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Translating Cancer Genomes and Transcriptomes for Precision Oncology

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Abstract

Understanding the molecular landscape of cancer has facilitated the development of diagnostic, prognostic, and predictive biomarkers for clinical oncology. Developments in next generation DNA sequencing technologies have increased the speed and reduced the cost of sequencing the nucleic acids of cancer cells. This has unlocked opportunities to characterize the genomic and transcriptomic landscapes of cancer for basic science research through projects such as The Cancer Genome Atlas. The cancer genome includes DNA-based alterations such as point mutations or gene duplications. The cancer transcriptome involves RNA-based alterations including changes in messenger RNAs. Together the genome and transcriptome can provide a comprehensive view of an individual patient's cancer and is beginning to impact real-time clinical decision-making. We discuss several opportunities for translating this basic science knowledge into clinical practice including a molecular classification of cancer, heritable risk of cancer,

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eligibility for targeted therapies, and the development of innovative genomic-based clinical trials. In this review, we outline key applications and new directions for translating the cancer genome and transcriptome into patient care in the clinic.

Keywords

Genomics; Transcriptome; Neoplasm; Gene fusion; Patient care (nlm.nih.gov/mesh)

Introduction

The molecular classification of cancer has informed novel approaches for clinical practice in oncology such as diagnosis, prognosis, and treatment decisions. In 2011, the National Research Council convened a committee to develop a framework for the precision taxonomy of human disease, with molecular classification as the foundation for advancing personalized or precision cancer medicine[1, 2]. Since 2003, new DNA sequencing technologies called next generation sequencing (NGS) have increased the speed and reduced the cost of sequencing cancer by nearly one million fold each[3]. These technologies have advanced our understanding of various subtypes of cancer through several national and international large-scale basic science cancer profiling efforts[4, 5]. Since 2011, clinicians have begun to translate genome and transcriptome sequencing approaches for patients with cancer in the clinic[6]. In this review, we will discuss the current and future impact of genome and transcriptome sequencing for patient care in clinical oncology.

Omics and defining the cancer genome and transcriptome

Through technology innovations that have miniaturized and parallelized laboratory tests to allow testing of thousands of molecules, we have entered the “Omics Era.” Omics refers to collection and analysis of large data sets of biologic variables or phenomenon, and is regularly applied to genes (genomics), proteins (proteomics), and their subunits including nucleotides, amino acids (metabolomics). Prior to the Omics Era, researchers have studied the traditional paradigm where genes encode a sequence of nucleotides that is transcribed into a messenger RNA (mRNA). These mRNA are processed by ribosomes where the sequence information is translated into a protein with the sequential addition of transfer RNAs (tRNA) and their associated amino acids. Collectively, proteins can affect biological processes such as metabolism by functioning as enzymes, structural proteins, or by regulating genes themselves through transcription or translation. Today, rather than studying a few genes, transcripts, or proteins, researchers can utilize new technologies to rapidly test or evaluate 1,000 to 10,000’s of data points.

The human genome includes two haploid sets of 23 chromosomes each comprised in total of six billion nucelotides[7]. One haploid set of chromosomes encodes approximately 20,000 genes that are transcribed into RNAs including the classical messenger RNAs (mRNA), ribosomal RNAs (rRNA), and transfer RNAs (tRNA). Collectively, these RNAs constitute the transcriptome. The mRNA sequence provides the recipe for a protein and is translated through ribosome machinery (including rRNA and ribosomal proteins) by consecutively adding amino acids carried by tRNAs. Additional RNAs have been discovered that do not

encode protein, termed non-coding RNAs (ncRNA). These ncRNAs include microRNAs and long ncRNAs and have more recently been proven to have regulatory functions affecting gene expression and protein function[8]. Prior to 2001, the scientific community did not have a complete road map or dictionary of the entire human genome[9, 10]. The Human Genome Project was initiated by the National Institutes of Health and coordinated the sequencing of the Human Genome over 14 years at a cost greater than three billion U.S. dollars[11]. The Project provided the necessary reference for researchers to study the role of genetics in human diseases such as cancer. The Project also inspired the development of new technologies that have changed the landscape of genomics research by accelerating the speed and reducing the cost of DNA sequencing by one million fold[12].

Historical impact of Omics on clinical medicine

The impact of Omics data on precision medicine can already be seen in clinical practice today. Clinical practices includes the application of data from genes, transcripts, and proteins towards diagnosis, disease monitoring, risk determination, counseling, and development of novel therapies[13]. In an early application for metabolomics, Koenig *et al.* described the value of assessing glycated hemoglobin (hgb A1c) as an every day metabolic measure of long term glucose levels for patients with glucose intolerance or diabetes[14]. Factor V Leiden is a genetic risk factor that occurs in 5% of North American Caucasians as a heterozygote mutation and is routinely applied towards risk assessment for thrombosis, and has led to the avoidance of prothrombotic drugs or prophylaxis recommendations in high-risk situations for patients who are at increased risk. Genetics has also made an impact on the most common form of dementia, Alzheimer's dementia, with the discovery of several genetic factors involved in this disease[15]. Mutations in the gene for Apolipoprotein E have been identified as a risk factor for late-onset Alzheimer's dementia, and research efforts are underway to study other genetic factors[16]. The genetic basis of cystic fibrosis was described in 1989 and 3–4% of Caucasians are carriers for this disease. Early on genetic testing has been important for counseling and diagnosis, more recently metabolic research has led to the development of novel therapies[17]. In cancer, Omics research led to the application of chromosome karyotyping of leukemias that guides diagnosis, risk stratification, and therapy selection. The discovery of the Philadelphia chromosome In chronic myeloid leukemia and subsequent characterization of the BCR-ABL1 gene translocation would pave a path for the development of imatinib, the first smart drug for cancer[18]. The majority of these Omics discoveries preceded the Human Genome Project (1989–2002) which has opened new doors for genomic medicine.

Next generation sequencing technologies and cancer

DNA sequencing has consistently relied on utilizing one strand of DNA as a template to synthesize the other strand by adding complementary nucleotides with the enzyme DNA Polymerase. The first method to determine which nucleotides are added was described in 1977 and involved termination of the sequencing reaction with nucleotides that cannot allow further DNA synthesis (so called Sanger or dideoxy termination)[19]. This strategy relied on radiographic detection of radioactively-labeled nucleotides and gel electrophoresis, and over time was supplanted for practical reasons by fluorescent-labeled nucleotides and

electrophoresis in small capillary tubes. Thus, the Human Genome Project was completed with capillary sequencers. In 2005, new technologies known as next generation sequencing (NGS) miniaturized and parallelized the sequencing process to improve yield and reduce cost[3, 12]. All NGS methods start by fragmenting DNA into small segments, followed by the addition of adaptors that allow the fragment to be sequenced. The addition of specific nucleotides can be measured by light (Illumina, Pyrosequencing) or pH changes (Ion Torrent). The resulting sequence data is comprised of millions of pieces ranging in length from 50 to 250 base pairs and must be matched and assembled to a reference genome or map and this is akin to a jigsaw puzzle. Base pairs that are different from the expected reference may be mutations. This field is called bioinformatics data analysis and utilizes high performance computing to process this so-called big data set. In addition to DNA sequencing, RNA can be similarly sequenced by first converting RNA into complementary DNA (cDNA) using the reverse transcriptase enzyme, and then following the same procedure for DNA. Today, there are third generation technologies for NGS that have effectively decreased the cost of sequencing by one million fold since the Human Genome Project. Technologies for NGS are further detailed and reviewed elsewhere[3].

Cancer genome (DNA) sequencing

Two major collaborative efforts, The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), have utilized NGS to profile the landscape of the 30 most common cancer types. Each of these efforts has collected primary tumors from surgical resections for tumor sequencing. In addition to these collaborative multi-center projects, there are numerous independent research groups that have contributed to cancer genomic profiling for cancers subtypes with lower prevalence. In general, rather than sequencing the whole genome, these profiling projects have focused on 1% of the genome containing the approximate 20,000 known genes, also known as the whole exome. Exome sequencing uses probes or baits to allow NGS platforms to focus on DNA fragments containing the 20,000 known genes, thereby reducing the cost of sequencing by 100-fold. Despite the improvements in cost and throughput for NGS, whole genome sequencing remains expensive for both basic and clinical research applications. Today, the majority of basic and clinical applications use a targeted or whole exome approach[20].

These large-scale cancer-profiling projects have revealed a landscape of the cancer genome that includes a diverse variety of genomic alterations including point mutations, copy number variation, and translocations. These genomic alterations can affect a variety of cellular processes from cell signaling and metabolism to gene expression[21]. Point mutations are single base pair substitutions that can change the function of a gene's protein product. One example is the clinically relevant *BRAF* oncogene mutation V600E (amino acid change from valine to glutamic acid) that occurs commonly in melanoma and leads to constitutive activation compared to the wild type gene[22–25]. Copy number variation refers to either extra or missing copies of a gene. Tumors with copy number amplification have extra copies beyond the expected two genes, such as 50–100 copies of HER2 (*ERBB2*) as seen in approximately 20% of breast cancer[26–28]. Alternatively, loss of gene copies, also known as deletion, often occurs in tumor suppressor genes such as *PTEN* in prostate and other cancers[29]. Translocations or gene rearrangements involve two genes that are brought

together and have a new function as a chimera. These events can create new functions for the gene fusion or inactivate functions. Gene fusions in cancer commonly involve kinases (enzymes that have phosphorylating activity) and transcription factors that are deregulated by the fusion event. With the advent of chromosomal karyotyping and banding in the 1960s, the first appearances of chromosomal rearrangements were uncovered in hematological malignancies and sarcomas due to the ability to easily obtain tumor tissue and metaphase chromosomes in these cancers. Visible chromosomal rearrangements in lymphomas, leukemias, and sarcomas facilitated the identification of novel oncogenes and tumor suppressors in cancer and characterization of their role in cancer biology and clinical applications[30]. As an example, chronic myeloid leukemia, characterized by the Philadelphia chromosome and *BCR-ABL1* gene rearrangement[31, 32], would later become a model for understanding kinases and the application of targeted therapies for treatment of cancer[33]. Meanwhile, outside of sarcomas and select solid tumors, there initially was a paucity of oncogenic gene fusions or translocations recognized in solid tumors. This was largely due to limited tissue access and technological restrictions, however it was predicted that gene fusions would be recurrent genomic alterations in solid tumors[34]. Since 2005, cancer genome and transcriptome sequencing has revealed additional clinically relevant novel gene fusions in solid tumors[35].

Cancer transcriptome profiling

The transcriptome is comprised of “classical” RNAs (mRNA, rRNA, and tRNA) as well as there are multiple subtypes of noncoding RNA (microRNAs and long ncRNAs) that have been discovered to have novel regulatory functions in cell biology[8, 36]. Gene expression can be characterized using earlier microarray technology or the more recent transcriptome sequencing (RNAseq) methods. Transcriptome sequencing has significant advantages including precise detail about base pairs and ability to detect novel RNAs that cannot be detected on microarrays. For clinical applications of the cancer transcriptome, efforts have focused on using gene expression to classify cancer subtypes (that differ with regard to prognosis and response to specific treatments) and detection of gene fusions or rearrangements. In routine clinical practice, fluorescence in situ hybridization (FISH) and RT-PCR is used to detect gene rearrangements but is limited by only testing for one gene at a time. Hence, the advantage of sequencing approaches is the ability to detect multiple gene rearrangements as well as novel ones. Although genome sequencing can detect fusions, whole genome sequencing of cancer remains costly, and RNAseq is a fraction of the cost of whole genome sequencing, and has been applied with new bioinformatics approaches to detect fusions. Using a paired-end sequencing approach of RNA, gene fusions that are expressed at the transcript level can be detected[37, 38]. More recently, Stransky *et al*., performed a comprehensive analysis of publically available tumor RNA sequence (RNAseq) data for nearly 7000 cancers in The Cancer Genome Atlas to catalog a diverse landscape of known and novel candidate kinase gene fusions[39]. Furthermore, Klijn *et al*., performed RNAseq on 675 cancer cell lines and similarly cataloged kinase fusions[40]. The application of RNAseq to detect novel clinically relevant gene fusions in cancer is still in its infancy, and we anticipate additional fusions will be discovered as the number of cancers profiled increases, especially in rare or previously uncharacterized cancer subtypes. Importantly,

detection of novel gene fusions involving kinases has also lead to novel treatment opportunities and therapeutic benefit with kinase inhibitors in patients with advanced cancer[35]. In pediatric B cell acute lymphoblastic leukemia, Roberts *et al*., recently identified kinase fusions involving genes such as *ABL1*, *JAK2*, *CSFR1*, and *NTRK3* that have corresponding targeted therapies, which opens up new treatment hypotheses for patients with this type of leukemia to be tested in clinical trials[41, 42].

Gene expression signatures can be utilized to classify cancer types into molecular subsets that have clinical relevance. For example, early studies applied transcriptome profiling of B cell lymphoma using microarray technologies to further classify this disease into clusters of activated B cell (ABC) and germinal center B cell (GCB) subtypes[43]. ABC lymphoma bears poorer prognosis compared to GCB[44]. In another pivotal study of breast cancer, Perou *et al*., used microarray-based transcriptome profiling on primary breast cancer samples and classified this disease into five molecular subsets with biological and clinical relevance[45]. Moving beyond microarray technologies, RNAseq has the potential to enable study of other diverse components of the cancer transcriptome.

For example, in addition to the classical elements of the transcriptome including messenger (mRNA), ribosomal (rRNA), and transfer (tRNA) RNAs, multiple subtypes of RNA have been discovered with novel regulatory functions in cell biology. In fact, the majority of the transcriptome are non-protein encoding RNAs (ncRNAs) including but not limited to microRNAs (miRNAs)[46–48], small interfering RNAs (siRNAs)[49], and long noncoding RNAs (lncRNAs)[36]. Beyond the classical function for mRNA that encode proteins, these novel RNAs can play multiple roles in cell biology ranging from regulation of transcription, post-transcriptional events, gene silencing, translation, and protein level function[50]. Much like prototypical genes encoded by DNA, miRNAs are subject to genomic alterations including mutation, deletion, amplification and epigenetic modifications[51]. Similarly, miRNAs can function as tumor suppressor or oncogenes[52]. siRNA are small RNAs that mediate a highly specific gene-silencing mechanism that is conserved from nematodes and plants to mammalian biology[49] and have emerged as tools for biomedical research and potential strategies for gene-silencing therapies[53]. Newly described lncRNA are ubiquitous in cancer, have diverse regulatory functions, and are only recently being systemically characterized[54–56].

Cancer genomes and transcriptomes: What have we learned?

Over 10,000 cases of cancer have undergone DNA sequencing and published through collaborative projects and this has revealed diverse heterogeneity within and across cancer types classified by tissue-of-origin (*e.g*. breast, lung). We have also learned that signatures of mutational patterns for point mutations can aid classification through association with an underlying mechanism such as defects in DNA repair, radiation exposure, tobacco exposure[57, 58]. As an example, several cancer types such as lung and melanoma have abundant point mutations due to carcinogen exposures of tobacco smoke and ultraviolet radiation, respectively[59]. (Figure 1. *With permission from Lawrence et al., reproducing* Figure 1 *from Nature 2012*). In contrast, acute myeloid leukemia and prostate cancer generally have few point mutations, and rather have more copy number variation and gene

fusions[59]. Ciriello *et al*., retrospectively assessed over 3000 cases from the TCGA and evaluated 12 types of cancer as having predominantly point mutations (M class), copy number variations (C class), while the majority are mixture of both M and C within a single disease type[60]. Along with the genome, the cancer transcriptome has informed classification of lymphoma and breast cancer into clinically relevant molecular subsets[61]. Lung cancer is an ideal example where genome and transcriptome profiling have affected measureable clinical outcomes, through moving from histology to a genomics-based classification based on point mutations (BRAF V600E), copy number alterations (MET amplification), and gene fusions(ALK fusion) that lead to treatment with matching targeted therapies[62].

Moving forward to the clinic, there are several lessons to consider for translation. It has become clear that clinical decision making for cancer will require a personalized approach based on an individual's cancer that is likely to be unique compared to other patients even with the same histologic type of cancer. Second, with slightly more than just 10,000 cases analyzed, researchers have only detected the clinically relevant mutations that represent more than 20% of common cancer types[63]. Therefore, based on limited sampling, we have not yet uncovered clinically relevant mutations with an estimated prevalence less than 20% in common cancers or in rare cancers that have simply not yet been sequenced yet. Therefore, more cancer sequencing data is necessary to advance a comprehensive catalog of cancer. Third, the majority of cancer genome data available are based on primary tumors rather than metastatic or advanced cancers, which may have acquired additional mutations, are cancers that behave more aggressively, and display more heterogeneity due to selective pressures from therapy. With this knowledge in hand, how are we proceeding to apply cancer genome and transcriptome biomarkers in the clinic?

Types of Molecular Biomarkers Applied in the Clinic: Diagnostic, Prognostic, and Predictive

Research discoveries derived through cancer genome and transcriptome studies have the potential for clinical impact as biomarkers[64]. There are three key types of biomarkers employed for clinical decision-making including diagnostic, prognostic, and predictive biomarkers. Diagnostic biomarkers facilitate identification of a cancer type or subtype. Prognostic biomarkers aid clinicians in determining the risk of relapse or disease progression after therapy, wherein patients with high risk are selected for aggressive screening or adjuvant therapy to prevent recurrence. Clinicians utilize predictive biomarkers to select one therapy over others, based on associations between biomarker results and likelihood of response to certain therapies. In practice, predictive biomarkers often identify the molecular targets of relevance to targeted anticancer drugs.

Each type of biomarker could be assayed to detect changes in a tumor's genome (DNA), transcriptome (RNA), proteome (protein), or by phenotypic characteristics (such as histopathologic classification). As examples of methods to detect these biomarkers, *BRAF* gene mutation testing for melanoma is a DNA-based predictive biomarker that can guide therapy selection. For RNA-based biomarkers, FISH methods are standardly used for diagnostic subtyping of lymphoma to measure Epstein-Barr virus RNA expression. Immunohistochemistry is utilized to detect estrogen receptor protein (ER) in breast cancer

and is an example of a biomarker that has both predictive and prognostic value. Oncotype Dx testing for breast cancer assesses the expression of 21 transcripts in women with node-negative estrogen-positive breast cancer is another example of a biomarker that is both predictive and prognostic, facilitating identification of patients after surgery who need further therapy (prognosis) and are most likely due to benefit from adjuvant chemotherapy (predictive)[65].

However before any biomarker can be translated to the clinic for use in standard practice, the clinical utility of the biomarker must be tested through clinical trials to establish its impact and association with clinical outcomes[66]. The presence of *ALK* gene fusions in patients with metastatic lung cancer is an example of predictive biomarker for clinical response to ALK inhibitors[67, 68]. Preceding the use of NGS, the clinical utility of ALK gene fusions was completed in pivotal clinical trials using standard FISH methods to detect the gene fusion. However, it costly to develop FISH and Sanger sequencing tests for single genes and their relevant mutation, and NGS has been translated to the clinic to cost-effectively broaden the number of genes and type of mutations tested.

Clinical examples: whole genome (DNA) sequencing

Early efforts to employ whole genome sequencing for patients with cancer began as case-by-case research endeavors. In 2011, Welch *et al*., applied whole genome sequencing for a patient with acute promyelocytic leukemia (APL) which is characterized by having a gene fusion involving the retinoic acid receptor or *RARA*[69]. Patients with APL and *RARA* fusions are typically very sensitive to therapy (predictive biomarker) with oral all-trans retinoic acid with a significantly improved long-term survival[70]. However, this patient's standard of care testing with cytogenetics and FISH did not detect the expected chromosome 15 and 17 translocation or the RARA fusion. Welch and team hypothesized that the *RARA* gene might be involved in a cryptic fusion that is not visible by standard cytogenetics or FISH methods and therefore evaluated the leukemia via NGS. They chose whole genome sequencing over exome sequencing since they were trying to detect potential chromosomal breakpoints that might not involve the exons tested by whole exome methods. After seven weeks of sequencing and analysis, they were able to identify a *PML-RARA* gene fusion, and this subsequently changed the course of treatment for the patient who received all-trans retinoic acid therapy instead of allogeneic stem cell transplantation.

In another clinical application, whole genome sequencing was able to identify a germline or heritable risk of cancer in a 37-year old woman who had personal history of ovarian cancer, breast cancer, and secondary therapy-related acute myeloid leukemia. While the patient did not have a significant family history of cancer, her clinicians were suspicious of her multiple primary cancers. Based on the personal history that suggested hereditary breast and ovarian cancer syndrome, gene testing for *BRCA1* and *BRCA2* was completed but no heritable genetic cause was identified. Link *et al*. performed whole genome sequencing of the patient's skin biopsy and bone marrow leukemia sample, and identified a deletion in the *TP53* gene which can confer a heritable risk of cancer as part of the Li-Fraumeni syndrome[71]. This case is illustrative of the advantages of NGS approaches to detect multiple genomic alteration types when the etiology is not apparent based on clinical presentation or

standard testing. As a comparison, conventional comprehensive sequencing of BRCA1 and BRCA2 in 2011 would have cost $4,000 alone. This information has substantial clinical impact for the patient's family where early screening measures to detect cancer are standard of care for relatives carrying the same mutation.

More recently, Demeure *et al*., evaluated a patient with papillary thyroid cancer whose disease was progressing despite thyroidectomy, radical neck dissections and radioiodine treatment. They performed whole genome sequencing of the patient's thyroid tumor and identified a gene fusion involving *EML4-ALK*, which is a targetable fusion seen in 3–5% of lung cancer but it not commonly found in thyroid cancer[72]. This led to treatment with crizotinib, an oral ALK inhibitor approved for lung cancer, and stabilization of the patient's tumor growth. This case example illustrates the advantage of NGS approaches in finding uncommon genetic changes in cases tests for the common genetic changes were negative.

These represent a sample of case reports demonstrating how whole genome sequencing technologies can affect the care of patients by providing an individualized treatment or screening plan that could affect the patient and even the family.

Clinical examples: integrating whole exome (DNA) and transcriptome (RNA) sequencing

Several groups have developed trials for clinical tumor sequencing to offer patients with advanced cancer new molecular diagnostic tests to classify their tumors, gain molecular eligibility for investigational therapies in trials, and track clinical outcomes[6, 73–75]. In 2011, we completed a pilot study offering a combination of whole genome, whole exome, and transcriptome sequencing for patients with advanced cancer and returned clinically significant results within a clinically relevant time frame[6] (Figure 2: Figure 1C+1D *adapted from Roychowdhury et al., Science Translational Medicine 2011*). This study demonstrated the feasibility of offering cancer genomic testing and addressed some of the early logistical challenges related to informed consent, incidental findings, and interpretation. The study also demonstrated the need for multi-disciplinary team effort required by oncologists, genomics scientists, bioinformaticians, pathologists, and genetic counselors. Currently, the majority of NGS assays are focusing on targeted DNA sequencing for 25–300 gene panels (discussed below[74, 76–79]), but several academic cancer centers continue to study the merits of whole exome (~20,000 genes) and transcriptome sequencing. Nevertheless, there are several advantages to be gained by incorporating RNA sequencing concurrently with DNA sequencing, including data on gene expression, enhanced variant calling, splice variants, novel RNAs, noncoding RNAs, and gene fusions[6, 80 81]. For example, whole transcriptome sequencing lead to the discovery of novel gene fusions involving fibroblast growth factor receptors (FGFR) occurring in an estimated 5–7% of solid tumor cancers[82, 83]. FGFR signaling is an important pathway for cancer biology and there are multiple inhibitors of FGFR in clinical development. The discovery of FGFR fusions subsequently led to the development of clinical trials of tyrosine kinase inhibtors ponatinib and BGJ398 to treat patients whose tumors have *FGFR* fusions with FGFR inhibitors (NCT02272998, NCT02160041).

To expand on this pilot study of integrative sequencing approach for clinical tumor sequencing, we collaborated with others on efforts to expand clinical cancer genomics in pediatric oncology and also overcome logistical barriers to launch a multi-center clinical study in adults. For pediatric oncology, we addressed issues for provided informed consent and assent to minors and their guardians, obtaining permission for research biopsy and tumor sequencing. This study enrolled 102 patients with refractory cancer, performed exome and transcriptome sequencing of tumor, demonstrating feasibility for pediatric oncology, and was able to identify clinically relevant alterations in 46% of patients[84]. In one case example, an infant who was diagnosed with spindle cell sarcoma was found to have a novel translocation involving *NTRK1*, which lead to treatment with crizotinib resulting in a partial response, followed by stable disease. To demonstrate feasibility for multi-center trials, our collaborative team designed a study for advanced prostate cancer deployed across multiple clinical sites and evaluated 150 men with metastatic castration-resistance prostate cancer[85]. These patients had progressed after receiving standard anti-androgen hormonal therapies for prostate cancer. We consented patients to tumor biopsy, tumor and germline testing with whole exome and transcriptome sequencing approaches. The study has identified pathways with known mutations, but also pathways that were previously not observed in prostate cancer including WNT pathway signaling and somatic defects in DNA repair. This international study has demonstrated multi-center feasibility and brought attention to additional considerations for scaling the volume of patients in the study related to testing (turnaround time, use centralized testing, and quality control) and availability of therapies in clinical trials.

EXPANDING CANCER GENOMIC MEDICINE

Clinical Sequencing Exploratory Research (CSER) Program: Systematically advancing genomic medicine

To help address the need to systematically apply genomics to the practice of medicine beyond a case-by-case basis, the National Human Genome Research Institute established and funded the CSER program to study and provide guidelines for bringing genomics to clinical practice (https://cser-consortium.org). The consortium includes sites that study adult and pediatric cancer, cardiovascular disease, and hereditary diseases. The projects include expertise in clinical specialties, laboratory scientists, bioinformaticians, clinical genetics, legal experts, bioethicists, and patient advocates. CSER has implemented working groups to evaluate specialized issues related to return of results, the electronic medical record, genetic counseling, informed consent, outcomes, pediatrics, phenotype measures, standards for sequencing, and cancer.

Physician and patients attitudes on genomic testing

As academic cancer centers begin to deploy clinical trials to evaluate how to deliver genomic medicine, it is vital to assess how both physicians and patients view genomic testing approaches. Gray *et al*. surveyed 160 physicians at an academic cancer center about use of somatic testing, and their genomic confidence. Interestingly 22% of physicians reported “low confidence in their genomic knowledge” and the authors suggested a need for guidelines and education to support understanding of genomic tests for physicians[86]. Miller

et al. completed structured interviews with 17 physicians about genomic testing in practice and they similarly observed a need for decision tools and education to aid physicians[87]. Gray and Blanchette *et al*., completed studies by interview and questionnaire respectively of patients with cancer about genomic testing and found that patients are interested and motivated to undergo genomic testing and potentially improving their cancer care, but also learned that some patients had concerns about incidental findings, discrimination, and a need for more information or genetic counseling[88, 89]. Studies such as these and through the CSER Program are critical to address barriers for translating cancer genomic medicine into the larger clinical oncology and patient community.

Clinical interpretation of tumor sequencing results

Interpretation of mutations is critical for translation of genomic testing results into the clinic. There are many software tools to predict or model the potential impact of mutations in basic science research[90], but there is a need for expert clinical annotation of specific mutations that provides the exact level of clinical or pre-clinical evidence that a physician will need for decision-making. When physicians receive genomic test results, they are faced with mutations in a large number of genes, across multiple pathways, and this is a significant obstacle for busy practicing oncologists who cannot keep up with the vast volumes of data that are emerging. Not all mutations are driver mutations that confer a selective advantage (to the cancer) for its survival, growth, or spread, and the majorities are so called passenger mutations or variants of unknown significance. Several databases and websites have been developed as clinical decision support tools to aid in the interpretation of mutations including MyCancerGenome (mycancergenome.com), Knowledge base for precision oncology (pct.mdanderson.org), and Cancer Driver Log (candl.osu.edu). Moving forward, CSER's Tumor Working Group and ClinGen's Somatic Working Group are working together to provide overarching infrastructure and leadership to support a more comprehensive database and framework for clinical interpretation of somatic mutations.

Genomic tests that inform clinical decision-making

Bringing NGS-based cancer genomic testing up to clinical grade standards to support clinical decision-making equates to understanding and following standards for molecular diagnostics. While NGS is relatively new to the molecular pathology and diagnostics community, several groups have already offered guidelines to address quality for NGS-based cancer genomic testing[91–93]. Assays must undergo analytic validation that includes determination of the assay's sensitivity and specificity for detecting mutations using standards. Clinical validation of the assay refers to the broader application of the assay on clinical samples such as formalin-fixed, paraffin-embedded or frozen tumors, and association of the test results with real world clinical diagnoses[66]. This often includes confirmation of the test result by another assay such as Sanger sequencing, PCR, or FISH. All of this is performed in a clinical grade laboratory that has been inspected by a certifying body such as a State Department of Health or the College of American Pathologists to ensure that the labs have standard operating procedures, training, and quality assurance programs in place to deliver quality tests[93]. Demonstrating clinical utility of the assay is separate from building the tests with analytic validity and is subsequently completed through clinical trials that may look at clinical outcomes retrospectively or prospectively[66].

Cancer gene panels and case reports

Developing NGS-based genomic tests in clinical grade laboratories has considerable constraints that include ensuring a rapid turnaround time, keeping costs of the assay down, and limiting the complexity (size) of the assay for analysis. As a consequence, many commercial and academic laboratories have developed targeted gene panels focused on 25–400 genes that are known to be important for cancer biology or disease management (Table 1). Because of the ease of testing for gene panels, thousands of patients are undergoing genomic testing with cancer gene panels in the U.S. since 2012. Ou *et al*., reported a patient with lung cancer who was found to have a novel ROS1 gene fusion based on a NGS test, that was missed by standard FISH or PCR approaches because it involved a novel fusion partner with *TMEM106B*[94]. Unfortunately, testing was not initiated until the patient had progressed on standard chemotherapy, and they passed from disease before receiving a ROS1 inhibitor. Chalmer *et al*., found a patient with a myeloid neoplasm with eosinophilia to have a novel gene fusion involving *PDGFRa, who* subsequently benefited from therapy with imatinib[95]. Once again, standard FISH testing missed this particular gene fusion since it is designed to detect a specific fusion only. Ali *et al*., observed a patient with metastatic kidney cancer with a *TSC1* mutation, which is predicted to result in activation of MTOR signaling, and who clinically responded and benefited from MTOR inhibitors[96, 97].

Unmet needs: Cancers of unknown primary

Cancers of unknown primary (CUP) represents 2–3% of adult cancers, up to 80,000 cases per year in the U.S., and are defined by the inability to identify the anatomic organ or tissue of origin for these patients using traditional radiologic imaging and immunohistochemical (IHC) assessment of the tumor[98]. Without knowledge of the tissue of origin (TOO), it is challenging for oncologists to select the appropriate treatment for these patients, and overall survival outcomes for these patients are poor[99]. In clinical practice, the focus has been to identify the most probable TOO based on clinical presentation and available pathologic data, recognize favorable subsets of cancer when possible, and choose therapies that match the suspected disease. Up to 20% of CUP may be favorable subsets including prostate cancer, ovarian cancer, breast cancer, germ cell tumors, or neuroendocrine cancers, all of which have established effective therapies. However, most CUP patients have tumors with poorly differentiated histology, limited markers, and no clear evidence of primary tumor origin. Consequently, empiric chemotherapy has been the standard of care with generally poor survival outcomes[100]. Several assays have been developed to classify the TOO on the basis of mRNA or miRNA expression signatures and may aid in choosing chemotherapy[101, 102]. More recently, NGS-based genomic testing assays have revealed potentially actionable genomic alterations in patients with CUP that could more directly guide selection of a targeted therapy[103].

DESIGN OF CLINICAL TRIALS

Genomics-based classification of cancer has changed the outlook on how clinical trials are designed. Traditionally, an investigational agent is developed for a specific cancer type such as lung or breast cancer. Since these cancers can now be characterized and split into different molecular subsets, there is a rationale for enrolling patients for treatment based on

molecular eligibility. As an example, for early trials of BRAF inhibitors in melanoma, the initial Phase 1 trial included patients with any solid tumor to determine dosing and assess toxicity and they observed that patients with *BRAF* V600E activating mutations were more likely to respond. In the subsequent expansion phase, only patients with *BRAF* mutations were enrolled, and 80% of patients had a response based on overall response rate[24]. Similarly, molecular eligibility with *ALK* gene fusions was a requirement for entry into early trials of ALK inhibitors in lung cancer[104].

A special challenge for conducting such clinical trials is that the number of eligible patients with molecular eligibility is dramatically reduced, and the traditional approaches for statistical trial design may not be feasible. For example, a clinical trial for a mutation with prevalence of 1% in a common cancer type will be difficult to accrue and complete. In contrast, this may in fact represent an advantage, as a molecularly enriched trial may be more likely to have patients who respond to therapy and may display a greater magnitude of response. Traditionally, patients are randomized to receive the targeted therapy and either previous best standard therapy or placebo, but there may be insufficient numbers of patients to complete these trials. Alternative endpoints such response rate and magnitude of response[105] may be necessary for rare mutations or rare cancers such as the non-randomized trial for imatinib, as a CKIT inhibitor, in patients with gastrointestinal stromal cell tumors[106]. Meeting the demands of trial accrual for patients with rare mutations may be partially accomplished via screening across many clinical sites and multi-center trials through networks such as the National Cancer Institute and its cooperative groups. One limitation for multi-center trials is the substantially increased regulatory cost, and reduced funding, for institutions to open trials for potentially enrolling only 0–2 patients per year, and difficulty in implementing complex correlative studies within those trials. Nevertheless, there are several examples in this regard for lung cancer. The Lung Cancer Mutation Consortium developed over a dozen pathway-based trials for lung cancer across 16 clinical sites (www.golcmc.com). Selected trials may ultimately be more feasible than others towards meeting accrual goals based on gene or mutation prevalence. More recently, the LungMap trial for squamous cell carcinoma similarly includes pathway-based trials for FGFR, PI3-Kinase, cyclin-dependent kinase pathways, and an immunotherapy arm for patients who lack an actionable driver mutation (www.lung-map.org).

Another emerging approach for trials is exclusively mutation-based and pathway-based eligibility and thus truly tumor site agnostic. One example is a so-called “basket trial” for patients with any solid tumor that has alterations in FGFRs including point mutations, amplifications, or fusions. In this Phase 2 study, patients receive an oral pan-FGFR inhibitor (ponatinib) and the endpoints are to identify clinical responders in disease or mutation subsets to guide future trials and drug development (NCT02272998). The National Cancer Institute has recently laid out a strategic plan for precision medicine trials including the MATCH program (Molecular Analysis for Therapy Choice)[107]. The NCI-MATCH Trials can be opened at any NCI-designated cancer center and entail centralized tumor testing for each patient at one of four genomic testing labs, and multiple trials each with eligibility for a targeted therapy that is mutation-based. While a majority of trials are tumor site agnostic,

some trials could be focused on a mutation pathway in a disease group such as MTOR signaling in genitourinary cancers.

Learning from exceptional responders in trials

In addition to prospective trials to match patients to targeted therapies based on the mutations in their cancer, other efforts from clinical trials are learning from rare patients who experienced an exceptional response to a therapy, but the mechanism for that response is unknown[107, 108]. In this approach, clinicians make phenotypic observations in rare patients who have complete responses to a therapy for their metastatic disease, and retrospective genomic and transcriptome sequencing of the patients archival tumor can potentially reveal the underlying biology. For example, Iyer *et al*., evaluated a patient with metastatic bladder cancer who had a sustained complete response to an MTOR inhibitor clinical trial, while the majority of patients in that trial did not[109]. They performed whole genome sequencing on the patient's archival tumor and identified a point mutation in *TSC1*, a tumor suppressor gene that negatively regulates MTOR signaling. Subsequently, they identified other patients who had *TSC1* mutations who were more likely to have response to MTOR inhibition. In another example, Wagle *et al*., observed an exceptional response in a patient with metastatic urothelial cancer receiving an MTOR inhibitor and identified alterations in another gene, specifically activating point mutations in *MTOR*[110]. Each of these exceptional responder evaluations has now identified mutations that represent new treatment hypotheses for the development of MTOR inhibitors in patients with mutations in *MTOR* and *TSC1* genes. On a national level the NCI has established an Exceptional Responders Initiative to utilize genomic sequencing to facilitate drug development for advanced cancer[107]. The mission of the NCI Exceptional Responders Initiative is to identify and confirm patients who have had remarkable responses to systemic therapy, and use genomic technologies to characterize their tumor to study the molecular mechanisms underlying why these patients benefit from systemic therapy, particularly chemotherapies[111].

Challenges and opportunities

While the new framework for precision medicine in cancer has great promise, the full realization of this approach has several challenges as well as opportunities. Beyond the molecular characterization of cancer through whole and transcriptome sequencing, there are additional complexities for cancer biology to consider. First, basic science research on epigenetic alterations are continuing to reveal how methylation regulates gene expression, and can contribute to our view of individual cancers[112]. Second, new omics approaches for the proteome and the metabolome can reveal additional layers to cancer biology. As new "Omics" approaches became more practical, we should consider new standards for integrating data analysis and transparency in clinical trials as recommended by the Institute of Medicine's recent assessment of translational omics[113]. Third, additional aspects of cancer biology including tumor heterogeneity[114], mechanisms of drug resistance[115, 116], the tumor microenvironment[117], and stem cell properties[118] of cancer can influence how patients respond to therapy. Together, these challenges can be met with new opportunities created by investment in science. Beyond genomics-guided therapy, we envision combination therapies with other modalities including immunotherapy, oncolytic viruses,

and stem cell/metabolism targeting inhibitors. For immunotherapy, genomic sequencing approaches can be applied to identify the burden of neo-tumor antigen or molecular defects in DNA repair such as mismatch repair genes that may predict response to novel therapies that inhibit immune regulatory checkpoints to boost the immune response against cancer[119–121]. These challenges can be met through a collaborative network of innovative clinical trials with systematic collection of tumor tissue, clinical data, and transparency (Figure 3).

Future directions

Integrative profiling through DNA and RNA sequencing opens new doors for both basic and clinical cancer research. Molecular classification of cancer based on genomic and transcriptome alterations may reveal novel biomarkers for diagnosis, prognosis, and predicting response to therapies. Translating the cancer genome and transcriptome for patients will require continued multi-disciplinary collaboration between oncologists, pathologists, basic scientists, and computational biologists (Table 2). Additional resources and funding are necessary to support the ongoing profiling efforts for basic genomics research, tumor sequencing in the clinic, and data sharing networks to enable precision cancer medicine.

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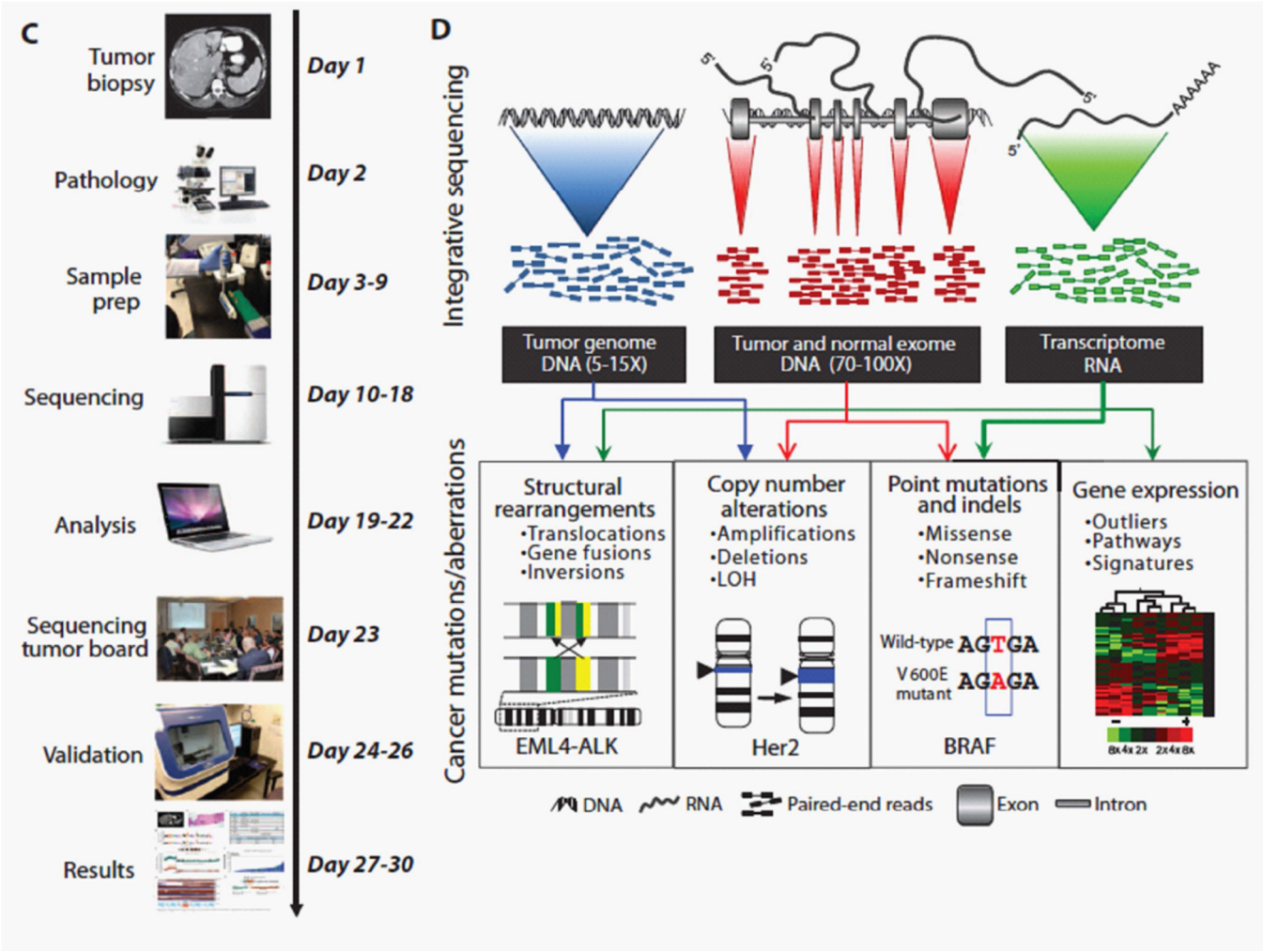

Figure 1. Mutational heterogeneity with and across cancer

(*Adapted from Lawrence, Nature 2013, Figure 1*). Authors assessed over 3000 cases of cancer from The Cancer Genome Atlas and plotted the number of mutations identified, patterns of mutations, and grouped them by tumor tissue-of-origin. The plot illustrates that variation of point mutation burden across different cancer types and also within a given cancer type such head and neck cancers, and the importance of performing personalized genomic testing. Further, some cancers appear to be hyper-mutated with 100s of mutations such as lung and melanoma cancers, while others such as acute myeloid leukemia have few point mutations.

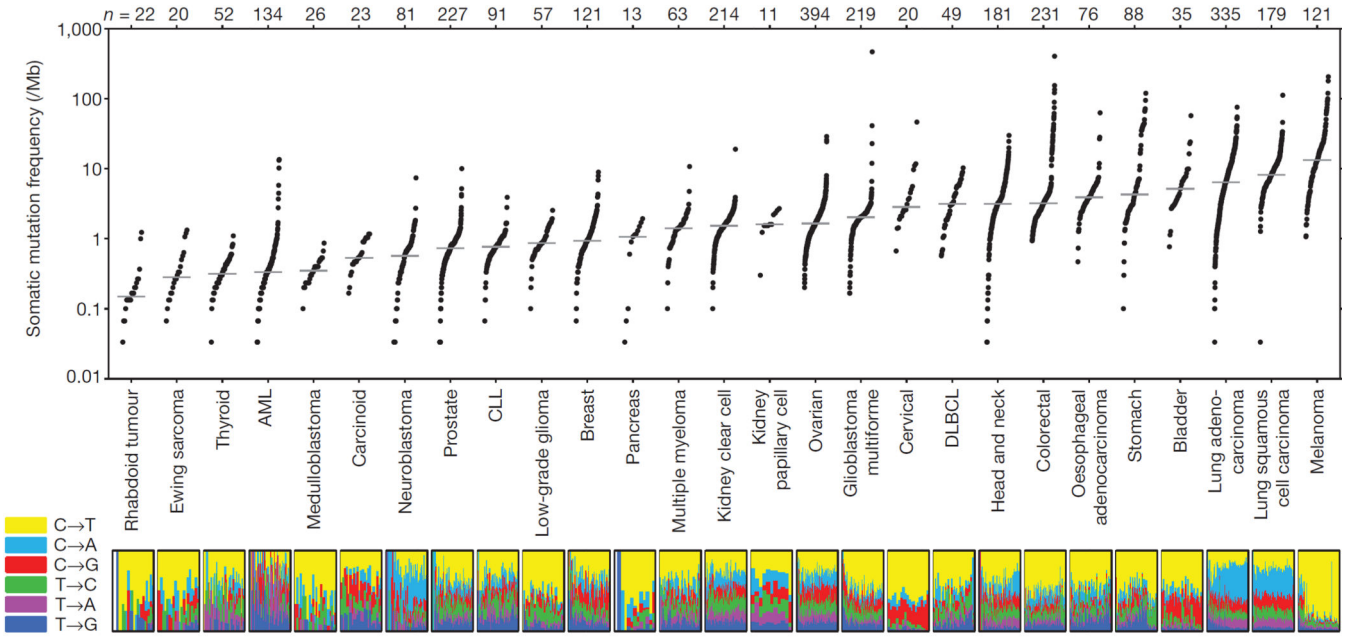

Figure 2. Strategies for integrative clinical tumor sequencing

(*Adapted from Roychowdhury, Science Translational Medicine 2011*, Figure 1C, 1D). This pilot study for clinical tumor sequencing demonstrated the feasibility and the need for multi-disciplinary collaboration. **A**, Shows a clinical relevant timeline that is dependent collaboration with oncologists, radiologists, pathologists, genetics labs, and bioinformaticians. **B**, Several strategies for sequencing tumor DNA and RNA can contribute to characterizing the landscape of alterations in an individual's cancer. Whole genome, whole exome, and transcriptome sequencing can be integrated to evaluate for point mutations, copy number alterations, gene fusions, and gene expression.

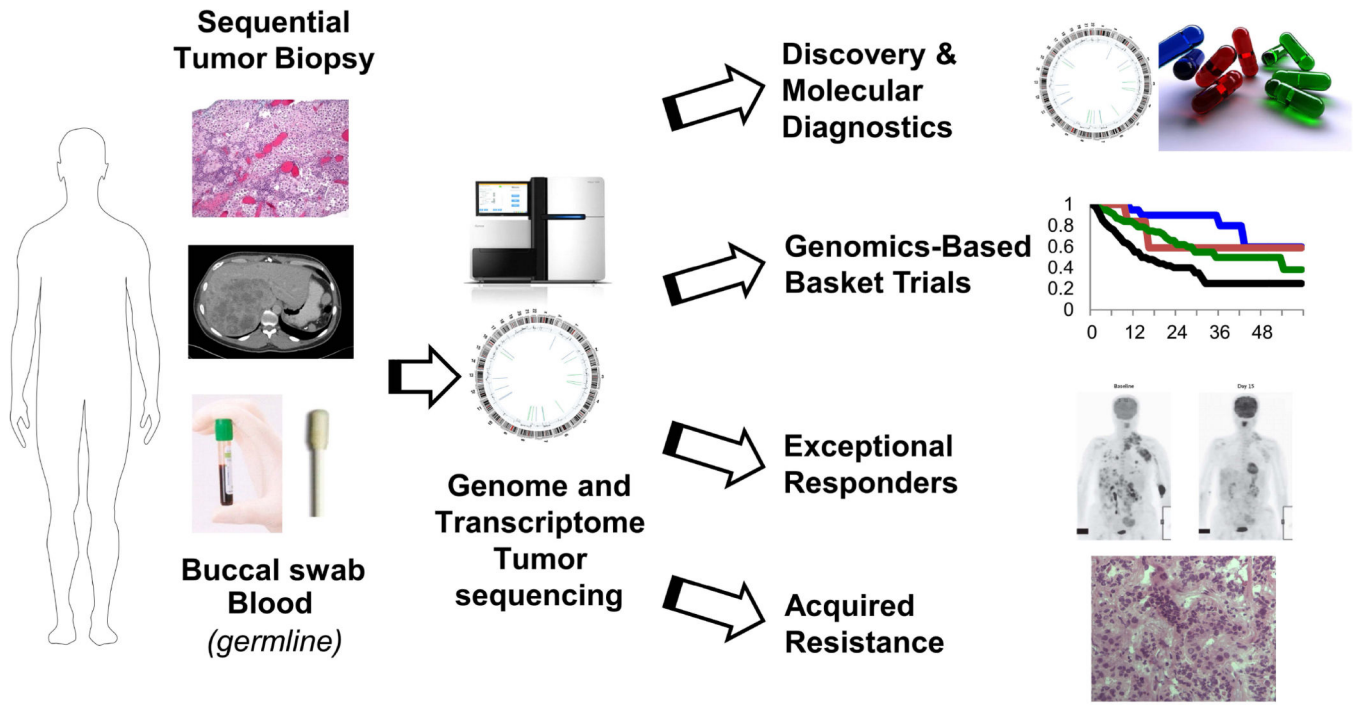

Figure 3. Research and clinical opportunities for precision cancer medicine through genomic and transcriptome sequencing

A systematic framework for implementing precision cancer medicine through genome and transcriptome sequencing can support multiple clinical and research efforts. Genomics can support the development of molecular diagnostics, drug target discovery, innovative genomics-based trials, evaluation of exceptional responders, and study of mechanisms of acquired resistance.

Table 1

Commercial Targeted DNA Pan-Cancer NGS Assays

Vendor	Assay Name	Number of genes	Results	Turnaround Time
Foundation Medicine	Foundation One	315	SNVs, CNVs, Fusions	12–14 days
University of Washington	UW-Oncoplex	234	SNVs, CNVs, Fusions	6 weeks
ParadigmDx	PCDx	114	SNVs, CNVs, Fusions	4–5 days
Washington University GPS	Solid Tumor Gene Set	48	Hot spot mutations, 6 fusions	3 weeks
ARUP Labs	Solid Tumor Mutation Panel	48	Hot spot mutations	14 days
Caris Life Sciences	MI Profile	46	Hot spot Mutations	14 days
Knight Diagnostic Labs	GeneTrails Solid Tumor Panel	37	Hot spots mutations	10–14 days

Abbreviations: SNV, single nucleotide variation or point mutation; CNV, copy number variation; NGS, next generation sequencing.

Table 2

Summary Points (Box)

- We have only a partial snapshot of the genomic landscape of cancer, and tens of thousands of patients with cancer must be profiled
- Cancer genome and transcriptome profiling have demonstrated clinically relevant impact on cancer biology
- Genome and transcriptome applications include diagnostic, prognostic, and predictive biomarkers
- Implementing precision cancer medicine will require multi-disciplinary collaborations to novel molecular diagnostics for cancer genomic testing in the clinic
- Clinical trials for precision cancer medicine will require an integrated network to coordinate tumor samples, clinical data, and offer access to novel therapies