Precision oncology in the age of integrative genomics

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Abstract

Precision oncology applies genomic and other molecular analyses of tumor biopsies to improve the diagnosis and treatment of cancers. In addition to identifying therapeutic options, precision oncology tracks the response of a tumor to an intervention at the molecular level and detects drug resistance and the mechanisms by which it occurs. Integrative genomics can include sequencing specific panels of genes, exomes, or the entire triad of the patient's germline and tumor exome plus tumor transcriptome. Although the capabilities of sequencing technologies continue to improve, widespread adoption of genomics-driven precision oncology in the clinic has been held back by logistical, regulatory, financial, and ethical considerations. Nevertheless, integrative clinical sequencing programs applied at the point of care have the potential to improve the clinical management of cancer patients.

The earliest documented examples of targeting the underlying mechanisms driving tumor growth to treat cancer might be George Beatson's treatment of breast cancer patients by oophorectomy in 1896[1], and Charles Huggins use of castration to treat prostate cancer half a century later[2]. Although the mechanisms that underlie cancer have been investigated for more than a hundred years, clinical management remains rooted in morphological and histopathological methods to diagnose and estimate prognosis, while treatments rely on surgery to remove tumors followed by chemo- and/or radiation therapy to stop uncontrolled cell proliferation[3,4].

Insights gained from the molecular characterization of aberrant genes, cell surface markers, hormonal/endocrine mediators, and signaling pathways associated with cancer have been incorporated into diagnostic and treatment strategies (Figure 1). The application of targeted

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therapies matched to specific aberrations for some cancers[5–15], synthetic lethal targeting of DNA repair machinery in *BRCA*-deficient ovarian cancers[5], and recent progress with immune checkpoint inhibitors in cancers with hypermutation/neo-antigen signatures[6] have collectively fueled optimism that identification of molecular targets in individual cancers to enable targeted therapeutics could represent a general paradigm for cancer care. This optimism has been tempered by inconsistent responses to targeted therapies and emergence of drug resistance in many patients.

Over the past decade, multiple large-scale genomic studies have identified genomic, transcriptomic, and proteomic aberrations that are specific to one cancer type or common among different cancers. These findings have suggested to many researchers that clinical management of individual cancer patients should be routinely informed by comprehensive molecular analyses of their tumors. Fortunately, policy and funding have kept pace with science, as exemplified by the Precision Medicine[7,8, 9] and National Cancer Moonshot Initiatives[10].

Precision medicine initiatives are poised to transform the paradigm of population-based clinical studies to define treatments for *average* patients into biomarker-driven clinical trials to identify the best treatments for *individual* patients [24,11]. Initial clinical efforts have mainly focused on sequencing panels of well-validated therapeutic target genes, and have gradually expanded to include broader panels of cancer-associated genes. Less frequently, whole exome sequencing or comprehensive, integrative sequencing encompassing germline, genomic, and transcriptomic sequencing have been performed (Figure 2).

We review the application of precision oncology by sequencing gene panels, the use of whole exome capture or genome sequencing, as well as RNA sequencing, in clinical trials and routine clinical practice. We also review analytical setup and operational workflow of current integrative clinical sequencing programs. This is followed by a brief discussion of salient issues and directions for future developments.

Sequencing of gene panels for precision therapeutics

Specific aberrations in approximately 40 different cancer genes are represented in FDA (Food and Drug Administration)-approved targeted therapeutics and detected by FDA-mandated diagnostic assays that use cytogenetics, PCR, microarrays, or Sanger sequencing to detect mutations. High-throughput sequencing can, in principle, detect all of these mutations with sufficient sensitivity, and, thus, sequencing of panels of therapeutically targetable genes has emerged as an entry point for precision oncology [12, 13]. For example, in a clinical trial that featured sequencing of 10 cancer genes in lung adenocarcinoma tissues from 733 patients, one or more oncogenic mutations were identified in 466 patients (64%), and 24 patients (3%) had mutations in two or more genes[14]. 260 patients received targeted therapy and achieved median survival of 3.5 years, compared with 2.4 years for 318 patients that did not receive matched therapy[15].

Seizing the opportunity for immediate impact on patient care, the National Cancer Institute (NCI) initiated several clinical trials to test the application of biomarker-driven therapeutic

approaches[16]. These trials include the Lung Master Protocol (Lung-MAP, S1400), which aims to target therapies for lung squamous cell carcinoma (SCC). SCC has no approved targeted therapies, and the frequency of actionable somatic aberrations in these cancers is so low (5%–20%) that traditional clinical trials are impractical[17]. In the Lung-MAP, NGS (next-generation sequencing) is used to identify actionable molecular abnormalities, and patients are randomized to targeted therapy or standard of care. Another example is the Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial (ALCHEMIST) in which early-stage lung cancer patients are screened for *EGFR* or *ALK* mutations by sequencing[18, 19]. In the Molecular Analysis for Therapy Choice (NCI-MATCH) clinical trial, biopsies of adult solid tumors and lymphomas are sequenced to screen for mutations in a panel of defined actionable genes, and patients are matched with either approved or investigational (Phase II) drugs[20–23]. The NCI-MATCH trial received such an enthusiastic response upon launch that enrollment had to be paused from January to April/May 2016 to allow for expansion of lab capacity, as well as addition of more than a dozen new treatment arms[24]. Similar trials were initiated for advanced solid tumors (NCI-MPACT; NCT01827384), pancreatic cancer (IMPaCT trial in Australia[25]), and thoracic malignancies (CUSTOM trial, NCT01306045[40, 41]), all involving sequencing of select target genes to be matched with precision therapies.

Sequencing extended panels of cancer genes

Following promising studies to identify hotspot mutations and single-nucleotide variants (SNVs) in specific genes, extended panels have been incorporated into recent analyses to detect SNVs, copy number variants (CNVs), structural rearrangements, and gene fusions. For example, the Memorial Sloan Kettering (MSK)-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) project used targeted sequencing of exons and selected introns of 341 cancer genes[26] and was expanded to 410 genes in a follow-up study[27]. Likewise, Foundation Medicine sequenced exomes of 287 cancer-related genes plus intronic sequences from 19 genes involved in rearrangements or other aberrations[28]; later, this was expanded to 315 exomes, plus intronic sequences from 28 genes. Perhaps one of the largest such efforts is the University of Michigan's MI_Oncoseq program, where exomes for a panel of 1700 cancer-related genes are captured for parallel sequencing of tumor and germline DNA[29]. Sequencing gene panels helps identify a broad range of cancer-associated aberrations but retains the advantages of cost effectiveness, fast output, and the use of limiting amounts of starting material, all preferred attributes for the application of NGS in clinical practice. Hand-in-hand with increasing participation of patients and cancer centers, more patients are being matched with therapeutics that are targeted for specific molecular aberrations but not yet approved for cancer treatment by the FDA. The American Society of Clinical Oncology (ASCO) has capitalized on these findings by launching a Targeted Agent and Profiling Utilization Registry (TAPUR) study (https://www.tapur.org/), which is a non-randomized clinical trial that will formally test the potential utility for off-label targeted therapies in cancer.

Exome or genome sequencing for individuals

The data from gene panel sequencing are indeed limited by the selection of genes in the panel but may also be limiting in chromosomal ploidy aberrations, arm level gains/losses, and unknown/new "cancer genes". Whole exome capture sequencing analyzes the complete coding portion of the genome and provides a comprehensive genomic profile of aberrations in protein coding genes, arguably at relatively reduced coverage at individual loci; it is also costlier, takes longer, and is more resource and analysis intensive than using a limited panel of sequences.

Whole exome or genome sequencing has been particularly informative for analyzing exceptional therapeutic response or resistance. For example, a metastatic bladder cancer patient who showed an exceptional response to the mTOR inhibitor everolimus in a clinical trial categorized as "failed", NCT00805129, showed loss of function mutations in *TSC1* and *NF2*. These genes are associated with mTOR pathway activity but were not previously associated with therapeutic response[30]. Similar mutations were identified in additional bladder cancer cases, who may also potentially respond to everolimus. Similarly, activating mutations in *mTOR* were identified in an exceptional responder to everolimus and pazopanib[31]; mutation in *RAD50* associated with loss of *ATM* signaling[32] was found in an exceptional responder to treatment with *CHK1* inhibitor in combination with DNA-damaging agent irinotecan; and an exceptional response to an *IGF-1R*-specific antibody was observed in a patient with *ALK* fusion-positive lung cancer[33].

The underlying mechanisms for drug sensitivity or resistance are not always straightforward. For example, a pre-treatment tumor sample from a patient with stage IVA head and neck squamous cell carcinoma who showed a near complete histologic response to erlotinib revealed no *EGFR* alterations as expected; instead, the tumor harbored an activating mutation in *MAPK1* (p.E322K) that enhanced *EGFR* phosphorylation, resulting in erlotinib sensitivity[34]. Further, sequencing of *BRAF*-mutant colorectal cancer biopsies pre- and post-treatment with *RAF* inhibitors, identified *KRAS* amplification and overexpression in one patient, *BRAF* amplification and overexpression in another case, and a putatively activating mutation in the RAF family protein, *ARAF* p.Q489L, plus a resistance mutation in *MAP2K1* p.F53L in a third patient[35]. In a study to investigate markers associated with resistance to PD-1 immune checkpoint blockade in metastatic melanoma, whole exome sequencing identified loss of function mutations in *JAK1, JAK2,* or *B2M*[36].

Remarkably, a review of 10 years of unpublished data from phase II clinical trials by the NCI Cancer Therapy Evaluation Program has estimated that as many as 10% of patients were "exceptional responders" in phase II clinical trials of therapies that failed to receive FDA approval[37]. NCI launched the Exceptional Responder Program in 2014 to systematically re-analyze these trials with the aim of identifying new combinations of aberrations and therapeutics[37–39]. These analyses will require comprehensive whole exome/genome analyses.

Despite evidence of utility, clinical sequencing programs have been deterred from application of whole exome or whole genome sequencing in routine clinical settings owing

to the extra time, cost, resources, data storage, and analysis requirements. Instead, most sequencing centers sequence targeted panels that can be gradually expanded to incorporate additional genes/sequences of interest.

Precision oncology in routine clinical practice

Sequencing-based clinical precision oncology programs have only recently been implemented and only a few have reported results so far. Among these representative programs, the University of California San Diego (UCSD) Moores Cancer Center reported findings from the Profile Related Evidence Determining Individualized Cancer Therapy trial (PREDICT-UCSD; NCT02478931), wherein 347 patients with advanced solid malignancies were analyzed using Foundation Medicine exon capture panels; of these, 87 patients (25%) were treated with a matched therapy and had a slightly longer median progression-free survival compared with unmatched patients[40]. Mentioned above, the MSK-IMPACT[27] project described an exome capture-based 410 gene panel assay for solid cancers (with matched germline samples), wherein more than 10,000 patients with advanced cancer have been analyzed so far, with up to 11% of patients enrolled in genomically matched clinical trials[29]. The MSKCC study prioritizes the use of targeted gene panels over whole exome sequencing to maximize throughput and depth of coverage, and, additionally, to reduce costs. The precision oncology program at the MD Anderson Cancer Center[41] reported sequencing results from 1200 patients with advanced cancer, in which targeted sequencing of 201 genes or hotspot mutation analysis of 11 to 50 genes were performed. At least one alteration in a potentially actionable gene was noted in 945 patients (79%) using the larger panel, compared to only 527 patients (44%) with hotspot testing, supporting the use of large panels in routine clinical tests[42]. Weill Cornell Medical College–New York applied whole exome sequencing in routine clinical practice, analyzing tumor-normal pairs of 97 metastatic cancer cases, with informative aberrations observed in 91 patients, of whom 5 went on to receive targeted therapies[43]. These early reports are indicative of the immense interest in the community to deploy precision oncology in routine cancer care, even as questions of cost, choice of optimal analytical platforms, standardization of the assays and reporting metrics, and potential efficacy of these efforts continue to be deliberated.

Integrative clinical sequencing in precision oncology

Combining exome sequencing of germline and tumor tissue DNA with RNA sequencing can interrogate a wide array of somatic and germline aberrations in parallel (Figure 2) and has been effective in discovering actionable aberrations in osteosarcoma[44], urothelial carcinoma[45], non-small cell lung cancer (NSCLC)[46], endometrioid endometrial carcinoma (EEC)[47], melanoma[48, 49], and a case of Sézary syndrome[50].

The University of Michigan has implemented an integrative clinical sequencing program called MI_Oncoseq[51] for all-comer advanced cancer patients with diverse tumor types in a hospital/academic setting. Briefly, as shown in Figure 3, participation in clinical sequencing is initiated by the attending physician. Following written informed consent from the patient, tumor biopsy and blood or buccal swab are used to extract DNA and RNA, which are sequenced and analyzed. These data are analyzed for potential clinical relevance and

actionability through extensive literature survey of the disease, tumor specific aberrations, and potential therapeutic matches (BOX 1). Finally, the integrative molecular analyses are summarized and any therapeutic insights discussed at a multidisciplinary tumor board meeting attended by the referring physician and various key personnel involved in the entire MI_Oncoseq process. A final summary clinical report with clinical recommendations is provided for the referring physician. From start to finish, this process takes three weeks in most cases.

Similar to the multidisciplinary cancer conferences ("tumor boards") that are currently mandated by the American College of Surgeons to facilitate access to expertise in evolving technologies in accredited cancer programs[52, 53], integrative precision oncology programs have implemented multidisciplinary molecular tumor boards to discuss molecular findings and make clinical recommendations[66,77–81]. MI_Oncoseq has implemented a multidisciplinary precision medicine tumor board (PMTB) comprising oncologists, cancer geneticists, genetic counsellors, pathologists, biologists, bioinformaticians, bioethicists, clinical study coordinators, and *ad hoc* expertise[51, 54]. Underscoring the vital importance of tumor boards in driving clinical sequencing efforts, and the need for standardized practices, ASCO is considering the development of a web-based, interactive molecular tumor board for educational purposes[55].

At MI_Oncoseq PMTB meetings, individual cases are presented with clinical history, family history, tumor pathology and histopathology of specimens used for sequencing, summary details of sequencing libraries, quality control metrics of sequencing data, and estimation of tumor content based on the proportion of copy neutral heterozygous SNVs in the data [see BOX 1 for details of these components of MI_Oncoseq PMTB meetings]. This is followed by a detailed assessment of germline and cancer aberrations, potential clinical implications thereof, and proposed follow-up action items, all topics expanded upon in the next sections. Clinical coordinators then continue to track the clinical course of patients in consultation with the clinicians.

Germline DNA sequencing of cancer patients

Sequencing of the cancer patient's germline DNA in parallel with tumor DNA has typically been undertaken to filter out germline polymorphisms from the somatic mutation data. However, identification of germline mutations in cancer predisposing genes has critical implications for the patient and their families, prompting active screening, surveillance, prophylactic actions, and preventative lifestyle adjustments, as well as can be informative with regard to treatment plans (Table 1). For example, germline aberrations in DNA repair pathway genes *BRCA1/2*, as well as *ATM*, *CHEK2*, and *PALB2*, have been associated with responsiveness to PARP inhibitor therapies in ovarian, breast, and prostate cancers[56–60]. Similarly, germline mutations in mismatch repair pathways have been associated with responsiveness to immune blockade therapy[61].

Furthermore, several recent cancer sequencing studies have observed a high frequency of germline mutations in cancer pre-disposition genes among sporadic cases with no family history of cancer, as suggested by a recent analysis of SNP (single-nucleotide

polymorphism) array-based GWAS (genome-wide association studies)[62]. Also, among 4,034 TCGA (The Cancer Genome Atlas) cancer cases representing 12 cancer types, rare germline truncations were noted in 114 cancer-susceptibility-associated genes, spanning 4% of acute myeloid leukemia (AML), 11% of stomach cancer, and up to 19% of ovarian cancer cases[63]. Not surprisingly, the germline mutation burden is high among pediatric cancer cases, where about 10% of the cases had notable germline findings in two studies[54, 64]. Incidentally, pathogenic germline mutations have been noted in a significant proportion of sporadic pediatric cancer cases[64]. Focusing on germline variants, the LCCC1108/UNCseq_ (NCT01457196) study involving 439 pediatric and adult cancer patients unselected for hereditary cancer predisposition identified 4.3% of the patients with pathogenic germline variants[65]. In addition to these pan-cancer studies, up to 11.8% of advanced prostate cancer cases were found to harbor pathogenic germline alterations[66,67], with a significantly higher rate of germline mutations observed in metastatic cases compared to patients with localized tumors[101]. The frequent germline mutations observed in sporadic cancer patients argues for germline sequencing to be included as an integral part of routine clinical sequencing workflows, not restricted to patients with family history of cancer.

A list of clinically actionable germline variants commonly identified can be found in Table 1, and Supplementary Table 1A details current clinical trials centered on germline aberrations. In MI_Oncoseq workflow, germline variants referenced as pathogenic in ClinVar are reviewed by a clinical geneticist for implications for disclosure to the patient/ family. Additionally, integration of the germline and somatic sequencing data helps define mutations showing loss of heterozygosity (LOH) in the tumor that may be missed if only the tumor was sequenced.

Copy number aberrations (CNAs):

Exome capture data from paired tumor and normal DNA is used to determine exome-wide somatic copy number aberrations by comparing the depth of coverage at all of the individual exons analyzed followed by segmentation analysis along the lines used for array cGH data, creating high resolution copy number profiles, while circumventing technical variations[68]. The genome-wide copy number profile at exon level resolution is plotted in a visually intuitive, color-coded linear chromogram to evaluate a variety of copy number aberrations, including focal or wider amplifications/deletions/copy losses or gains. Interestingly, in addition to identifying susceptibilities to classical therapies, aneuploidy and the burden of copy number loss have been associated with responsiveness to immunotherapy[69, 70]. These findings add another layer of potentially actionable information available from CNV analyses, and one that might be missed in highly-selective targeted gene panels. Analysis of data from cancer samples with low tumor content and extreme ploidy changes, however, continues to present analytical challenges still awaiting satisfactory resolution.

Somatic SNVs/indels:

Analysis of somatic mutations in cancers, many of which define canonical driver aberrations and therapeutic targets, likely represents the most emphasized output of clinical sequencing (Supplementary Table 1B). Pairwise analysis of tumor DNA samples compared with germline sequencing data helps distinguish germline polymorphisms from somatic mutation

calls. At MI_Oncoseq, we also determine the variant allele fraction (the ratio of variant/reference reads), zygosity mutations, total number of somatic mutations, and mutation burden (number of mutations/Mb) (considered in the context of the published range of cohort specific mutation numbers in TCGA data[71–73] and similar data from our compendium of more than 1,700 advanced cancer cases[29]).

To identify functionally relevant variants, hotspot, activating, or loss of function mutations based on recurrence in the COSMIC (Catalogue of Somatic Mutations in Cancer) database, as well as stop/gain SNVs or frameshifting insertions/deletions, are highlighted. Mutations close to hot-spots or involving functionally critical domains are also noted. Published literature on key mutations is reviewed manually with special attention given to therapeutic, prognostic, diagnostic, or mechanistic associations.

The level of expression of mutant genes often provides additional supportive evidence for the likely effect of the mutations. For example, splicing mutations show intron retention (e.g. *CBL, NF1, ATM, TP53* etc.) or exon skipping (e.g. *MET*), and in-frame expression of large indels (e.g. *NOTCH1, FOXA1, EGFRvIII* or its variants) can occur. Additionally, a locus sometimes shows chromosomal gain or amplification but no corresponding increase in expression levels of resident genes.

Mutational signatures:

Analysis of the patterns of somatic aberrations in cancers has emerged as a source of clinically-actionable insights. Distinct patterns of genome-wide mutations in tri-nucleotide units observed in genome/exome sequencing data from diverse cancers have helped define signatures of somatic mutations characteristic of different tumor types, defective DNA recombination/repair pathways, and those that provide insights into the mechanism of carcinogenesis through external exposures such as UV radiation, tobacco, or alkylating chemotherapeutics like temozolomide[71–76] (http://cancer.sanger.ac.uk/cosmic/signatures). Some hyper-mutated cancers, such as UV-induced malignant melanoma, have shown dramatic responsiveness to immunotherapies, associated with expression of neo-antigens by the cancer cells as a result of their increased mutational load[77–82]. Additionally, cases with a microsatellite instability (MSI) signature, typically but not always accompanied with loss of function mutations in mismatch repair (MMR) genes[83], have been associated with responsiveness to immune checkpoint inhibitor therapy[61, 84]. Along similar lines, the signature of homologous repair deficiency (HRD)[72] typically associated with mutations in *BRCA1, BRCA2,* and other fanconi anemia pathway genes[85], is also observed in some sporadic cancers said to display "BRCAness"[86, 87]. Similar to *BRCA* mutation carriers, cancers displaying BRCAness have been associated with responsiveness to platinum-based therapies as well as PARP inhibitors[88–93].

In addition to informing therapeutic avenues, mutational signatures of individual cases also help corroborate or qualify challenging diagnoses (e.g. a cancer of unknown primary showing distinct smoking signature characteristic of lung cancer[71]) or glean insights into specific mechanisms of tumor progression (APOBEC signature[94, 95], signature of temozolomide treatment, etc.). Notably, whole exome or larger exome capture panels are better suited for mutation signature analyses; for more selective targeted panels, it may be

useful to incorporate targeted probes to query for therapeutically informative signatures such as MSI and BRCAness.

Precision immunotherapy:

In recent years, a number of different immunotherapy approaches have shown promise in the clinic. Immune checkpoint blockade targeting CD28/CTLA4 or PD-1/PD-L1 has emerged as a promising therapeutic approach across diverse cancers[71, 102–105]. However, as only small subsets of patients benefit from the treatment, genomic or transcriptomic markers to predict response in genomes or transcriptomes are highly sought after (Figure 2B).

Neo-antigen peptide vaccines based on individual cancer mutanomes (all mutant protein coding sequences identified by high-throughput sequencing) have shown efficacy in protecting and treating the tumor in xenograft models[78, 96] and are being tested in several ongoing clinical trials (for example, NCT02287428, NCT02950766, NCT01970358).

Adoptive cell therapy uses ex vivo expanded tumor-infiltrating lymphocytes (TILs), based on identification of an immunogenic neo-antigen showing high affinity binding to the patient's MHC antigen[97, 98]. Unfortunately, the excitement of promising responses to immunotherapy across several cancer types is tempered by a relatively small percentage of patients achieving dramatic, durable responses and multiple modes of primary or acquired resistance[99].

Several markers of sensitivity, response, and resistance to the various immunotherapies have been identified, including the level of tumor neoantigens[17,140,141, 142], tumor genomic aberrations, gene expressions, profiles of TILs, and T-cell receptor (TCR) diversity[100]. Gene expression analysis tools like CIBERSORT[101] and TIMER[102] help define the profile of TILs from tumor RNA-seq data.

Transcriptome sequencing in the clinic

Many clinical sequencing workflows currently do not involve RNA sequencing of the tumor samples, possibly due to additional requirements of technical and analytical bandwidth as well as cost and time constraints. However, we and others have observed that a parallel analysis of genomic and RNA-seq data helps to identify expressed gene-fusions (including inactivating rearrangements involving tumor suppressors) and splicing aberrations and enables the detection of pathogenic viruses. Expression signatures of tumor biomarkers can help confirm/corroborate tumor diagnoses, and in cases of tumors of unknown primary origin, help predict the likely tissue/lineage of origin. Gene expression profiles also help assess the functional status of critical pathways. For example, expression levels of androgen receptor (AR) pathway genes, like *ACPP*, *KLK2/3*, *SLC45A3*, and *TMPRSS2*, help assess the status of AR pathway regulation in prostate cancer samples, irrespective of the status of *AR* gene or level of *AR* transcript. Similarly, specific mutations in cancer-associated pathway genes, like those of the *NOTCH*, *WNT-beta-catenin*, *SHH*, and *HIPPO* pathways, can be assessed for functional consequences in terms of expression levels of their downstream target genes. As mentioned in the previous section, RNA sequencing data has also found application in defining the expressed mutanome of cancer samples to nominate

candidate neo-antigens for immunotherapy[78, 81, 103]. In an interesting analysis, Newman et al. [101] have defined gene expression patterns corresponding to various cell types comprising cancer tissues, including tumor infiltrating immune cells, providing a powerful tool to assess the immune reactive status of different tumors. A fortuitous application of RNA-seq is also in highly sensitive and specific detection of cancer virus/pathogens in tumor tissues, such as human papilloma virus (HPV16/18), human herpesvirus-4 (EBV), human T-lymphotropic virus (HTLV), and merkel cell polyoma virus, which is important for the application of immunotherapy and cancer virus vaccines. These diverse observations afforded by RNA-seq provide critical diagnostic and therapeutic insights that are not available with DNA sequencing alone[104, 105].

Gene fusions:

A wide variety of gene fusions serve as diagnostic and prognostic biomarkers, as well as therapeutic targets, for several types of cancer[106, 107]. RNA-seq data is particularly useful in not only identifying gene fusions, but also providing an assessment of expression levels of the fusion transcripts. In MI_Oncoseq, we have identified *ETV6-ABL1*[54], *NAB2-STAT6*[108], and various *FGFR* gene fusions[109] using RNA-seq. Detection of chimeric RNAs involving tumor suppressor genes, showing loss of open reading frame/functional domains, although a relatively underexplored area of investigation, is another clinically-informative application of RNA sequencing data analyses[107]. Apart from gene fusions, RNA-seq can provide evidence of alternative splicing aberrations (*AR-V7* in prostate cancer[110, 111]), novel isoforms with therapeutic implications (*ALK* alternative transcription initiation, ATI[112]), or exon skipping events, including exon 14 skipping in *MET* reported in subsets of lung cancer[113, 114].

Gene expression analyses:

The RNA-seq data from tumor samples are assessed for expression of tumor type specific biomarkers/cell surface biomarkers, as well as additional biomarkers that are often part of the routine clinical work-up of patient samples (tested by immunohistochemistry, qRT-PCR, etc.). For instance, a readout of *ESR1/PGR* and *ERBB2* expression data can confirm or qualify immunohistochemistry status of ER/PR/HER2 in breast cancer samples; similarly, RNA-seq expression of *AR, KLK3, SLC45A3, ACPP, AMACR, TMPRSS2*, and *ERG* provides an informative readout of the status of AR signaling and/or ERG fusion status in prostate cancers.

The biomarker analysis is particularly useful in cases of diagnostically-challenging specimens, as well as advanced cancer cases with unknown primary tissue of origin[115–119]. Besides expression of tissue specific biomarkers, we nominate the tissue type of tumors of unknown origin using a machine learning algorithm[29], using a bootstrap aggregation of six different prediction models trained on RNA-seq data from 33 primary tumor types in TCGA and normal tissue expression data obtained from GTEX, TCGA, and the Human Proteome Atlas, based on a modification of a method by Vincent et. al[120].

Finally, some therapeutic target genes show exceedingly high outlier expression in certain samples, with or without an observed genomic aberration, and may represent therapeutic

avenues not derived from obvious genomic aberrations. For example, outlier expressions of *MET* in a case of esophageal carcinoma, *ROS1* in a non-small cell lung cancer, and *RET* in a neuroendocrine carcinoma of the larynx were found to represent potential therapeutic targets in our study[29].

Clinical report enables actionable recommendations

All of the potentially actionable or informative molecular aberrations in a patient's tumor or germline discussed in the PMTB are summarized and submitted to the attending physician with specific recommendations relating to the individual cases. While the essential report format is similar across different tumor sequencing programs, specific details vary based on types of analyses. Collectively, all of the different aspects of the germline, somatic, and/or expression data are represented among the different cases analyzed (examples in Table 3), highlighting the critical importance of integrative analyses, as singular focus on exome sequencing would likely miss many of the actionable observations revealed through integration. A formal comparison of the different modalities may be moot to consider for future programs.

Outlook for clinical sequencing in cancer

Currently, clinical sequencing programs largely focus on exome capture sequencing instead of sequencing the whole genome. However, it is increasingly apparent that recurrent aberrations in non-exonic regions of the genome, including promoters, enhancers, other regulatory elements, protein/RNA binding sites, intergenic loci of lncRNAs, and miRNA, need to be reconciled for a fuller assessment of cancer genomic aberrations. The recent discovery of hotspot mutations in the *TERT* promoter that lead to aberrant reactivation of telomerase was based on sequencing of a genomic locus defined by GWAS studies of familial melanoma[130] as well as whole genome sequencing of melanoma samples[131]. This was followed by its detection in urothelial carcinoma[132], brain cancer[133, 134], and thyroid cancer[135] using amplicon sequencing or targeted *TERT* promoter sequencing[136]. Fortuitously, the *TERT* promoter mutation hotspots happen to be located just upstream of the first exon and are, thus, captured by whole exome sequencing[137–139]. This example highlights the realm of somatic aberrations located outside the regions typically included in exon capture panels that are not being actively targeted by exome capture. Indeed, using whole genome sequencing data from TCGA and elsewhere, recurrent mutations in upstream regulatory elements have been described in *DPH3*, *PLEKHS1*, *WDR74*, and *SDHD*[140, 141].

Further highlighting the importance of gene regulation in cancer, sequencing efforts have revealed numerous aberrations in chromatin-related genes[142–148] across diverse tumor types. As an example, almost all cases of pediatric malignant rhabdoid tumors are characterized by the singular loss of SWI/SNF chromatic remodeling complex gene *SMARCB1*[142, 144, 149–151]. Similarly, almost 60% of bladder tumors show mutations in epigenetic modifiers[152, 153], and a majority of pediatric diffuse intrinsic pontine gliomas harbor mutations in histone *H3A/H3B*[154–157]. Therapeutic approaches targeting epigenomic aberrations have primarily included DNA demethylation (DNMTase) inhibitors such as azacytidine, which is FDA-approved for use in myelodysplastic syndromes, and histone

deacetylation (HDAC) inhibitors such as vorinostat (SAHA) and panobinostat, which are FDA-approved for cutaneous T-cell lymphoma and multiple myeloma, respectively. In this context, it is expected that high-throughput epigenomic profiling integrated with clinical sequencing will illuminate a mechanistic understanding of the molecular ramifications of aberrant DNA modification pathways in cancer to help inform wider application of precision therapeutic approaches targeting other epigenomic aberrations[158, 159]. Integration of methylome sequencing to profile epigenomic aberrations and proteomics will help further expand the field of actionable cancer aberrations.

Another exciting area of development with potential for immediate clinical impact is sequencing of minimally invasive "liquid biopsies", including blood, cerebrospinal fluid, or urine from cancer patients. Analysis of circulating tumor cells (CTCs), exosomes, or cell free DNA/RNA (ctDNA/RNA) transcends the issues of sampling bias, tumor heterogeneity, and metastases not amenable to biopsy, and can help assess disease progression, response to therapy, emergence of resistance, or new therapeutic targets[160–162]. Marking a tangible advance in this arena, the FDA has recently approved detection of *EGFR* mutations in the ctDNA from blood of lung cancer patients as a companion diagnostic assay for erlotinib treatment[163]. The next frontier may be sensitive and robust detection of panels of "hotspot" aberrations in liquid biopsies[164, 165].

Apart from genomic analyses, integration of gene-expression signatures with genes and small molecules[166], metabolomic assessments[167], and proteomic interactome maps[168] represent areas of future development. However, functional characterization and translation of these data to inform clinical decisions could be more challenging than matching somatic aberrations with therapies.

Evaluation of workflows for integrative precision oncology

Cancer is a long-term disease which means that sequencing a tumor once (current practice) provides only a snapshot of a dynamic process. As sequencing becomes routine, sequencing of tumor biopsies at diagnosis, resection, progression, and after therapy will help generate a more complete picture of cancer development. Common examples of treatment-emergent alterations that could be detected by sequencing include the acquisition of mutations in the ligand binding domain of *ESR1* following aromatase inhibitor therapies in breast cancer, *AR* amplification and mutations in prostate cancers following endocrine deprivation therapy, and mutations in receptor tyrosine kinases following treatment with TKIs. It is also important to determine if multiple targeting avenues are potentially available at an early stage in cancer. Eventually, clinical sequencing could supplant individual gene centric assays. First, we need evidence that sequencing provides a more sensitive and reliable detection modality than FDA-approved diagnostics. It is feasible that sequencing could serve as a primary diagnostic modality, along with histopathology and radiographic imaging.

Currently, the reported turnaround time for clinical sequencing analyses ranges from two to six weeks. Turnaround time is two weeks at Foundation Medicine, a month or less at MSKCC for the MSK-IMPACT study[27] and Clinical Genomics Program, Taussig Cancer Institute, and Cleveland Clinic[169], and between ten days to six weeks for the University of

Michigan MI_Oncoseq[54] study. This time frame may need to be further shortened to one to two weeks for routine clinical application.

Determining the efficacy of integrative pipelines is confounded by the fact that typical patients availing of clinical sequencing, such as at MI_Oncoseq, present with late stage, advanced disease who have received, and often failed, multiple therapies, have maximal mutational burden including therapy resistance mutations, and more or less arrived at a therapeutic *cul de sac*. In this setting, despite identification of compelling therapeutic leads, the patient's physical condition often makes them ineligible for trials or incapable of tolerating treatment. Unfortunately, in a number of cases, within a month of providing samples for analysis, patients moved to hospice care, were lost to follow up, or died.

Several unforeseen circumstantial contingencies can also mitigate potential benefits from the findings. Patients enrolled for a clinical trial following a specific therapeutic indication after sequencing analysis may get placed on the control arm of the study, denying them opportunity to benefit from the specific information about their cancer; a number of such cases occurred in the gene fusion study at the University of Michigan, UMCC 2012.022. Having varying eligibilities for clinical trials across different institutions is also problematic; for example, activating mutations in *PIK3CA* are common in breast cancer, but patients displaying hot-spot activating mutation in *PIK3CA* being treated at the University of Michigan cannot enroll in the ongoing PI3Ki SIGNATURE trial, as it excludes breast and prostate cancer. The need to negotiate with pharma and insurance companies to consider sequencing results as rationale for providing drugs for off-label use on compassionate grounds is also a constant hurdle.

Unlike the rigorous assessment that novel drugs or therapeutics are subjected to, precision oncology is fairly new and empirical evidence of its effectiveness remains equivocal. A systematic, multicenter randomized, controlled phase 2 trial (SHIVA; NCT01771458) directly comparing the efficacy of off-label molecularly-targeted therapies based on tumor molecular profiling with conventional therapy, observed no significant improvement in progression-free survival in the targeted therapy group in a cohort of heavily pre-treated cancer patients[12, 170]. Elsewhere, the NCI initiated comparative effectiveness research (CER) to systematically assess the efficacy of cancer genomics and precision medicine. Based on early findings of seven research studies and a follow-up workshop, they reported "insufficient evidence of clinical utility of precision medicine in translating genomic discoveries into clinical practice"[171]. Representing a skeptical position on the efficacy of precision oncology, the hematologist–oncologist Vinay Prasad at Oregon Health and Science University recently weighed the rather few reports of exceptional responses to targeted therapies against a preponderance of failed attempts. Given the paucity of randomized clinical trials formally testing the metrics of success, the very premise and promise of precision oncology was questioned[172, 173]. On a more positive note, in a recent prospective clinical trial to evaluate the clinical benefit of high-throughput genomic analyses (MOSCATO 01), actionable molecular alterations were identified in up to 48% of the cases analyzed (411 of 843 patients), of which 199 patients could be treated with a matched targeted therapy[174]. 7% of the successfully screened patients were assessed as having

benefited from this approach in terms of progression-free survival on matched therapy as compared to prior therapy.

In our opinion, the metrics of the utility of precision oncology should be considered in the context of adding value to the standard of care, not apart from it. Much of what is the standard of care is already part of 'precision oncology', including all the diagnostic/ prognostic markers and targeted therapies matched with specific aberrations currently in use. The latest high-throughput methodologies only help to scale up and expedite the assays over a broader range of cancers, providing access to molecular information that encompasses our collective knowledgebase. In this sense, the current forays in precision oncology would help generate an integrative knowledgebase of clinical, molecular, and therapeutic aspects of cancers that could usher in the next phase in the quest for a cancer cure.

Clinical sequencing data co-operatives

As numerous institutional efforts in precision oncology have grown, many initiatives to harness the information from collective datasets are underway. To formally test the suitability and efficacy of off-label use of targeted therapeutics, ASCO has launched a clinical trial (TAPUR) that will use genomic profiling data to match and test the utility of molecularly targeted cancer drugs outside the indications approved by the FDA and generate a registry of effective off-label usage[20]. In a different approach, AACR has launched the Project Genomics Evidence Neoplasia Information Exchange (GENIE), wherein seven independent clinical sequencing programs will pool their collective clinical, sequencing, treatment, and follow-up data to populate a public data repository reference. A joint research program undertaken by the National Human Genome Research Institute (NHGRI) and the NCI, called Clinical Sequencing Exploratory Research (CSER), is coordinating several research programs to help define optimal use and implementation of clinical sequencing tests[175, 176]. Under this conglomerate initiative, diverse issues such as considerations for validation of NGS variants[177], reporting germline findings[178–180], diagnostic yield of tumor sequencing data[181], classification of variants[182], incorporation of sequencing data in electronic health records[183], genetic counselling[184], and social and behavioral research[185] are explored. Another co-operative effort is exemplified by the Oncology Research Information Exchange Network (ORIEN), comprising 11 US-based cancer centers sharing clinical, molecular, and therapy related data to help match patients with appropriate clinical trials based on their molecular profile. Industry is also participating; Medical Evidence Development Consortium (Med-C), a non-profit organization floated by Genentech, Roche, and Eli Lilly, plans to develop uniform, standardized work flows for matching cancer mutations with targeted therapies, intuitive to clinicians and insurance companies. Similar initiatives are mooted in the international setting through the Clinical Cancer Genome Task Team of the Global Alliance for Genomics and Health[186], as well as European data centers[187]

Precision FDA was launched on December 15, 2015 to provide a private workspace in a public setting to make precision oncology studies available to users without access to big sequencing facilities (https://precision.fda.gov/). Users will have access to Genome in a Bottle, reference DNA for validating human genome sequences developed by the National

Institute of Standards and Technology. Users will also be able to compare their results to previously validated reference results and share their results with other users, track changes, and obtain feedback. See BOX 2 and Table 2 for more details on these and other resources.

Conclusion

The incorporation of clinical sequencing analyses in oncology represents the culmination of a long-standing quest to systematically link tumor specific molecular aberrations with mechanistically-targeted therapies to inform individual patient treatment. It is envisaged that widespread access to the high-resolution molecular data on individual cancer cases, along with attendant clinical data, therapy details, and follow-up information, should help close the gaps in our understanding of cancer progression and pave the way for improved cancer treatments, as well as anticipate and overcome resistance to drugs. A sobering disclaimer is due at this stage; we are not there yet.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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BOX 1

Precision Medicine Tumor Board

The actionable germline and somatic findings along with clinical implications and therapeutic options for individual cases are discussed in the context of the following background information. Please refer to the main text of this article for detailed discussion of the clinical data analyses.

Clinical History:

Brief clinical histories of the patients, from the time of cancer diagnosis, key clinical signposts, prior therapies, responses, status of imaging, histological and/or molecular markers, history of response to therapies, disease progression etc. leading up to the details of biopsy of the clinical sequencing, are noted.

Family History:

Incidences of cancer in the family of patients are documented by a clinical geneticist to assess each case as likely familial or sporadic. Detailed in the main text, in a subset of cases, pathogenic germline variants are noted irrespective of a family history of cancer.

Pathology:

Hematoxylin and eosin stained sections of the tumor biopsy specimens to be used for sequencing are assessed by a pathologist for histopathology of the tumor specimen as well as estimation of tumor content. Tissue blocks adjudged to represent the highest tumor content are used for making sequencing libraries.

Samples for Sequencing Libraries:

DNA and RNA are simultaneously isolated from the same tumor tissue sections to ensure concordance of the samples used for genomic and transcriptomic analyses. DNA for germline sequencing is typically derived from blood samples in the case of solid cancers and from buccal swabs for hematological malignancies. Although frozen tumor samples are preferred starting material for sequencing DNA or RNA, recent technical and analytical improvements have facilitated routine use of formalin fixed-paraffin embedded (FFPE) samples for clinical sequencing[28, 54, 66, 188–190]. Quality of RNA sequencing is particularly sensitive to the integrity of the starting RNA material. To improve the information content of RNA-seq using suboptimal quality RNA samples, we have developed a transcriptome capture methodology using standard exome capture probes[191].

QC of Sequencing Data:

Based on the guidelines proposed by the Standardization of Clinical Testing (Nex-StoCT) workgroup[192], quality metrics of the sequence data are assessed before launching into mutation/gene expression analyses. Exome capture data from tumor samples with approximately 400X average coverage, matched with normal (blood or buccal) samples with approximately 300X average coverage, are considered optimal for analyses. The tumor content is estimated using a set of high quality SNV candidates on 2-copy genomic regions[54]. Sequencing quality is determined by a number of standardized criteria[29], and

sequencing libraries failing any of the quality metrics are flagged and factored during biological analysis and interpretation of the data.

BOX 2.

Community resources and data repositories serving precision oncology

High-throughput, data-intensive applications of precision oncology have produced a plethora of databases, repositories, and online portals, catering to various distinct niches that are extensively utilized in clinical sequencing data analyses (Table 2). These include repositories of primary sequencing data, such as SRA (Short Reads Archive) and dbGAP, the database hosted by NCBI that stores and provides high-throughput genomic/ transcriptomic/methylome and other data relating to genotype and phenotype in humans. Public databases of germline variations, such as 1000 Genomes Project and ExAC, provide very useful compendia of genetic polymorphism in the human population. Of these, the 1000 Genomes Project, concluded in 2015[193, 194], provides a comprehensive reference of common human genomic variations compiled from 2,504 individuals representing 26 distinct populations worldwide. An even more expansive public resource called the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/) has aggregated exome sequencing data from multiple large-scale sequencing projects (including the 1000 Genome Study), spanning a total of 60,706 unrelated individuals from various disease-specific and population genetic studies. Reference databases with curated, annotated information on pathogenic germline aberrations associated with cancer include OMIM[195], Leiden Open Variation Database (LoVD)[196, 197], and NCBI ClinVar[198]. Primary cancer sequencing data repositories include The Cancer Genome Atlas (TCGA), International Cancer Genomics Consortium (ICGC), and University of California Santa Cruz (UCSC) Cancer Genomics Browser. These provide valuable references for assessing recurrence of rare somatic variants, estimation of tumor type specific mutation burden, mutation signature analyses, and comparisons of gene expression, among other applications. Data visualization portals include cBioportal for TCGA data and UCSC Xena Browser for data across multiple consortia. There is a compendium of somatic aberrations in cancer (COSMIC), as well as databases providing multidimensional assessment of somatic mutations, including the Turnkey Variant Analysis Project (TVAP) of National Human Genome Research Institute (NHGRI) that provides multiple popular open source bioinformatics tools for detection, interpretation, and visualization of high-throughput sequencing data, and database of curated mutations (DoCM). Finally, to explore models of "community" sharing of collective data repositories, the Genomic Data Commons (GDC) program of NCI and Project Genomics Evidence Neoplasia Information Exchange (GENIE) launched by AACR aim to foster unified data repositories that enable data sharing, analyses, and clinical interpretations across cancer genomic studies.
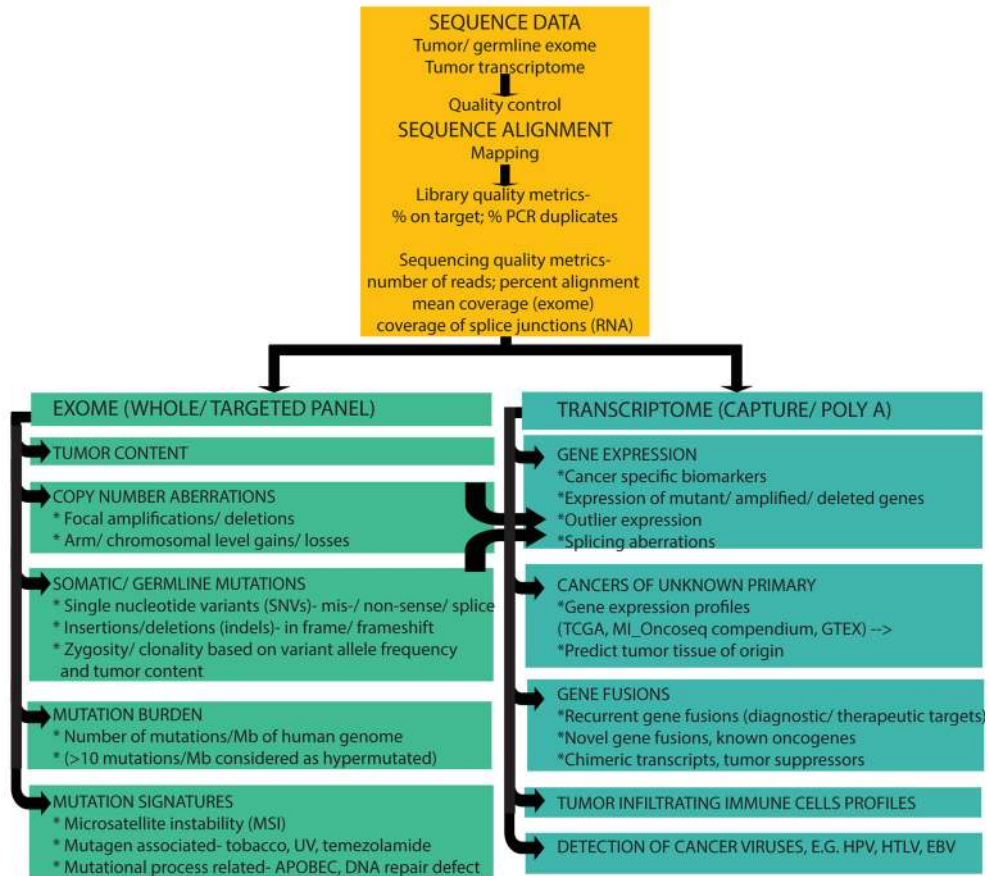
Year	Landmarks in the application of analytical tools to inform cancer diagnosis, prognosis, and therapy*
1847	**Microscopy** Formal description of "Leukemia" by Rudolf Virchow[1,2]
1941	**Cytopathology** Hematoxylin and Eosin (H&E) staining of Papanicolaou-smear, cervical cancer[3,4]
1956	*Improved karyotyping: accurate determination of human chromosome numbers[5,6]*
1960s	**Cytogenetics** Philadelphia chromosome, chronic myeloid leukemia (CML)[7] **Electron microscopy** Epstein Barr Virus (EBV) associated with Burkitt's lymphoma[8]
1970s	**Chromosome banding** Recurrent translocations in hematological malignancies[9-15] **Radioimmunoassay** Carcinoembryogenic antigen (CEA), colorectal cancer[16,17] *DNA sequencing[18-20], molecular cloning[21]*
1980s	**Chromosome banding** Recurrent translocations in sarcomas/ soft tissue tumors[22-25] **Radioactive probe hybridizations** Detection of BCR-ABL1, CML[26]; IgH-BCL2, B-Cell lymphoma[27]; TcR-MYC, T-cell leukemia[28]; human papilloma virus (HPV) in cervical cancer[29] **Fluorescence in situ hybridization (FISH)[30,31]** ERBB2 in breast cancer[32] **Flow cytometry** Acute promyelocytic leukemia (APML)[33], neuroblastoma[34], myelodysplastic syndrome (MDS)[35], multiple myeloma[36] *Oncogenes and tumor suppressors: identification and characterization eg. RAS, MYC, RB1[37-39]* **Radioimmunoassay** Estrogen receptor[40], prostate specific antigen[41] **Immunohistochemistry** Estrogen receptor[40,42], ERBB2[43,44] *Invention of PCR[45]* **Reverse transcriptase PCR (RT-PCR)** BCR-ABL1 in CML[46], PML-RARA in APML[47], AML1/ETO in AML (acute myeloid leukemia)[48]
1990s	*Human Genome Project[49,50]* **Positron emission tomography (PET), computed tomography (CT)[51-53]** **Microarray profiling for high-throughput genomic and transcriptomic profiling of cancers[54]** Expression profiles of cancers[55,56], diffuse large B-cell lymphoma (DLBCL) subtypes[57], breast cancer prognosis[58], hereditary breast cancer[59], biomarkers of prostate cancer[60], lung cancer[61], gene fusions in prostate cancer[62]
2000s	**PCR amplification and sequencing of "cancer genes"[63] from tumor specimens** Genomic landscapes of somatic aberrations in different cancers- breast, colorectal, pancreatic[64-67] **Massively parallel high-throughput/ next-generation sequencing[68-70]** *TCGA- The Cancer Genome Atlas[71-75], https://cancergenome.nih.gov/* Various modalities of precision oncology projects in research, clinical, and clinical trial settings discussed in this review *Precision Medicine Initiative[76-78]* *Cancer Breakthroughs 2020 (formerly, Cancer Moonshot), http://www.cancerbreakthroughs2020.org/*

* citations in Supplemental References

Figure 1. The progression of analytical tools in oncology.
The historical timeline highlights key developments in the assessment of cancer cells/tumor tissue, starting from the microscopic description of leukemia cells by Rudolf Virchow, up to the futuristic Cancer Breakthroughs 2020 project. It may be noted that the modern tool-kit for cancer analyses includes a range of old and new tools, and the high-throughput sequencing approaches add a highly informative component, complementary to other methods that include imaging, histopathology, and biochemical analyses.

A. Exome and transcriptome data analysis

SEQUENCE DATA
Tumor/ germline exome
Tumor transcriptome

Quality control
SEQUENCE ALIGNMENT
Mapping

Library quality metrics-
% on target; % PCR duplicates

Sequencing quality metrics-
number of reads; percent alignment
mean coverage (exome)
coverage of splice junctions (RNA)

EXOME (WHOLE/ TARGETED PANEL)

TUMOR CONTENT

COPY NUMBER ABERRATIONS
* Focal amplifications/ deletions
* Arm/ chromosomal level gains/ losses

SOMATIC/ GERMLINE MUTATIONS
* Single nucleotide variants (SNVs)- mis-/ non-sense/ splice
* Insertions/deletions (indels)- in frame/ frameshift
* Zygosity/ clonality based on variant allele frequency
  and tumor content

MUTATION BURDEN
* Number of mutations/Mb of human genome
* (>10 mutations/Mb considered as hypermutated)

MUTATION SIGNATURES
* Microsatellite instability (MSI)
* Mutagen associated- tobacco, UV, temezolamide
* Mutational process related- APOBEC, DNA repair defect

TRANSCRIPTOME (CAPTURE/ POLY A)

GENE EXPRESSION
*Cancer specific biomarkers
*Expression of mutant/ amplified/ deleted genes
*Outlier expression
*Splicing aberrations

CANCERS OF UNKNOWN PRIMARY
*Gene expression profiles
(TCGA, MI_Oncoseq compendium, GTEX) -->
*Predict tumor tissue of origin

GENE FUSIONS
*Recurrent gene fusions (diagnostic/ therapeutic targets)
*Novel gene fusions, known oncogenes
*Chimeric transcripts, tumor suppressors

TUMOR INFILTRATING IMMUNE CELLS PROFILES

DETECTION OF CANCER VIRUSES, E.G. HPV, HTLV, EBV

B. Prediction and validation of neo-antigens for immunotherapy

TUMOR MUTANOME   PATIENT HLA TYPE
9-mer neo-epitopes   PHLAT/ HLA reporter
(SNVs, indels, gene fusions)

NEO-EPITOPE-MHC BINDING AFFINITIES, IN SILICO PREDICTION
IEDB consensus algorithm (IC$_{50}$<500nM)

Mutant/wild type MHC binding IC$_{50}$ (fold change)

NEO-EPITOPE EXPRESSION, RNA-SEQ DATA

MHC BINDING /ELISPOT ASSAY
Experimental validation of candidate
immunogenic neo-epitopes
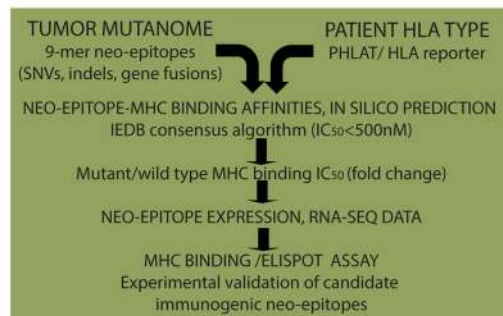
Figure 2. Integrative sequencing analysis to define the spectrum of cancer aberrations.
A. Bioinformatic workflow and classes of cancer aberrations identified. The primary
sequencing data is subjected to different quality control metrics and aligned to the reference
genome to define the indicated genomic and transcriptomic aberrations. **B. Prediction of
neo-antigens for immunotherapy**. The flow chart indicates primary steps involved in *in
silico* prediction of immunogenic cancer specific neo-antigens in tumor samples, for
potential use in developing personalized peptide vaccines.
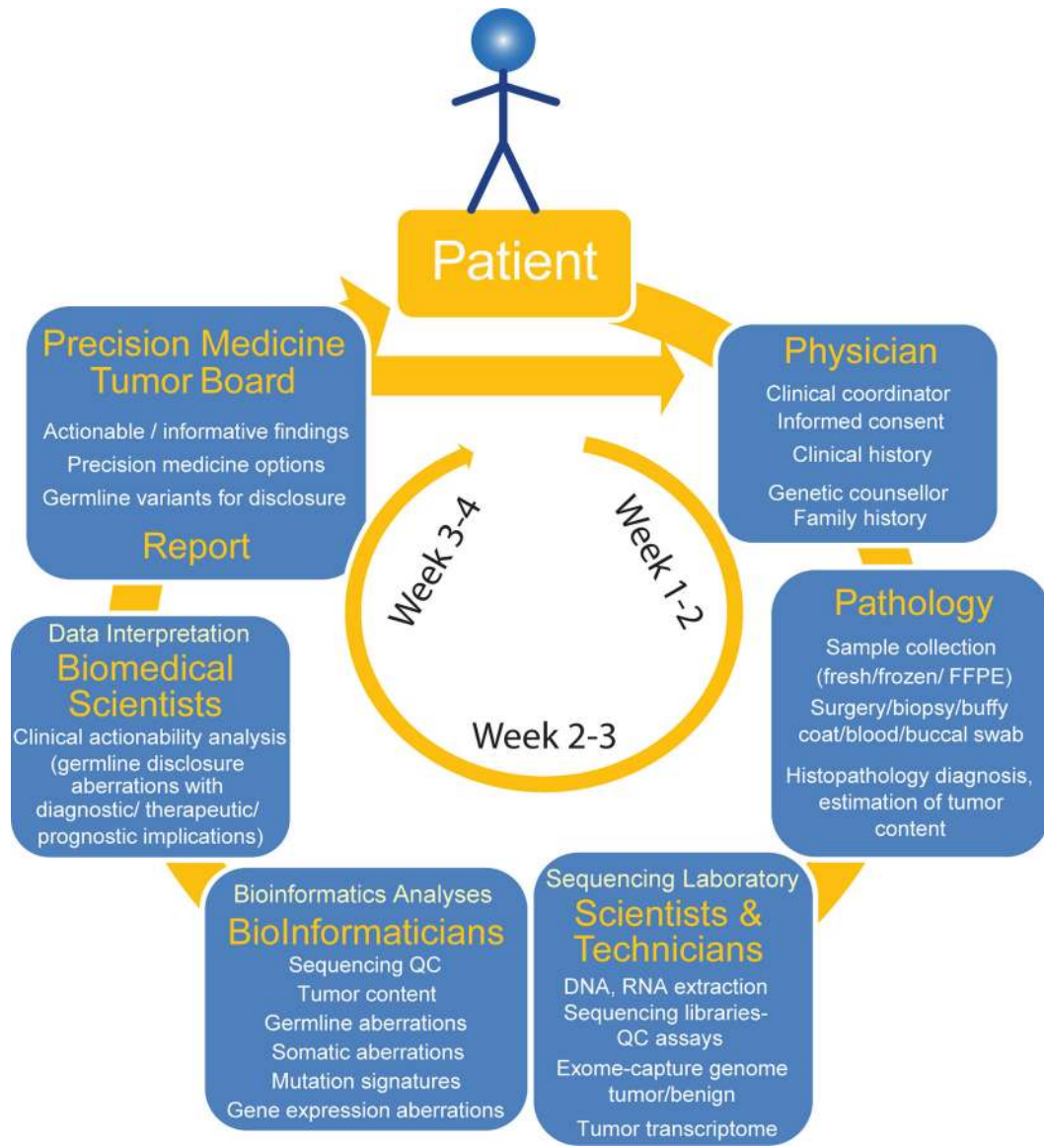
Figure 3. Workflow of integrative clinical sequencing for precision oncology.
The patient, in consultation with the cancer physician, enters the clinical sequencing program upon signing the informed consent. Along with documentation of detailed clinical history, a genetic counselor obtains family history of the patient to assess likely hereditary predisposition to cancer. Patient's tumor biopsy is flash frozen in OCT blocks, and, along with blood or buccal swab, the samples are sent to the CLIA-certified sequencing laboratory. Histology sections of the tumor biopsy blocks are evaluated by a clinical pathologist for diagnosis and tumor content. DNA and RNA from tissue blocks with the highest tumor content and DNA from blood/buccal samples are used to generate sequencing libraries. Exome capture libraries from germline and tumor samples and the transcriptome library from tumor RNA are analyzed for germline and somatic aberrations. Potentially actionable molecular observations are identified and discussed at the multidisciplinary precision

medicine tumor board (see also BOX 1), and a summary report of clinical recommendations is provided to the attending physician.

Table 1.

Summary of actionable germline aberrations in cancer predisposition genes.

Genes associated with predisposition or susceptibility to cancer included in NCCN guidelines, ACMG recommendations for reporting (Green et al, Genet. Med. 2013;15(7):565–574), currently being tested in clinical trials (see Supplementary Table 1A for details), or considered for reporting in MI_Oncoseq study.

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype*	NCCN guidelines	ACMG recommendation	Ongoing clinical trials**	MI_Oncoseq
611731	APC	5q22.2	5:112707504–112846238	Adenomatous polyposis coli	Colorectal cancer, somatic, 114500; Brain tumor-polyposis syndrome 2, AD, 175100; Hepatoblastoma, somatic, 114550; Adenomatous polyposis coli, AD, 175100; Gastric cancer, somatic, 613659; Adenoma, periampullary, somatic	X	X	X	X
607585	ATM	11q22.3	11:108222483–108369101	Ataxia-telangiectasia mutated (includes complementation groups A, C, D, and E)	Lymphoma, B-cell non-Hodgkin, somatic; Breast cancer, susceptibility to, AD, 114480; T-cell prolymphocytic leukemia, somatic; Lymphoma, mantle cell, somatic	X		X	
601215	ATR	3q23	3:142449234–142578825	Ataxia-telangiectasia and Rad3-related (FRAP-related protein-1)	Cutaneous telangiectasia and cancer syndrome, familial, AD, 614564			X	
601593	BARD1	2q35	2:214725644–214809710	BRCA1-associated RING domain 1	Breast cancer, susceptibility to, AD, 114480			X	
601299	BMPR1A	10q23.2	10:86755785–86927968	Bone morphogenetic protein receptor, type IA	Polyposis, juvenile intestinal, AD, 174900; Polyposis syndrome, hereditary mixed, 2, 610069; Juvenile polyposis syndrome, infantile form, AD, 174900	X			
113705	BRCA1	17q21.31	17:43044294–43125482	Breast cancer-1 gene	Pancreatic cancer, susceptibility to, 4, 614320; Breast-ovarian cancer, familial, 1, AD; Multifactorial), 604370	X	X	X	X
600185	BRCA2	13q13.1	13:32315479–32399671	BRCA2 gene	Breast cancer, male, susceptibility to, AD, 114480; Prostate cancer, AD, 176807; Wilms tumor, SM; AD, 194070; Pancreatic cancer 2, 613347; Medulloblastoma, AD, 155255; Glioblastoma 3, AR, 613029; Breast-ovarian cancer, familial, 2, AD, 612555	X		X	X

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype*	NCCN guidelines	ACMG recommendation	Ongoing clinical trials**	MI_Oncoseq
605882	BRIP1	17q23.2	17:61679185–61864119	BRCA1-associated C-terminal helicase 1	Breast cancer, early-onset, AD, 114480			X	X
192090	CDH1	16q22.1	16:68737289–68835541	Cadherin-1 (E-cadherin; uvomorulin)	Ovarian carcinoma, somatic, 167000; Gastric cancer, familial diffuse, with or without cleft lip and/or palate, AD, 137215; Endometrial carcinoma, somatic, 608089; Prostate cancer, susceptibility to, AD, 176807; Breast cancer, lobular, AD, 114480	X	X		
604373	CHEK2	22q12.1	22:28687742–28741865	Checkpoint kinase 2, S. pombe, homolog of (RAD53, S. cerevisiae, homolog of)	Prostate cancer, familial, susceptibility to, AD, 176807; Breast cancer, susceptibility to, AD, 114480; Osteosarcoma, somatic, 259500	X		X	X
606241	DICER1	14q32.13	14:95086227–95158009	Dicer, Drosophila, homolog of, 1	Rhabdomyosarcoma, embryonal, 2, 180295; Pleuropulmonary blastoma, AD, 601200				X
131550	EGFR	7p11.2	7:55019031–55207337	Epidermal growth factor receptor	Non-small cell lung cancer, susceptibility to, AR, 211980; Non-small cell lung cancer, response to tyrosine kinase inhibitor in, AR, 211980; Adenocarcinoma of lung, response to tyrosine kinase inhibitor in, AR, 211980			X	
185535	EPCAM	2p21	2:47369147–47387027	Epithelial cellular adhesion molecule	Colorectal cancer, hereditary nonpolyposis, type 8, 613244	X			
607139	FANCA	16q24.3	16:89737550–89816657	Fanconi anemia, complementation group A	Fanconi anemia, complementation group A, AR, 227650			X	X
136850	FH	1q43	1:241497556–241519784	Fumarate Hydratase	Leiomyomatosis and renal cell cancer, AD, 150800		X	X	X
610290	GALNT12	9q22.33	9:988807698–98850080	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12	Colorectal cancer, susceptibility to, 1, 608812	X			
604607	HOXB13	17q21.32	17:48724762–48728748	Homeobox B13	Breast cancer, 114480; Prostate cancer, 610997				X
613733	MEN1	11q13.1	11:64803513–64811293	Menin	Adrenal adenoma, somatic; Parathyroid adenoma, somatic; Multiple endocrine neoplasia 1, AD, 131100; Lipoma, somatic; Carcinoid tumor of lung; Angiofibroma, somatic		X		
164860	MET	7q31.2	7:116672358–116798385	Oncogene MET	Renal cell carcinoma, papillary, 1, familial and			X	

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype*	NCCN guidelines	ACMG recommendation	Ongoing clinical trials**	MI_Oncoseq
					somatic, 605074; Hepatocellular carcinoma, childhood type, somatic, 114550				
120436	MLH1	3p22.2	3:36993349–37050845	mutL, E. coli, homolog of, 1	Colorectal cancer, hereditary nonpolyposis, type 2, 609310; Mismatch repair cancer syndrome, AR, 276300	X	X	X	X
600814	MRE11A	11q21	11:94415569–94512700	Meiotic recombination 11, S. cerevisiae, homolog A of	Ataxia-telangiectasia-like disorder, AR, 604391		X	X	X
609309	MSH2	2p21-p16	2:47403066–47634500	mutS, E. coli, homolog of, 2	Mismatch repair cancer syndrome, AR, 276300; Colorectal cancer, hereditary nonpolyposis, type 1, AD, 120435		X	X	X
600678	MSH6	2p16.3	2:47783081–47806952	MutS, E. coli, homolog of, 6	Endometrial cancer, familial, 608089; Colorectal cancer, hereditary nonpolyposis, type 5, AD, 614350; Mismatch repair cancer syndrome, AR, 276300	X	X	X	
604933	MUTYH	1p34.1	1:45329241–45340924	MutY, E. coli, homolog of	Adenomas, multiple colorectal, 608456; Gastric cancer, somatic, 613659; Colorectal adenomatous polyposis, AR, with pilomatricomas, SM, 132600	X	X		X
602667	NBN	8q21.3	8:89933335–89984732	Nibrin	Nijmegen breakage syndrome, AR, 251260; Leukemia, acute lymphoblastic, 613065; Aplastic anemia, 609135			X	X
607379	NF2	22q12.2	22:29603555–29698599	Merlin	Neurofibromatosis, type 2, AD, 101000; Meningioma, NF2-related, somatic, 607174; Schwannomatosis, AD, 162091		X	X	
191315	NTRK1	1q23.1	1:156815749–156881849	Neurotrophic tyrosine kinase, receptor, type 1	Medullary thyroid carcinoma, familial, AD, 155240		X		
610355	PALB2	16p12.2	16:23603161–23641356	Partner and localizer of BRCA2	Pancreatic cancer, susceptibility to, 3, 613348; Breast cancer, susceptibility to, AD, 114480; Fanconi anemia, complementation group N, 610832			X	X
600259	PMS2	7p22.1	7:5970924–6009105	Postmeiotic segregation increased, S. cerevisiae, 2, homolog of	Mismatch repair cancer syndrome, AR, 276300; Colorectal cancer, hereditary nonpolyposis, type 4, 614337	X	X	X	

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype*	NCCN guidelines	ACMG recommendation	Ongoing clinical trials**	MI_Oncoseq
601728	PTEN	10q23.31	10:87863437–87971929	Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	Glioma susceptibility 2, 613028; Endometrial carcinoma, somatic, 608089; Squamous cell carcinoma, head and neck, somatic, 275355; PTEN hamartoma tumor syndrome; Prostate cancer, somatic, 176807; Malignant melanoma, somatic, 155600; Meningioma, AD, 607174	X	X	X	
604040	RAD50	5q31.1	5:132556923–132644620	RAD50, S. cerevisiae, homolog of	Nijmegen breakage syndrome-like disorder, 613078			X	
602774	RAD51C	17q22	17:58692537–58734341	RAD51, S. cerevisiae, homolog of, C	Breast-ovarian cancer, familial, susceptibility to, 3, 613399; Fanconi anemia, complementation group O, AR, 613390			X	X
602954	RAD51D	17q12	17:35099791–35119868	RAD51, S. cerevisiae, homolog of, D	Breast-ovarian cancer, familial, susceptibility to, 4, 614291			X	
614041	RB1	13q14.2	13:48303746–48481889	Retinoblastoma-1	Small-cell cancer of the lung, somatic, 182280; Retinoblastoma, trilateral, SM; AD, 180200; Retinoblastoma. SM; AD, 180200; Osteosarcoma, somatic, 259500; Bladder cancer, somatic, 109800		X		X
604610	RECQL3	15q26.1	15:90717326–90815461	DNA helicase, RecQ-like 3	Bloom syndrome, AR, 210900	X			
164761	RET	10q11.21	10:4307068–43130350	RET transforming sequence; oncogene RET	Multiple endocrine neoplasia IIB, AD, 162300; Multiple endocrine neoplasia IIA, AD, 171400; Medullary thyroid carcinoma, AD, 155240; Pheochromocytoma, AD, 171300		X		
613019	SDHAF2	11q12.2	11:61430124–61446766	Succinate dehydrogenase complex assembly factor 2	Paragangliomas 2, AD, 601650		X		
185470	SDHB	1p36.13	1:17018721–17054169	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	Pheochromocytoma, AD, 171300; Paragangliomas 4, AD, 115310; Paraganglioma and gastric stromal sarcoma, 606864; Gastrointestinal stromal tumor, AD; Isolated cases, 606764		X		
602413	SDHC	1q23.3	1:161314375–161364750	Succinate dehydrogenase complex, subunit C, integral membrane protein, 15kD	Paragangliomas 3, AD, 605373; Paraganglioma and gastric stromal sarcoma, 606864; Gastrointestinal		X		

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype*	NCCN guidelines	ACMG recommendation	Ongoing clinical trials**	MI_Oncoseq
					stromal tumor, AD; isolated cases, 606764				
602690	SDHD	11q23.1	11:112086823–112095800	Succinate dehydrogenase complex, subunit D, integral membrane protein	Carcinoid tumors, intestinal, AD, 114900; Paragangliomas 1, with or without deafness, AD, 168000; Paraganglioma and gastric stromal sarcoma, 606864; Merkel cell carcinoma, somatic; Cowden syndrome 3, 615106; Pheochromocytoma, AD, 171300		X		
600993	SMAD4	18q21.2	18:51030212–51085041	Mothers against decapentaplegic, Drosophila, homolog of, 4	Polyposis, juvenile intestinal, AD, 174900; Pancreatic cancer, somatic, 260350; Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome, AD, 175050	X			
602216	STK11	19p13.3	19:1205798–1228434	Serine/threonine protein kinase-11	Pancreatic cancer, (SM; AD; Multifactorial), 260350; Melanoma, malignant, somatic; Testicular tumor, somatic, 273300	X	X		
191170	TP53	17p13.1	17:7668401–7687549	Tumor protein p53	Glioma susceptibility 1, (SM; AD), 137800; Hepatocellular carcinoma, SM, 114550; Basal cell carcinoma 7, 614740; Colorectal cancer, AD, 114500; Pancreatic cancer, (SM; AD; Multifactorial), 260350; Choroid plexus papilloma, AD, 260500; Osteosarcoma, AR, 259500; Breast cancer, AD, 114480; Nasopharyngeal carcinoma, 607107; Adrenal cortical carcinoma, AR, 202300	X	X		
605284	TSC1	9q34.13	9:132891347–132945268	Hamartin (tuberous sclerosis 1 gene)	Tuberous sclerosis-1, AD, 191100; Lymphangioleiomyomatosis, 606690		X		
191092	TSC2	16p13.3	16:2047803–2088719	Tuberin (tuberous sclerosis 2 gene)	Tuberous sclerosis-2, AD, 613254; Lymphangioleiomyomatosis, somatic, 606690		X		
608537	VHL	3p25.3	3:10141634–10153669	VHL gene	Renal cell carcinoma, somatic, 144700; Pheochromocytoma, AD, 171300; Hemangioblastoma, cerebellar, somatic		X		

Gene MIM#	Gene	Cytoband	Chr coordinates (GRCh38)	Gene name	Phenotype[*]	NCCN guidelines	ACMG recommendation	Ongoing clinical trials[**]	MI_Oncoseq
607102	WT1	11p13	11:32387774–32435534	Wilms tumor-1	Mesothelioma, somatic, 156240; Wilms tumor, type 1, (SM; AD), 194070		X		

[*]
AD: Autosomal dominant; AR: Autosomal recessive; SM: somatic mutation

[**]
See Supplementary Table 1A for details

Table 2.

Online resources for precision oncology studies.

A list of online portals and databases catering to different data analysis requirements for precision oncology.

Data portals for mutations and/or germline variations	Website
1000 Genomes Project, global reference for human genetic variation	http://www.1000genomes.org/
Cancer genomics data portal_ICGC	https://dcc.icgc.org/
Cancer genomics data portal_TCGA	https://tcga-data.nci.nih.gov/docs/publications/tcga/
cBioPortal, visualization, analysis and download of cancer genomics data	http://www.cbioportal.org
ClinVar, Database of genomic variants related to human health	http://www.ncbi.nlm.nih.gov/clinvar
COSMIC, Catalog of somatic mutations in cancer	http://cancer.sanger.ac.uk/cosmic/
dbGAP, Database of Genotypes and Phenotypes	http://www.ncbi.nlm.nih.gov/gap
dbNSFP, Database of Functional Predictions for SNVs	https://sites.google.com/site/jpopgen/dbNSFP
dbSNP, Database of Single Nucleotide Polymorphisms (SNPs)	http://www.ncbi.nlm.nih.gov/snp
dbVar, Database of genomic structural variations	http://www.ncbi.nlm.nih.gov/dbvar
DoCM, Database of Curated Mutations	http://docm.genome.wustl.edu/about
Ensemble Variant Effect Predictor	http://useast.ensembl.org/info/docs/tools/vep/index.html
ExAC, Exome Aggregation Consortium	http://exac.broadinstitute.org/
Exome Variant Server	http://evs.gs.washington.edu/EVS
Genome Modeling Tools, Washington Univ. St. Louis	http://gmt.genome.wustl.edu
Human Gene Mutation Database	http://www.hgmd.org
IARC (WHO) TP53 mutation Database	http://p53.iarc.fr
Intogen mutational cancer drivers database	https://www.intogen.org/search
IGV, Integrative Genomics Viewer	http://software.broadinstitute.org/software/igv/
LOVD, Leiden Open Variation Database	http://www.lovd.nl
MuSiC, Mutational significance in cancer genomes	http://tvap.genome.wustl.edu/tools/music/
Pediatric Cancer Genome Project	http://explore.pediatriccancergenomeproject.org/
SRA, Short Reads Archive	http://www.ncbi.nlm.nih.gov/sra
The Turnkey Variant Analysis Project	http://tvap.genome.wustl.edu/
UCSC Cancer Genome Browser	https://genome-cancer.ucsc.edu/
Xena, Integration/visualization of in-house data with public data	http://xena.ucsc.edu/
Data portals for integration of -omics data with clinical interpretation/resources	
AACR_Project Genomics Evidence Neoplasia Information Exchange (GENIE)	http://www.aacr.org/Research/Research/Pages/aacr-project-genie.aspx#.WMs4uWfau70
Cancer Commons knowledgebase	https://www.cancercommons.org/patients-caregivers/
Cancer Resource	http://data-analysis.charite.de/care/
CancerLinQ	http://cancerlinq.org/
CIViC, Clinical interpretations of variants in cancer	https://civic.genome.wustl.edu

Data portals for mutations and/or germline variations	Website
Clinical Trials	http://clinicaltrials.gov
CollabRx	http://www.collabrx.com/
Electronic Medical Records and Genomics (eMERGE)	https://emerge.mc.vanderbilt.edu/
Gene cards	http://www.genecards.org/
GeneInsight	http://geneinsight.com/
Genetic Testing Registry	https://www.ncbi.nlm.nih.gov/gtr/
Genomic Data Commons	https://gdc.cancer.gov/
GTEX, genotype-tissue expresssion data	http://www.gtexportal.org/home/
Malacards	http://www.malacards.org/
My Cancer Genome, Vanderbilt-Ingram Cancer Center	http://www.mycancergenome.org/
N-of-One, clinical interpretation service	http://n-of-one.com/
Personalized Cancer Therapy	https://pct.mdanderson.org/#/
Pubmed Clinical	https://www.ncbi.nlm.nih.gov/pubmed/clinical
NIH Collaboratory	https://www.nihcollaboratory.org

Data portals providing gene-drug knowledgebase	
Cancer Driver Log	https://candl.osu.edu/
DGIdb, The drug gene interaction database	http://dgidb.genome.wustl.edu/
Drugs@FDA: FDA Approved Drug Products	http://www.accessdata.fda.gov/scripts/cder/daf/index.cfm
Drug Bank	http://www.drugbank.ca/about
Gene Drug Knowledge Database	https://www.synapse.org/#!Synapse:syn2370773/wiki/
IUPHAR/BPS, guide to pharmacology	http://www.guidetopharmacology.org/download.jsp
NCI Drug Dictionary	https://www.cancer.gov/publications/dictionaries/cancer-drug
Personalized cancer Therapy, MD Anderson Cancer Center	https://pct.mdanderson.org
Pharmacogenomics Research Network sequence platform	http://www.pgrn.org/
PharmaGKB	https://www.pharmgkb.org/
SuperTarget	http://bioinf-apache.charite.de/supertarget_v2/
TARGET, Tumor alterations relevant for genomics-driven therapy	http://www.broadinstitute.org/cancer/cga/target
TTD, Therapeutic Targets Database	http://xin.cz3.nus.edu.sg/group/ttd/ttd.asp

Table 3.

Integrative sequencing provides an array of actionable observations.

Potentially actionable observations in a representative set of cases following integrative sequencing analyses highlights the spectrum of different kinds of aberrations including germline, somatic, and gene expression changes.

		MO_1311	TP_2132	MO_1329	MO_1233	TP_2130	MO_1102	MO_1315	MO_1331	MO_1547	MO_1177
	Cancer	Renal Cell Carcinoma	Glioblastoma Multiforme	Chondrosarcoma	Adenoid Cystic Carcinoma	Sphenoid Sinus Squamous Cell Carcinoma	Thymic Carcinoma	Non-Small Cell Lung Cancer	Tongue Squamous Cell Carcinoma	Parotid Gland Cancer	Non-Small Cell Lung Carcinoma
	Gender/Age	M65	M53	F59	F44	M70	M54	F54	F68	F66	M63
Germline	Germline_Pathogenic SNV/indels	FH p.K477dup non-frameshift insertion	PMS2 p.R802*	-	-	-	MLH1 splicing mut.	-	-	-	-
Tumor exome/genome	Copy number_Amp	-	FGFR3-ΔKIT	-	-	-	-	-	MCL1, ARNT	MDM2, CDK4, CCND1	PDGFA, BRD4
	Copy number_Del	-	PTEN	-	copy loss: CDKN1C, CDKN2A, CDKN2B, ARID1A, ARID1B, SMARCA2	-	MLH1 copy loss	copy loss: CDKN2A, CDKN2B, SMARCA2, PTEN, TP53	-	-	CDKN2A/2B
	SNV_gain of fn	-	-	IDH p.R132G	-	-	-	-	-	-	-
	SNV_loss of fn	-	-	-	KDM6A p.Q1311* (homozygous)	TP53 p.R273C, p.T125M	TP53 p.R282Q	TP53 p.S260T; KEAP1 p.R470H	IQGAP2 (G833*)	NOTCH1 p.L1678R; p.Q2444* (PEST domain); TP53 p.R175H	TP53 p.E285K; BAP1 p.W52* (homozygous)
	Indel_gain of fn	-	-	-	*NOTCH1 non-frameshift deletion (hotspot) activating	EGFR p.D770delinsDGF non-frameshift insertion (activating)	CDKN2A fs insertion	-	-	-	-
	Indel_loss of fn	-	PTEN	-	-	FAT1 p.T1818fs deletion	-	-	-	-	-
	Mutation signature	-	-	-	-	-	MSI Signature	-	-	MSI Signature	-
Transcriptome	Gene Fusion	-	-	-	NOTCH1, FGFR2, ERBB3	-	-	KIF5B-RET	-	-	NF2-OSBP2 (loss of NF2)
	Gene Expression_Outlier	-	-	-	-	-	PD-L1	-	MCL1, ARNT	-	PDGFA, BRD4
	Gene Expression_Biomarkers	-	-	-	-	-	-	-	-	-	UPK3B, LRRN4, CALB2, WT-1, MSLN, PDPN
Pathogens	Cancer Virus	-	-	-	-	-	-	-	HPV16	-	-
	Clinical Action	FH p.K477dup is associated with Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC).	TKIs such as Nilotinib/Ponatinib. Ongoing Counseling	IDH inhibitors	Clinical trial with Notch inhibitor	Eligible for FDA approved Cetuximab	PD-1/PD-L1 targeting immunotherapy	Cabozantinib in Patients With RET Fusion Positive Advanced Non-Small Cell Lung Cancer; NCT01639508	T Cell Receptor Immunotherapy. Targeting HPV16/18 for HPV-Associated Cancers (NCT02280811)	Immunotherapy, NOTCH inhibitor, CDK4/6 inhibitor	Biomarkers supporting diagnosis of Mesothelioma. Clinical trial with BRD inhibitor (NCT01587703)

Therapeutically actionable aberration

Diagnostically/prognostically actionable aberration