Clinical Outcomes of *TP53* Mutations in Cancers

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High-throughput sequencing of cancer genomes is increasingly becoming an essential tool of clinical oncology that facilitates target identification and targeted therapy within the context of precision medicine. The cumulative profiles of somatic mutations in cancer yielded by comprehensive molecular studies also constitute a fingerprint of historical exposures to exogenous and endogenous mutagens, providing insight into cancer evolution and etiology. Mutational signatures that were first established by inspection of the *TP53* gene somatic landscape have now been confirmed and expanded by comprehensive sequencing studies. Further, the degree of granularity achieved by deep sequencing allows detection of low-abundance mutations with clinical relevance. In tumors, they represent the emergence of small aggressive clones; in normal tissues, they signal a mutagenic exposure related to cancer risk; and, in blood, they may soon become effective surveillance tools for diagnostic purposes and for monitoring of cancer prognosis and recurrence.

In the 5 years since our earlier review (Robles and Harris 2010), next-generation sequencing (NGS) has become the new standard for mutation testing that is rapidly being adopted in the clinic. In addition, the comprehensive characterization of human cancer genomes led by The Cancer Genome Atlas (TCGA) project (cancergenome.nih.gov) and the International Cancer Genome Consortium (ICGC) project (icgc.org) has ushered in the era of clinical genomics, in which molecular features are becoming an essential tool for cancer taxonomy. These studies have provided an unprecedented amount of information on *TP53* mutations and their association to important exposure and clinical variables. Herein, we provide an

update on the state of *TP53* mutation testing in the context of clinical genomics and explore recent advances in the field and emerging areas of application.

METHODOLOGIES USED IN ASSESSMENT OF p53 STATUS IN CLINICAL AND EPIDEMIOLOGICAL STUDIES

Sanger sequencing relies on the chain-termination method, which uses labeled termination nucleotides (ddNTPs) during DNA synthesis by DNA polymerase to generate DNA fragments varying by one base from each other and then separated by electrophoresis (Sanger et al. 1977). Variations and improvements on

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this technique were the gold standard for sequencing for >30 years after its development. Although much less used, Sanger sequencing still remains one of the most reliable, fail-proof, and simple methods to assess the nucleotide sequence in a given DNA sample. Even today, it is not uncommon for molecular alterations observed by NGS to be validated using Sanger sequencing (Jelinic et al. 2014; Streppel et al. 2014).

In the past decade, the cost and turnaround time (TAT) of NGS have decreased significantly, and the technology has matured to make it practical to use NGS platforms for gene mutation testing in routine clinical settings. NGS-based platforms use a massively parallel strategy capable of concurrently interrogating hundreds of thousands to millions nucleotide sequences of a given sample at the resolution of single molecules. The generated sequences are mapped to the reference genome and computationally summarized to reveal the quality of the read, type of the nucleotide, and the number of read evidence at each base position. Genomic status of *TP53* can be identified as part of whole-genome or whole-exome sequencing efforts such as those performed by TCGA (Kandoth et al. 2013). It can also be done through targeted approaches that involve either multiplex polymerase chain reaction (PCR)-based gene panels or hybrid capture of targeted genes followed by NGS sequencing, such as Life Technologies' AmpliSeq (Tsongalis et al. 2014), Foundation Medicine (Frampton et al. 2013), Personal Genome Diagnostics (PGDx, Baltimore, MD), and others. In clinical settings,

NGS-based *TP53* sequence analysis is usually done using targeted approaches because of the relatively lower cost and faster TAT for data generation and analysis. A comparison of these approaches is summarized in Table 1.

GERMLINE *TP53* MUTATIONS AND CANCER PREDISPOSITION

The cancer predisposition syndrome that would come to be known as Li–Fraumeni syndrome (LFS; OMIM #151623, omim.org) was initially identified among families with a high incidence of childhood cancers, particularly sarcomas, early-onset cancers, and multiple primary tumors (Li and Fraumeni 1969; see also the review by Guha and Malkin 2016). An epidemiological link between germline *TP53* mutations and LFS was made shortly after the discovery of p53, as it was realized that sporadic forms of the same types of cancers observed in patients with clinical features of LFS carried somatic inactivation of *TP53* (Malkin et al. 1990; Srivastava et al. 1990). Diagnostic testing for germline *TP53* mutations is based on criteria that take into account the presence of early-onset tumors within the LFS tumor spectrum as well as familial aggregation and multiplicity of cancers (Li et al. 1988; Kamihara et al. 2014). Recently, testing recommendations have been broadened to include women with early-onset breast cancer in the absence of family history or *BRCA1/BRCA2* mutations (McCuaig et al. 2012), as they would be suspected of carrying de novo *TP53* germline mutations (Gonzalez et al. 2009). In addition, germline *TP53* mutations

Table 1. Comparison of methods used to sequence *TP53*

Sequencing technology	Platform	Sample requirement	Data analysis	Turnaround time	In clinical use
Sanger	ABi 7300	FFPE, FF 10–100 ng	Easy	1–2 days	Yes
Targeted gene panels ^a	Illumina MiSeq, Ion Torrent, PacBio RS	FFPE compatible 10–500 ng	Moderate	1–2 weeks	Yes
Whole genome/exome	Illumina HiSeq 2000 or higher	FF (mostly), FFPE 0.5–1 µg	Complex	≥4–6 weeks	No

^aExamples of targeted gene panels are those provided by Foundation Medicine, Personal Genome Diagnostics, and Life Technologies' AmpliSeq.

FFPE, Formalin-fixed paraffin-embedded; FF, fresh-frozen.

have now been uncovered in patients who did not fulfill the clinical criteria for LFS testing, but presented with rare pediatric cancers including hypodiploid acute lymphoblastic leukemia (Holmfeldt et al. 2013), melanoma (Lu et al. 2015), gastric adenocarcinoma (Chang et al. 2013), early-onset colorectal cancer (Yurgelun et al. 2015), early-onset osteosarcoma (Mirabello et al. 2015), or multiple early-onset malignancies (Yamada et al. 2009; Chak et al. 2015). The wide application of NGS technologies will undoubtedly lead to more such findings and require continued reassessment of the role of *TP53* germline variants in cancer predisposition and the need for intense surveillance of mutation carriers and their families (Villani et al. 2011).

THE *TP53* MUTATION LANDSCAPE AS A FOOTPRINT OF THE EXPOSOME

The patterns and spectra of *TP53* mutations have long been recognized as molecular links to the exposome, a term coined by Christopher Wild to globally refer to environmental exposures (e.g., smoking and diet) and internal consequences (e.g., obesity and inflammation) during an individual's lifetime (Wild 2005). Genotoxic exposures to environmental carcinogens and endogenous mutagens promote specific sequence base changes that provide clues to the etiological origins of cancer (Hollstein et al. 1991; Hussain and Harris 1999; Olivier et al. 2010; Schetter and Harris 2012). *TP53* somatic mutations and the exposome were first linked with the discovery that dietary exposure to aflatoxin B1, a chemical carcinogen found in crops contaminated with the mold *Aspergilla flavus*, caused a specific G:C > T:A transversion at codon 249 of *TP53*, R249S, in hepatocellular carcinoma from geographical areas with endemic hepatitis B virus infection (Bressac et al. 1991; Hsu et al. 1991). Exposure to ultraviolet light from the sun was found to cause characteristic CC > TT tandem transitions in *TP53* in non-melanoma skin cancers (Brash et al. 1991). Similarly, cigarette smoke is molecularly linked to G:C > T:A transversions in *TP53* in lung cancers (Suzuki et al. 1992). Recently, exposure to

aristolochic acid was found to cause A:T > T:A transversions in *TP53* in urothelial carcinomas (Moriya et al. 2011; Chen et al. 2012). Signatures of these mutational processes that were first described by their characteristic footprint on *TP53* have now been confirmed and generalized by recent comprehensive sequencing studies of cancer genomes (Alexandrov et al. 2013; Lawrence et al. 2013; Poon et al. 2013; Schulze et al. 2015). Importantly, accumulation of somatic *TP53* mutations in histologically normal tissues can be readout for exposure to agents etiologically linked to cancer and serve as an early biomarker for risk (Hussain et al. 2000a,b, 2001). Examples of the powerful application of NGS are the identification of patients at high risk for developing cancer by the presence of low-abundance somatic *TP53* mutations in gastric mucosal tissues with *Helicobacter pylori*-induced gastritis (Shimizu et al. 2014), or in Barrett's esophagus tissues (Weaver et al. 2014).

CLINICAL IMPLICATIONS OF SOMATIC *TP53* MUTATION

Chromosomal losses involving chromosome 17p, where *TP53* resides, occur less frequently than deletions of other tumor-suppressor genes (Fig. 1) (Weinberg 1991). Instead, somatic alteration of *TP53* is most commonly characterized by recurrent nonsynonymous missense or nonsense mutations and small deletions along the entire gene (Fig. 2). For more than two decades, it has been known that somatic mutations in *TP53* occur frequently in human cancers arising from diverse tissue types (Nigro et al. 1989; Hollstein et al. 1991). NGS sequencing of cancer genomes revealed the rate of somatic *TP53* mutation to be even higher than previously thought, depending on tissue of origin (Kandoth et al. 2013). *TP53* is the most frequently mutated gene across 3281 tumors from 12 cancer types analyzed by unbiased high-throughput sequencing, showing sequence alterations in 42% of tumors (Kandoth et al. 2013). Overall, the frequency of *TP53* alterations varies widely, depending on tissue of origin and histological subtype (Fig. 3). Large-

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Figure 1. Frequency of mutation types found in the tumor-suppressor genes *TP53*, *PTEN*, *RBI*, and *APC*. Mutations are classified as substitution, deletion, insertion, or complex mutations. The “other” category contains all those mutations that fall outside the defined categories or for which there is no information on nucleotide changes. Color codes are indicated. Data was downloaded from COSMIC (cancer.sanger.ac.uk) (Forbes et al. 2015) and includes only tumor samples.

scale sequencing efforts have also brought renewed interest in the prognostic value of *TP53* mutations in the context of clinical genomics. These studies have confirmed the association of *TP53* mutations with more aggressive tumors and poor overall outcome in various cancer types (Cleary et al. 2013; Kandoth et al. 2013; Churi et al. 2014; Tirode et al. 2014; Moreira et al. 2015; Parry et al. 2015). The clinical value of *TP53* mutation is strongest in hematological malignancies. In these cancers, *TP53* mutations, although relatively infrequent overall (5%–15%), are correlated with abnormal karyotypes (including loss of 17p) and clearly associated with poor outcome (Zenz et al. 2010; Rucker et al. 2012; Kulasekararaj et al. 2013; Malcikova et al. 2014; Stengel et al. 2014; Parkin et al. 2015).

FUNCTIONAL CLASSIFICATION OF *TP53* MUTATIONS AND THEIR CLINICAL IMPACT

p53 is a transcription factor activated by cellular stress that regulates gene expression via sequence-specific recognition of a responsive element consisting of variations of a consensus comprised of two decamers (PuPuPuC(A/T)(T/A)GPyPyPy) separated by a variable-length spacer (el-Deiry et al. 1992). Missense substitutions affect p53's transcriptional activity to various degrees, depending on the specific location of the substitution, the amino-acid change, and the cellular context (Kato et al. 2003). These mutations can be broadly categorized on the basis of the type of molecular disruption and the biological consequences on the wild-type (WT) p53 protein. *TP53* mutations may lead to complete loss of tumor-suppressor properties (loss of function), enable mutated p53 to form a heterodimer with the remaining WT protein and block its activity (dominant-negative), or confer a conformational change on the protein that allows it to interact differently with its downstream effector proteins or bind entirely new DNA target sequences gain of function (GOF) (Oren and Rotter 2010; Bisio et al. 2014). A more detailed picture of the impact of *TP53* somatic mutations on the outcome of cancer patients has started to emerge through the systematic classification of missense *TP53* mutations based on DNA binding and transcriptional and oncogenic properties of mutant p53. In breast cancer, for example, it is well established that missense mutations affecting DNA binding are associated with worse patient survival than missense mutations outside of DNA-binding motifs (Olivier et al. 2006). It has since become apparent that the specific functional properties of p53 mutant proteins play a key role in conferring several tumor types with poor prognosis and therapeutic resistance (Poeta et al. 2007; Govindan and Weber 2014; Molina-Vila et al. 2014; Brachova et al. 2015). Crystallographic studies focused on the interaction of p53 and its cancer-associated mutants with DNA have led to a classification system that defines mutations occurring in the p53-DNA-binding surface as contact mutations

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Figure 2. Distribution of *TP53* mutations in cancer genomes analyzed by exome sequencing. Figure was generated by the cBio Cancer Genomics Portal (cbiportal.org) (Cerami et al. 2012). Mutation diagram circles are colored with respect to the most frequent mutation type at that position (green, missense; red, truncating; black, in-frame deletion (del)/insertion (ins); gray, splice-site; purple, different mutation types at the same proportion). P53_TAD, P53 transactivation motif (5–29); P53, P53 DNA-binding domain (95–289); P53_tetramer, P53 tetramerization motif (318–359).

Figure 3. Frequency of *TP53* mutations in 24 cancer genomes analyzed by The Cancer Genome Atlas (TCGA) using exome sequencing. The cBio Cancer Genomics Portal (cbiportal.org) (Cerami et al. 2012) was used to interrogate *TP53* mutation frequency in each tumor data set, except for esophageal carcinoma data, which was downloaded from TCGA Data Portal (tcga-data.nci.nih.gov).

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(e.g., those involving residues R273 and R248) and those that cause conformational instability of the p53 protein as structural mutations (e.g., those involving residues R175, G245, R249, and R282) (Joerger et al. 2006). It has recently been proposed that missense mutations resulting in oncogenic properties that are independent of WT p53 (such as those occurring at codons R248, R273, and R175) should be named “oncomorphic” mutations, to emphasize the fact that they lead to loss of WT function concomitant with gain of oncogenic function (Brachova et al. 2013). In patients diagnosed with advanced serous ovarian carcinoma oncomorphic, *TP53* mutations predict resistance to chemotherapy (Brachova et al. 2015). Another mutation classification system is based on the degree of perturbation they impose on p53’s transcriptional activity. Mutations are considered “disruptive” if they lead to complete or near complete loss of transcriptional activity, and “nondisruptive” if they do not (Poeta et al. 2007). Disruptive mutations include those that introduce a STOP codon, as well as certain mutants within DNA-binding domains that result in changes in polarity of amino-acid residues. Nondisruptive *TP53* mutations include some of the most frequent hotspot mutations, such as R175H, R273H, and R273C, and will often lead to p53 mutant proteins that show GOF properties (Molina-Vila et al. 2014). Disruptive and truncating mutations were associated with worse prognosis in patients with surgically resected squamous cell carcinoma of the head and neck (Poeta et al. 2007; Lindenberg-van der Plas et al. 2011; Skinner et al. 2012). To the contrary, nondisruptive *TP53* mutations were associated with shorter survival in patients with advanced non-small-cell lung cancer (Molina-Vila et al. 2014).

Synonymous or silent mutations (those that do not lead to a change in the coded amino acid) have generally been overlooked because they were perceived as being neutral. However, it has long been suspected that silent *TP53* mutations may alter splicing patterns (Hongyo et al. 1995; Lamolle et al. 2006). A large genomic study of matched cancer exomes and RNA sequencing has recently confirmed that *TP53* is unique among tumor-suppressor genes in that

recurrent synonymous mutations inactivate splice sites (Supek et al. 2014). This finding could be clinically relevant, as instances of silent *TP53* mutations associated with poor survival have been reported in the literature (Sturm et al. 2003). Moreover, the patterns of p53 protein isoform expression, as further detailed below, may also be altered by mutations that affect splicing.

***TP53* ISOFORM EXPRESSION AND CANCER OUTCOME**

The vast majority of studies describing abnormal p53 function in cancers have so far focused on its main full-length isoform (i.e., p53FL, p53 α , TAp53 α). However, *TP53* encodes at least 13 different isoforms through alternative promoter usage, alternative splicing, and alternative translation start sites (Bourdon 2007; Marcel et al. 2010; Senturk et al. 2014). Evidence indicating that patterns of isoform expression can have prognostic value is starting to surface. So far, these studies have focused on quantifying p53 isoforms at the mRNA level. Expression of p53 γ , but not p53 β , in breast cancer patients bearing *TP53* mutant tumors resulted in overall survival that was similar to that of patients with *TP53* WT tumors (Bourdon et al. 2011), whereas a recent study showed that expression of the p53 β but not the p53 γ isoform was protective in patients bearing *TP53* mutant tumors (Avery-Kiejda et al. 2014). Expression of the $\Delta 40$ p53 isoform has been associated with triple-negative breast cancer, an aggressive breast cancer subtype (Avery-Kiejda et al. 2014), and mucinous ovarian cancer, but it conferred a favorable prognosis in the latter (Hofstetter et al. 2012). Studies that combine transcriptomic and sequencing analysis will enable the generation of hypothesis regarding the interplay between p53 isoforms and *TP53* mutations. Silent mutations may affect the ratio at which protein isoforms are expressed, and truncated variants may affect the function of mutant p53FL. An additional level of complexity is found at the protein level. $\Delta 133$ p53 protein isoforms are regulated by selective autophagy (Horikawa et al. 2014); thus, evaluating mRNA alone is insufficient to fully

characterize the expression of p53 isoforms and their prognostic value.

EMERGING AREAS FOR CLINICAL APPLICATIONS OF *TP53* MUTATION TESTING

Cancer Stem Cells

Recent reports have revealed that p53 regulates stem-cell homeostasis and pluripotency. WT p53 negatively regulates proliferation and self-renewal of neural (Meletis et al. 2006) and hematopoietic (Liu et al. 2009) stem cells. In mice, loss of p53 leads to expansion of the mammary stem-cell pool, through increased frequency of symmetric cell divisions (Cicalese et al. 2009). Moreover, p53 suppresses somatic cell reprogramming and its inactivation results in enhanced efficiency of induced pluripotent stem (iPS) formation (Hong et al. 2009; Kawamura et al. 2009; Marion et al. 2009). Mutant p53 can further enhance somatic cell reprogramming; however, iPS cells generated in the presence of mutant p53 give rise to malignant tumors (Sarig et al. 2010). Surprisingly, mutant p53 did not affect the quality of reprogramming of fibroblasts from LFS patients (Lee et al. 2015). However, the R175H GOF mutant promoted the expansion of mammary stem cells and cancer initiation (Lu et al. 2013). Thus, mutant p53 appears capable of tipping the balance between pluripotency and tumor formation (Rivlin et al. 2015). Consistently, the presence of *TP53* mutations correlates with stem-cell-like gene-expression patterns in diverse types of cancer (Mizuno et al. 2010; Villanueva and Hoshida 2011; Woo et al. 2011; Schwede et al. 2013). The hypothesis that mutant p53 or a compromised p53 network can promote the development of dedifferentiated cells with self-renewal properties (also known as “cancer stem cells”) has clinical implications. Cancer stem cells are conceptually at the top of the hierarchy within a tumor and bear the capacity for sustaining tumor growth, and regenerating tumor heterogeneity. Cancer stem cells can be resistant to therapies that reduce tumor bulk, and are, therefore, responsible for micrometastatic disease

and local recurrence. Their existence challenges the current paradigm of cancer treatment by implying that the fundamental test of most therapies, that is, their ability to reduce tumor size, may be inadequate unless it is ensured that the subpopulation of cells within a tumor with self-renewal properties is completely eradicated. Knowing that tumors bearing mutated p53 may be rich in cancer stem cells may inform treatment modalities so that they are aimed at eradicating such subpopulations of cells.

Liquid Biopsies

Another emerging area of opportunity for clinical application is the evaluation of *TP53* mutations in tumor components shed into the bloodstream (also known as “liquid biopsies”). Examples of this application include targeted deep sequencing of *TP53* for longitudinal analysis of circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), or circulating tumor cells (CTCs) to monitor cancer recurrence (Forsheve et al. 2012; Dawson et al. 2013; Fernandez et al. 2014; Rothe et al. 2014; Hamakawa et al. 2015). Recently, Bettegowda et al. (2014) showed that digital PCR can detect tumor-related mutations in the plasma of patients with multiple different types of cancer suggesting that ctDNA is potentially a sensitive and specific biomarker that can be used for a variety of clinical and research purposes, including the determination of clinically relevant mutations. This comprehensive study that included >600 patient samples also established two important caveats for this analysis: that abundance of ctDNA is determined by tumor type (i.e., some tumors “shed” more DNA into the bloodstream) and tumor burden (i.e., size and stage), and that ctDNA can be found in samples without detectable CTCs.

Intratumor Evolution and Heterogeneity

Systematic characterization of intratumor heterogeneity through deep sequencing of multiple regions of a tumor has revealed a dynamic process of clonal evolution that affects therapeutic response, metastatic spread, and, ultimately, patient outcome (Burrell et al. 2013). Although

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intratumor heterogeneity of *TP53* mutations and p53 staining has long been appreciated, these studies have shed new light on the role of p53 alterations for shaping a tumor's subclonal architecture. The association of *TP53* mutations with clonal diversity is of particular interest in chronic lymphocytic leukemia (CLL), as p53 alterations have unambiguously been linked with adverse outcome of this disease (Malcikova et al. 2014). Sequencing of samples collected longitudinally through cycles of chemotherapy revealed that chemotherapy resulted in the expansion of clones with driver mutations (including *TP53* mutations) and this expansion can hasten disease progression (Landau et al. 2013; Rossi et al. 2014; Malcikova et al. 2015). This finding has important implications for clinical management of CLL patients, as the sensitivity afforded by NGS allows detection of small subclones bearing *TP53* mutations in pretreatment samples, possibly identifying patients at high risk for clonal expansion and acquisition of therapeutic resistance.

***TP53* AT THE CROSSROADS OF CLINICAL GENOMICS AND CANCER THERAPY**

The advent of comprehensive genomic characterization through NGS has brought to the forefront the often complex and idiosyncratic genetic changes associated with cancer development. In the clinical setting, these alterations provide new opportunities to help physicians select more appropriate drugs or ongoing clinical trials for a given patient. This approach is aligned with the oncology "precision medicine" initiative recently put forward by the National Institutes of Health (Collins and Varmus 2015). Precision oncology trials, most notably the just-launched NCI-MATCH (cancer.gov/nci-match) trial (McNeil 2015), aim to evaluate the extent to which genomics-based treatments will be able to improve patient performance. However, the large set of data associated with NGS-based reports is generally very lengthy, inconclusive, and not readily understandable by clinicians not intimately familiar with the technology and the rapidly evolving field. In response to these challenges, Genomic Tumor Boards that bring to-

gether an interdisciplinary group of experts, including internists, oncologists, surgeons, biostatisticians, radiologists, pathologists, clinical geneticists, basic and translational science researchers, and bioinformaticians, convene to discuss the intricacies of tumor genetics and tailor a personalized treatment plan for patients who often have advanced cancer or have exhausted standard therapies. Being one of the most commonly altered genes in cancer, *TP53* mutations are frequently observed in advanced tumors. Their presence is discussed in the context of other molecular changes for determining a potentially more effective treatment strategy. Not infrequently, a recommendation can be made when there is an appropriate and effective drug for a given genomic alteration, such as *EGFR* gene mutations or *ALK* fusions in lung cancer, or *BRAF* V600E in melanoma (Kelleher et al. 2012). However, obstacles abound because of the experimental nature of the clinical trials, the lack of a clear targetable drug for the vast majority of mutations found through large-scale sequencing, and the absence of strong evidence that a particular treatment might actually be effective for an individual patient. *TP53* mutations, in particular, are not currently "clinically actionable" (Meric-Bernstam et al. 2015); thus, making use of the molecular genetic information, including the tumor status of *TP53*, to effectively improve patient care remains a challenge in clinical settings. In the not-too-distant future, however, the presence of *TP53* mutations may indeed inform targeted therapeutic approaches. Therapeutic strategies aimed at restoring p53 pathway function are showing promising results in clinical trials (refer to the literature for further details). These include approaches to disrupt the interaction of p53 with MDM2 (Khoo et al. 2014), replace its key downstream microRNA target miR-34 (Bader 2012), or directly restore function to mutant p53 (Lehmann et al. 2012).

Analogous to sensitivity tests for antibiotics routinely used in clinical practice for management of infections and for tumors without a targetable driver mutation, it would be ideal if the individual tumor being profiled for genomic alterations could also be rapidly expanded

ex vivo to enable functional screenings against a panel of all available antitumor drugs before being recommended for the patient. These patient-derived tumor xenografts (PDXs) are currently a valuable resource for study (Tentler et al. 2012) and increasingly performing as predictors of response to targeted therapy (Stewart et al. 2015). Because *TP53* is ubiquitously altered in cancers, *TP53* mutations are a tool to confirm the provenance of PDX (Park et al. 2013; Walters et al. 2013; Dodbiba et al. 2015). Moreover, selection pressures that lead to expansion of aggressive clones on implantation of primary tumors appear to replicate metastat-

ic colonization such that *TP53* mutations found in PDX can serve as a beacon to track the origin of metastases back to minor subclones present in the primary tumors (Bousquet et al. 2015). Additional resources and further development are needed before PDX can become an integral part of a cancer patient's clinical care as a platform to help define therapies based on the molecular make-up of an individual tumor.

CONCLUDING REMARKS

The precision medicine strategy includes four basic premises (Fig. 4) (National Research

Figure 4. A Precision Medicine research strategy. As outlined in the 2011 Institute of Medicine's National Research Council report "Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease" (National Research Council 2011), Precision Medicine starts with the creation of an Information Commons that interactively houses multiple "-omics" data types (genomics, epigenomics, transcriptomics, metabolomics, proteomics) along with historical exposure and lifestyle information from individual patients. Bioinformatic integration of these data will lead to the development of a Knowledge Network that will be used to improve disease taxonomy, the application of clinical medicine and the study of molecular mechanisms of disease. An iterative process of acquiring information in individuals or cohorts of patients, making improvements in taxonomy and using that knowledge to care for patients and design new studies that further feed the Information Commons will refine the molecular taxonomic classifiers and improve clinical medicine.

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Council 2011). First, the “Information Commons” for each cancer type has to be populated with a variety of “-omic” (exposome, genome, epigenome, transcriptome, metabolome, microbiome) analyses, as well as clinical information and epidemiological data from individual patients. Second, these data are integrated into a “Knowledge Network” that examines the interconnectivity of each layer of data from the Information Commons. Third, this knowledge network is used to develop new “Taxonomic Classifiers” with the goal of improving patient diagnosis, decisions on therapeutic strategies, and cancer-related health outcomes. Finally, this knowledge is used to guide biomedical, prevention, and clinical research to perform relevant mechanistic and observational studies. These data are used to validate previous observations in new cohorts to ensure the integrity of the Knowledge Network and to make decisions about what new data should be added to the information commons to further improve and refine the molecular taxonomic classifiers representing the intrinsic biological distinctions. p53 and its functions contribute to the “Hallmarks of Cancer” (Hanahan and Weinberg 2011), as discussed in other articles in this collection, and can be analyzed within the frame-

work of “omics” Precision Medicine to improve disease taxonomy and medical care of individual patients, and guide disease prevention and biomedical research. As shown in Figure 5, *TP53* mutations can potentially affect, and be modulated by, multiple OMICS in the Precision Medicine paradigm. Within the “exposome,” *TP53* somatic mutations can be linked to dietary and environmental exposures (e.g., aflatoxin B1, cigarette smoke, etc., as described earlier). *TP53* mutations with less-understood etiology comprise most of the common somatic mutations found in the “genome” of human cancers. Some of these have characteristic GOF phenotypes and can predict poor patient prognosis (Brosh and Rotter 2009). In addition, *TP53* bears common germline polymorphisms that affect certain biochemical properties of the protein (Whibley et al. 2009). As a transcription factor activated by cellular stress, p53 regulates the expression of an ever-growing list of protein-coding and non-coding mRNAs in the “transcriptome,” mainly involved in cell-cycle checkpoint, DNA repair, senescence, and apoptosis (Li et al. 2012). Mutant p53 can also manifest GOF phenotypes through unique transcriptional effects (Oren and Rotter 2010). p53 also modulates the “epigenome,” via up-regulation of the miR-34 fam-

Figure 5. p53 and its functions affect multiple layers of “-omics” data in the Precision Medicine paradigm.

ily of microRNAs (Bommer et al. 2007; Chang et al. 2007; Corney et al. 2007; He et al. 2007; Hermeking 2007; Raver-Shapira et al. 2007; Tarasov et al. 2007; Tazawa et al. 2007). The role of p53 in tumor metabolism is central to the “metabolome” (Berkers et al. 2013) (see also the review by Humpton and Vousden 2016). Mutant p53 promotes metabolic changes that support tumor growth including enhanced tumor lipid metabolism (Freed-Pastor et al. 2012) and glucose uptake (Zhang et al. 2013). Emerging evidence indicates that compromised p53 function also affects the tumor “microbiome” by reducing the mucosal barrier that prevents bacterial infiltration (Schwitalla et al. 2013). Conceivably, the presence of mutant p53 could modulate the extent, and even the type of microbial changes observed in tumors. With this in mind, it should be clinically useful to consider *TP53* mutations in the context of each of the other layers of information available for an individual patient, whether or not acquired as part of a comprehensive screen.

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