 2009; Slamon et al., 2001). In contrast to high concordance seen for *KRAS* and *EGFR* mutations, a recent study in breast cancer reported discordant *PIK3CA* mutations in 32% of 103 cases (Dupont Jensen et al., 2011), indicating that concordance may be mutation and/or tissue-type specific and may be influenced by prior therapy. At this time, whether to use archived diagnostic samples versus samples obtained at the time a new treatment is indicated is driven by convenience, costs, and standard practices rather than by data. Additional studies are needed to address the feasibility of biopsying of metastatic lesions for genomic profiling and whether treatment decisions based on this approach lead to improved outcomes compared with genomic profiling of archival samples of the corresponding primary tumor. In the current environment, serial biopsying of patients is not scalable to large clinical trials or current clinical practice environments. However, this important question can and should be addressed through a coordinated effort of committed investigators and academic cancer research centers.

Genomic Technologies and Data Management

At all stages of development and adoption, companion diagnostics used to identify somatic mutations to inform real-time clinical decisions need to meet clinical workflow speed requirements and high levels of test accuracy to not only detect mutant alleles, but also provide quantitative measure of their abundance. So-called ''second-generation'' deep-sequencing instruments (Natrajan and Reis-Filho, 2011; Wong et al., 2011) currently used by cancer genome centers to sequence entire genomes, exomes, transcriptomes, and methylomes often require weeks for sample template preparation, sequence generation, and data analyses. Generating and assembling the massive number of relatively short sequence reads into usable data that specify genes and mutations remains complex, which partly explains why this generation of instruments is mostly used in research facilities with sophisticated databases and highly qualified and diversified scientific staff. Because these technologies have had minimal use in diagnostic settings, additional validation of potential candidate mutations is required using clinical-grade sequencing assays in certified diagnostic laboratories. The addition of extensive genomic sequencing and follow-up mutation validation introduces significant stress to the clinical workflow (Figure 3). The advent of ''third-generation'' sequencers such as Pacific Biosciences PacBio RS and Life Technologies' Ion Torrent PGM provides increased speed of sequencing due to their use of sensors that detect nucleotides as they are added to DNA molecules in synthesis, although parallelization and machine throughput currently is much lower than with second-generation technologies (Eid et al., 2009; Korlach et al., 2010; Rothberg et al., 2011).

In contrast to germline DNA mutations, which represent 50% or 100% of alleles in heterozygous or homozygous individuals, respectively, clinically relevant somatic mutations may only be present in a small percentage of cells and thus represent less than 5% of sequence reads, either as a result of high percentages of nontumor cells in biospecimens or because some mutations are only present in a subpopulation of tumor cells. To achieve this needed sensitivity, protocols can be adapted to obtain high depth (i.e., to generate many overlapping sequence reads such that every nucleotide is detected multiple times). Although the typical depth requirement for normal diploid genomes is usually 20–30×, tumor coverage requirements may need to exceed 100× to detect clinically relevant somatic mutations. Important factors for determining depth include the relative proportions of tumor versus nontumor cells in the sample extracted for DNA analyses (which can be quite low, for example, in pancreatic cancer due to high stromal cell content) and tumor heterogeneity. The latter reflects the mosaic nature of tumors, whereby multiple subclones diverge in their mutation load (Figure 1), leading to different proportions of mutant alleles in the same tumor. The clinical implications of low-abundance mutations remain unclear.

Capturing sequence information on all nucleotides in genomes, exomes, or large sets of target genes is challenging using all current technologies, and the extent of cancer genome sequence that is needed to inform clinical decisions is debatable. There is a small subset of genes that is currently deemed to be actionable because mutations in these genes already have diagnostic, prognostic, or predictive implications. Potentially actionable cancer genes should be sequenced in their entirety. Published coverage estimates for whole-genome and exome datasets are below 90% (Cancer Genome Atlas Research Network, 2011), which is inadequate for genes associated with actionable mutations. Near complete coverage of all protein-coding bases in important genes can usually be achieved using polymerase chain reaction (PCR)-based strategies and optimized through trial and error. However, PCR-based approaches consume relatively more tumor DNA and do not scale well. In addition, there are thousands of genes that are known to harbor somatic mutations (see ICGC database). Though the consequence of most of these mutations is unknown, it would be useful to prospectively archive all mutation data in databases that can be shared among cancer organizations to accelerate the expansion of knowledge regarding clinical and functional significance of these new mutations. The cost of sequencing a few hundred genes, exomes, and whole genomes has and will continue to decrease, with high-throughput laboratories currently achieving costs in the $1,000–$2,000 range for large gene panels as well as exomes when using pooling

strategies (Kozarewa and Turner, 2011). Whole-genome sequencing is approximately five to ten times more expensive; given the complexity in their analyses and that most of the clinically interpretable mutations are confined to protein-coding genes, whole-genome data sets will likely not become routine studies to be conducted in clinical trials and patient management in the next decade. The trade-offs between rapidity of analyses, depth, coverage, cost, and acquisition of new information on somatic mutations will continue to change in lockstep with continued improvements in technologies.

Sequencing and other genomic technologies used to detect somatic mutations are data intensive. The management and delivery of clinically useful and easily interpretable information to healthcare providers will need to address several issues, including data standards, integration and linkages with clinical and laboratory data and other external data warehouses, and data security. Some of these issues are generic; for example, the rapid increase in genomic data generation rate exceeds the corresponding growth rates in data storage technologies, network bandwidth, or processing speeds. Robust data pipelines are needed to track data associated with the sequence information, including instruments, protocols, mutation calling software, quality metrics, etc. Resolving privacy concerns around integrating clinical sequence data sets with electronic medical records requires further efforts.

Informatics challenges related to cancer sequencing arise as a result of tumor heterogeneity and interpretation of mutation data. All bases (with or without variants) need to be tracked in regard to depth, coverage, and base-calling method and confidence score. Because sequence data is ideally generated in tumor and matched normal samples, parallel data capture and analysis is needed to classify variants as germline and somatic. Germline variants can also be screened electronically via polymorphism databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Interpreting the clinical significance of somatic mutations is challenging unless the mutation has previously been shown to be recurrent and actionable. Rapid access to curated information on cancer mutations and genes is the logical first step in this process, as a match with a previously characterized mutation that is known to predict response to a targeted therapy is the simplest scenario. Informatics systems are thus needed to query mutation and cancer gene databases (such as COSMIC and the NCI Gene Index) and large-scale data sets generated by The Cancer Genome Atlas (Cancer Genome Atlas Research Network, 2011) and the ICGC, as well as the literature to determine what is known about identified variants. Novel somatic mutations require careful interpretation that relies on informatics systems that provide information on the identity of the gene, the functional domain, and the extent of evolutionary conservation of the affected amino acid. Software such as SIFT (sorting intolerant from tolerant) (Ng and Henikoff, 2003) can be used to predict whether an amino acid substitution affects protein function. Though each clinical study or cancer center may benefit from storing novel mutations in its local databases to inform subsequent cases with similar mutations, many mutations will be too rare to recur in the same organization, which argues for the establishment of international databases with mutation, function, patient demographic, treatment, and outcome data to ensure robust statistical analyses.

Reporting Data to Clinicians and Patients

Current cancer treatment decisions are informed by knowledge of a limited number of disease-modifying genes. As the cost of genome sequencing technologies rapidly declines, it is conceivable that oncologists will have knowledge of an individual patient's complete cancer genome in the near future. The ultimate goal of such comprehensive profiling is to benefit the individual patient being profiled and, for mutations in germline, family members. However, for the foreseeable future, the ability to generate genomic data will supersede the capacity to decipher patterns across complex data sets, draw inferences from prior experiences, and make informed treatment recommendations that will benefit the profiled individual patient. Novel tools to integrate genomic information with traditional clinical and pathological data in an iterative manner are needed, as are tools that present complex results to clinicians and patients in understandable formats.

Given the complexity of the data, the high number of somatic mutations that can be detected using large-scale sequencing, and the many unique situations that will be encountered, there is a place for establishing expert panels to review the mutation data, deliberate clinical significance, and offer a multidisciplinary perspective regarding the consequences of mutation profiles observed in patients. Multidisciplinary representation allows for input from experts having different training and background, including genome scientists, clinicians, ethicists, clinical geneticists, and genetic counselors to provide balanced interpretations of the potential functional and clinical significance of mutations in the foreseeable future when information from diverse sources will rapidly evolve. Expert panel reports to clinicians should include the rationale for decisions, the degree of consensus, and the level of evidence supporting the decision. Clinical significance should be based on publications reporting on prognostic or predictive role and whether there are clinical trials of targeted agents for the protein product of the mutation, the gene, or pathway. This approach is scalable if it leverages existing and emerging databases and informatics tools that generate draft physician reports that can be reviewed and modified by the expert panel as new information arises. Cursory review will be needed for frequent actionable mutations, and more time will be devoted to deal with novel mutations of possible significance and incidental findings.

The content and format of reports to clinicians are important considerations. Data reports to clinicians must be understandable. Critical pieces of clinical and diagnostic information need to be prioritized according to their clinical utility and level of validation. There is a need for easily accessible smart user interfaces that provide the support for clinical decisions. These need to be structured around best practices and tailored to the level of expertise of the decision maker.

Monitoring and Evaluating the Utility of Clinical Genomics

It will be critically important to evaluate the utility of genomic results. For the foreseeable future, genomic sequencing will be

largely a research approach, and its value must be demonstrated prior to its broad adoption. The genomic information should be not only understood, but also used by clinicians to inform their discussions with patients and to modify treatment recommendation. Such treatment recommendations should result in improved clinical outcomes at affordable costs. Thus, genomic clinical trials should ascertain whether the genomic analyses and mutation-based treatment decisions result in greater survival, improved quality of life, and avoidance of toxicity.

It is important to highlight that genomic results will include somatic mutations, identifying (or failing to identify) a druggable tumor marker, and (where relevant) the receipt of germline genetic results identifying inherited risk of cancer, of other diseases, and of drug toxicity. Information on inherited risks of disease may have minimal impact on a treatment and outcome of a patient with advanced cancer; however, such information may be relevant to patients with potentially curable cancer and to their family members. What information should be conveyed—how, when, and to whom—are areas that require additional research to assess preferences of patients, clinicians, family members, bioethicists, and policy makers.

Data Sharing

Data from these diverse inputs, linked to information on treatment selection and response, should be broadly leveraged across research centers to generalize knowledge and increase the likelihood that genomic profiling will benefit individual patients in the future (Figure 4). This will require some degree of altruism among patients to make their personal genomic and medical information publicly available and a spirit of collaboration between researchers to share their data prior to publication (Mousses et al., 2008; Toronto International Data Release Workshop Authors et al., 2009). Balancing timely access to data with protection of sensitive personal health information of patients and their families is challenging. Four core bioethical principles have been established by the International Cancer Genome Consortium (ICGC) to guide data sharing: (1) participation of individual patients is voluntary; (2) a patient's care will not be affected by his/her decision regarding participation; (3) samples and data collected will be used for cancer research, which may include whole-genome sequencing; and (4) data generated will be made accessible to researchers through either an open or controlled access database under terms and conditions that will maximize participant confidentiality (Hudson et al., 2010).

Implications for Drug Development

Advances in the understanding of specific somatic mutations or amplifications and incorporation of single gene tests have had demonstrable impact on drug development and cancer treatment. The characterization of actionable mutations has already allowed: (1) selection of subsets of patients for clinical trial participation and ultimately for marketing authorization based on greater treatment benefit (i.e., trastuzumab for HER2 breast cancer) ; (2) restriction of labeled indications for targeted agents to avoid treating patients that do not benefit (i.e., lack of efficacy of EGFR antibodies for colorectal cancer patients with *KRAS* mutations); and (3) prediction of toxicity risk (i.e., neutropenia and diarrhea associated with irinotecan in patients for *UGT 1A1*28* homozygosity). Molecular testing will continue to impact

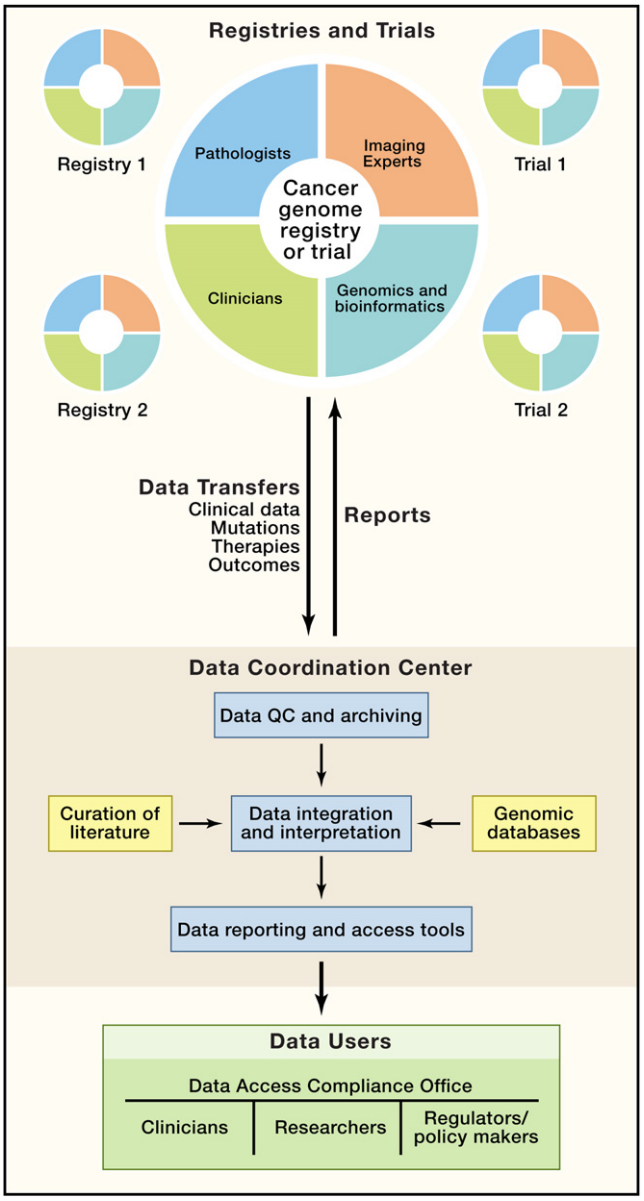

Figure 4. Model for Sharing Cancer Genome Data Sets from Registries and Clinical Trials with Clinicians, Researchers, Regulators, and Policymakers

Novel tools and data repositories are needed to integrate genomic information with traditional clinical and pathological data and to present complex results to clinicians and patients in understandable formats. Genomic clinical trials and registries provide patient demographics, germline and somatic variants, treatment, and outcomes. Informatics systems query mutation and cancer gene databases and curated literature to determine what is known about identified variants. Interpretation of novel somatic mutations may be based on information on the identity of the gene and the functional domain and extent of evolutionary conservation of the affected amino acid. Data reports to clinicians include clinical and diagnostic information on the gene(s) and mutation(s) according to their clinical utility and level of validation.

patient eligibility for clinical trials, study design, drug approval, market utilization, and reimbursement; however, the challenges to rational and practical utilization of complex genomic data are still not fully understood. The amount of genomic information becoming available is adding a high level of complexity to the process of drug development. Information technologies to manage extensive and diverse biological, chemical, and clinical data sets and computational methods to identify the most pertinent information are essential to guide the development of new drugs and establish priorities to generate scientific hypotheses that warrant clinical testing. Clinical development plans for new agents should aim at documenting major and unequivocal treatment benefit validating the hypothesis.

To date, pilot trials of molecular profiling have focused on patients with advanced disease to provide a molecular-based rationale for enrollment of cancer patients in phase I/II clinical trials and have used exploratory methodologies (Tsimberidou et al., ASCO, abstract; Von Hoff et al., 2010). The next wave of trials incorporating tumor DNA sequencing data should establish that genomic testing is associated with improvements in drug development processes that ultimately improve patient management and provide clinical benefit. Efficient clinical trial designs are needed to discriminate new agents and/or combination strategies that warrant further development in patients selected by genomic testing rather than solely by histological characteristics. Efficiencies in clinical trial conduct are gained by identifying patients who are unlikely to benefit from currently available treatment and thus have a relatively poor outcome and/or are more likely to benefit from the investigational agent due to the presence of tumor genetic abnormalities that confer drug sensitivity. In either of these situations, fewer numbers of patients are required to demonstrate an improved treatment effect. This approach requires the discipline to quickly discontinue development when a sufficient signal of activity is not detected in the trial population thought most likely to benefit from a new treatment or if such a subgroup cannot be identified. Definitive clinical trials leading to drug approval should be based on strong scientific hypotheses and robust signals of activity and should aim to show efficacy and safety in selected populations based on complex genomic testing. This will have a major impact on the drug approval process and will modify marketing expectations for new agents at least at the time of initial approval. This is, however, probably the only viable and sustainable approach to allow the rapid translation of the new genomic advances to the cancer patients.

Obviously, there are costs to incorporating genomic sequencing analyses in cancer therapeutic trials. The information gained from broad and deep assessments of the cancer genome may be greater than needed for a clinical trial evaluating the safety and activity of an agent of interest to achieve trial specific objectives. For a given therapeutic agent, the numbers of genes and associated mutations known to be relevant to the disease, drug target, and pathway may be relatively limited. Financial constraints and ethical concerns of administering agents to patients that may be inactive may also limit the ability to screen large numbers of patients and enroll them on an investigational drug trial to determine activity in rare genetic subgroups. Given the current costs of exome sequencing, an initial strategy is to ensure coverage of specific genes relevant to the drug/target, to expand to the top few hundreds of genes of possible clinical and biological interest, and then, as costs fall further, to include other emerging genes of interest. As the information gained from broad molecular screening is of value beyond the industry sponsors, there should be a willingness among public agencies, health insurers, philanthropy, and industry to fund these activities, with the proviso that the information gained will be made publicly available.

Implications for Regulatory Bodies

In many jurisdictions, regulatory authorities have adapted their drug development guidelines, approval decisions, and pharmacovigilance processes to incorporate the knowledge of specific gene alterations in individual patients. A recent review of the approval of 33 new oncology agents approved by the CHMP in Europe between 2000 and 2008 noted that pharmacogenetic biomarkers were mentioned in nine cases (EMEA Committee for Medicinal Products, 2008). Of interest, genetic testing was associated with prescription restriction of the new agent to a subset of patients expressing the biomarker. In addition, many of the regulatory decisions were based on the utilization of nonapproved tests and, in many cases, on retrospective analyses of subsets of patients enrolled in large phase III trials conducted in nonselected patient populations. This illustrates that, in the early days of the incorporation of genomic testing in oncology drug development, regulatory authorities had to be reactive to a rapidly moving field.

Several new guidance documents have been issued or are in development to inform the design of clinical studies incorporating genetic biomarkers and the development of companion diagnostic tests (FDA, 2005). An example of drug and companion diagnostic codevelopment paradigm is the recent approval of vemurafenib for patients with advanced melanoma harboring the BRAF V600 mutation. This represents the first FDA approval of a drug and a companion diagnostic mutation test that stipulates within the package insert the use of the approved test to determine patient eligibility for treatment. However, institutions may prefer to perform mutation analyses using more extensive profiling technologies in their own Clinical Laboratory Improvement Amendments (CLIA) facilities. Individual tests for single or limited numbers of mutations are unlikely to be efficient or cost effective for cancer patients or for drug development. Genomic profiling will generate more extensive data that could impact patient management to a greater extent than very selective tests approved by regulatory agencies. In the case of *BRAF*, the approved test only documents the presence of a single point mutation (V600E); however, assessing other *BRAF* mutations (i.e., V600K and V600D) and mutations in other genes may be relevant to understanding treatment resistance and could ultimately help patients get access to second-generation inhibitors of different *BRAF* variants or other relevant targets. There is a need for collaboration among regulatory agencies, industry, and academics at the forefront of genomic technology to develop new approaches for comprehensive genomic testing in the drug and test approval processes.

In addition to developing new approaches for genomic testing that will be not only acceptable to regulatory agencies,

but also reflective of the constant scientific progress, other key regulatory questions related to the evidence of safety and efficacy for drug and test approval need to be addressed. For example, in highly selected patient populations identified by genomics testing, are randomized trials needed for initial approval of a new target therapy, or should the concept of ''targeted approval'' be considered based on striking results from open label phase II studies (Chabner, 2011; FDA, 2011)? What is the size of the safety database that will be required for initial approval, knowing that genomic-based therapy will lead to relatively small patient subsets? Generating the large safety data set in accordance with regulatory guidelines will become more challenging prior to approval, and the solution may be the adoption of a risk management program in highly selected populations after a product has been approved for marketing. Finally, because tumor growth is usually driven by complex genomic alterations that cannot be controlled by inhibiting a single pathway, how should combinations of investigational agents be developed to document activity and safety as required by regulatory agencies? The FDA draft guidance on the development of investigational drug combinations is a first step to accelerating the early clinical testing of novel targeted agents (FDA, 2010). It is clear that regulatory authorities are adapting and providing guidance on issues associated with genomic testing and drug and diagnostic approvals. However, further dialog is needed to ensure an appropriate and dynamic regulatory framework as technological and scientific advances in cancer genomics and its role in drug development continue to evolve.

Implications for Patients, Healthcare Providers, and Payers

The implications of personalized cancer medicine are complex and can be viewed from the perspectives of the patient, the healthcare provider, and the society. From the cancer patient's perspective, the prospect of receiving specific targeted agents that match ''driver'' mutations offers an attractive therapeutic strategy even though toxicity due to off-target effects may remain relevant. Until molecular profiling becomes a standard of clinical care, there are many challenges for the healthcare provider, such as the access to CLIA-certified laboratories that can perform validated genomic evaluations, the assurance of timely turnaround of results to minimize treatment delay, and the responsibility of finding appropriate treatment for patients based on the returned results. There will be an expanded need for clinicians knowledgeable in cancer genetics and the interpretation of genomic results and for clinical geneticists to work alongside oncologists in multidisciplinary clinics to advise patients and family members on inheritable risks. This will require new curricula, training, and facile knowledge transfer and addressing critical shortages of geneticists and counselors (Cooksey et al., 2005; Cooksey et al., 2006). From the societal view point, the economic balance of personalized cancer medicine must take into consideration the benefits derived from the cost savings of avoiding empiric prescription of expensive medicines versus the expenditures of training personnel with appropriate expertise, setting up certified laboratories with close monitoring of quality control, and high-throughput screening.

Conclusions

The recent advances in DNA sequencing allow for the characterization of a large number of genes and, ultimately, of the entire cancer genome in a timeframe that is compatible with treatment decisions for the patient. This creates opportunities for the development of new agents but also results in challenges that will only be solved if scientists, clinical investigators, pharmaceutical companies, regulatory agencies, and third-party payers collaborate closely. A rigorous approach to developing a complete clinical workflow in which every component of the process is optimized prior to scale-up is essential.

Genomic analyses and results need to be accessible to guide management and clinical trial decisions throughout a patient's disease course. This means that the information should be available irrespective of the party who covered the cost of genomic testing and the initial research study that led to the analyses. It is essential that access to individual agents and to rational drug combinations be easier for both investigational agents as well as marketed drugs. This will require collaboration between pharmaceutical companies that control access to most of the new agents available for clinical testing and third-party payers. Data generated through repetitive genomic studies from individual patients at different stages of disease should be made publicly available to better understand the genomic evolution according to disease stage and therapeutic intervention. This information is required to define the clinical setting in which a therapy will be most effective and to elucidate mechanisms of therapeutic resistance. Many may argue that such complex and far reaching collaborations are not attainable; we would ask ''how can we not?'' given the overwhelming burden of cancer and the unprecedented opportunities for advancements in outcomes for cancer patients.

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