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Perspective

The next horizon in precision oncology: Proteogenomics to inform cancer diagnosis and treatment

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https://doi.org/10.1016/j.cell.2021.02.055

SUMMARY

When it comes to precision oncology, proteogenomics may provide better prospects to the clinical characterization of tumors, help make a more accurate diagnosis of cancer, and improve treatment for patients with cancer. This perspective describes the significant contributions of The Cancer Genome Atlas and the Clinical Proteomic Tumor Analysis Consortium to precision oncology and makes the case that proteogenomics needs to be fully integrated into clinical trials and patient care in order for precision oncology to deliver the right cancer treatment to the right patient at the right dose and at the right time.

INTRODUCTION

Precision oncology is changing faster than ever. This evolving ecosystem that relies on advances in technology refers to interventions in the cancer continuum that are based on the molecular pathology and pathogenesis of the disease. Although precision oncology approaches have led to significant advances in cancer prevention and screening such as human papillomavirus (HPV) vaccination and HPV-based screening for cervical cancer (Fontham et al., 2020; McClung et al., 2019), its use is far more common in the diagnosis and treatment of cancer. The recent development and widespread dissemination of cancer genomic applications (for simplification in this perspective, genomics will encompass DNA, epigenetic, and RNA [transcriptomics]) have had a major impact on our recognition of the molecular heterogeneity prevailing between different tumor subtypes within a tumor type (Bedard et al., 2013; Hanahan and Weinberg, 2011). Despite this heterogeneity, certain genetic lesions, especially those that result in the activation of oncogenes, have led to the development of clinically useful therapeutic inhibitors that successfully target such abnormalities (National Cancer Institute, 2020).

Over the past decade, it has been increasingly recognized that no two patients' cancers are exactly the same. Consequently, each cancer may have a different response to common treatments such as chemotherapy and radiation. A precision medicine approach to cancer treatment, or precision oncology, utilizes the molecular attributes of an individual patient's tumor to assess the probability of benefit or toxicity from a specific therapeutic intervention (a patient's response to a given therapy). This approach relies on the assumption that matching the molecular mechanism of action with a therapeutic agent based on the status of the molecular target for the agent in a patient's tumor will improve cancer treatment. As we detail below, cancer proteogenomics, the integration of proteomics with genomics, can successfully assign tumors to molecular subtypes that share oncogenic mechanisms and respond preferentially to targeted agents aimed at these mechanisms. In this vision of precision cancer medicine, proteogenomic profiling will provide a molecular diagnosis of a patient's cancer, which will carry recommendations for treatment regimens that are most likely to provide benefit.

The National Cancer Institute (NCI) at the National Institutes of Health (NIH) serves a vital role in supporting preclinical and clinical investigations of precision oncology through its multi-site, multi-disciplinary collaborative omics programs that utilize rapid improvements in technology for characterizing molecular aberrations in a patient's tumor to advance clinical trials, novel molecular diagnostics, and patient care. Establishing these activities, we focus on the NCI's key contributions from initial investments in The Cancer Genome Atlas (TCGA) to the Clinical Proteomic Tumor Analysis Consortium (CPTAC), whose results are laying the foundation to the next horizon (Figure 1) in precision oncology—proteogenomics.

DAWN OF A NEW ERA: MOLECULARLY TARGETED THERAPIES FOR CANCER

Precision oncology has made important contributions to the efficacy of cancer treatment. The use of specific genetic perturbations in a patient's tumor to select cancer therapy began in the

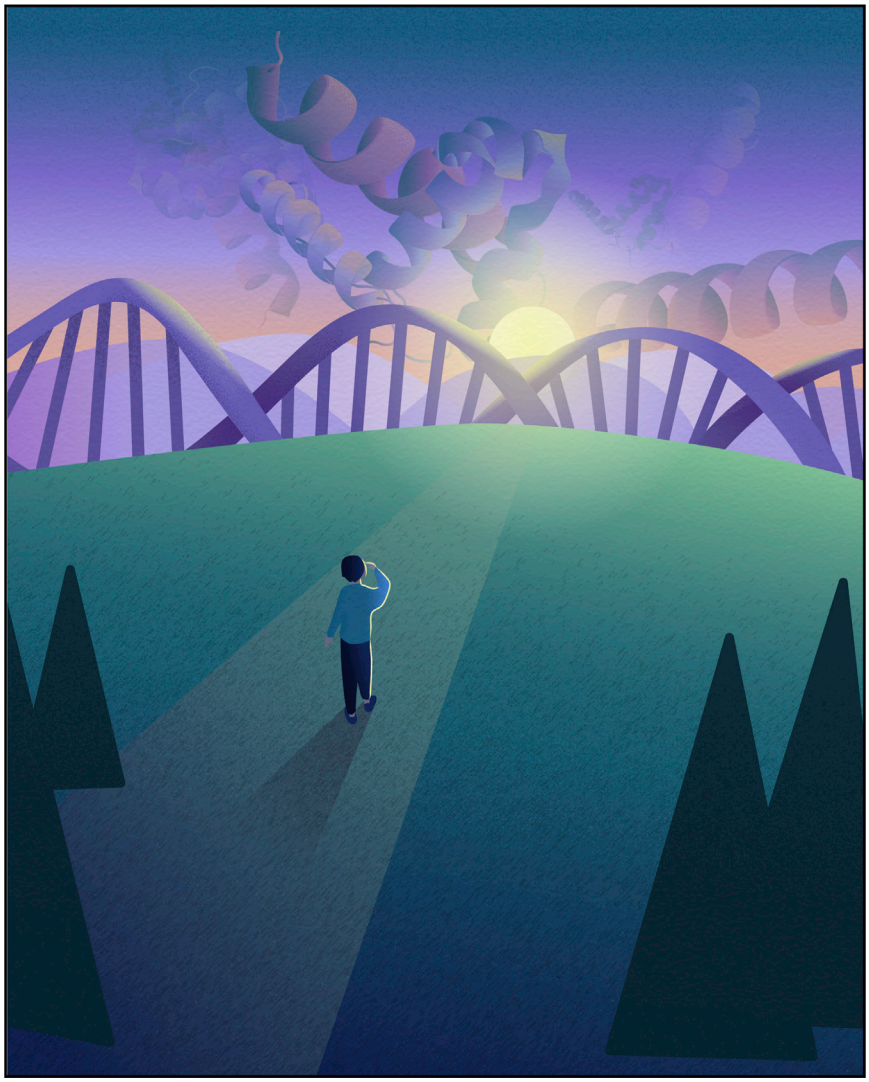

Figure 1. Proteogenomics in precision oncology

In this figurative piece, the role of proteogenomics in precision oncology is illustrated by a cancer patient looking out into the horizon. His horizon is framed by DNA and RNA hills (genomics) and protein clouds (proteomics), which together represent the keys to overcoming scientific mountains. The strong ray of sunlight embodies the current contributions of genomics to oncology, lighting the path to the next step in the journey, multiomics molecular characterization of patient tumors. The patient looks forward to a brighter horizon, one where genomic and proteomic molecular diagnoses are combined into clinical trials and patient care, one where patients and families are gifted with hope.

late 1990s with the demonstration of the efficacy of trastuzumab (Herceptin) for women whose breast cancers overexpressed the *HER2* oncoprotein as a result of gene amplification and the subsequent development of imatinib (Gleevec), which targets the constitutively active *BCR-ABL1* kinase produced by chromosomal translocation in patients with chronic myelogenous leukemia (Doroshow and Doroshow, 2020).

These early successes based on genomic markers demonstrated the impressive potential of target-specific drugs and suggested future therapeutic possibilities. Over the past 20 years, cancer patients have benefitted from the discovery of many new classes of both small-molecule and antibody therapeutics that selectively improve clinical outcomes in many common as well as rare types of cancer based on their ability to engage specific (usually somatically altered) molecular targets (Hyman et al., 2017; Moscow et al., 2018). For example, a genetic rearrangement in the anaplastic lymphoma kinase (ALK) drives tumor formation in about 5% of non-small cell lung cancers (Soda et al., 2007). This discovery led to the development of ALK inhibitors, including crizotinib and ceritinib, and US Food and Drug Administration (FDA) approval for their use in lung cancer patients who test positive for an ALK rearrangement. Such therapies provide a powerful validation of the precision oncology approach, which has transformed the lives of many patients and contributed to recent nationwide decreases in non-small cell lung cancer mortality rates (Howlader et al., 2020).

PRECISION ONCOLOGY: GENOMICS AND TCGA

Precision oncology would not exist without the major accomplishment of sequencing the human genome. The human genome project was a 15-year collaborative multinational effort whose first drafts were published in 2001 (Lander et al., 2001; Venter et al., 2001) and completed in 2003. Less than three years after completion of the Human Genome Project, the NIH officially launched the pilot stage of an effort to create a comprehensive catalog of the genomic changes involved in cancer—TCGA, a collaboration between the NCI and the National Human Genome Research Institute (NHGRI). Established in 2006 and expanded in 2010, TCGA was based on the premise that unique and reproducible genomic differences exist among patients' tumors and that these differences could lead to the development of individualized treatments (Hanauer et al., 2007; Heng, 2007).

TCGA has systematically characterized an unprecedented number of well-annotated human tumor samples as well as their matching normal tissues (approximately 11,000 cases from 33 tumor types) (Blum et al., 2018), including nine rare cancers, producing a vast amount of data that the cancer research community continues to mine, to identify genomic changes that may play a role in the oncogenic phenotype (Cancer Genome Atlas Research Network, 2014). For example, analysis of gastric

cancer by TCGA revealed four molecular subtypes, one of which harbored the Epstein-Barr virus, had PIK3CA mutations in 80% of cases, and recurrently amplified the genomic locus encoding PD-L1 and PD-L2, suggesting opportunities to evaluate inhibitors of phosphatidylinositol 3-kinase (PI3K) and immune checkpoints (Cancer Genome Atlas Research Network, 2014). The TCGA analysis of malignant brain tumors indicated that a subset of glioblastoma multiforme (GBM) tumors have genetic abnormalities that are shared by low-grade gliomas (Cancer Genome Atlas Research Network, 2015). Patients with such GBMs experience durable responses to therapy, unlike the majority of patients with GBMs. This molecular diagnosis of brain cancer subtypes has now been adopted as a standard by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) (Louis et al., 2017) and is being routinely implemented in clinical practice.

In another TCGA study (Cancer Genome Atlas Research Network, 2013), two endometrial cancer tumor subtypes—uterine serous tumors and high-grade endometrioid tumors—were shown to share several genomic alterations. Specifically, researchers found that approximately one-fourth of tumors classified as high-grade endometrioid had genetic features similar to uterine serous carcinoma tumors, including extensive copy-number alterations (CNAs) and frequent mutations in the *TP53* gene. Moreover, uterine serous carcinomas shared genomic features with ovarian serous cancers and basal-like breast carcinomas. The new data also suggested that the frequency of mutations in specific genes in a tumor could be used to help guide treatment decisions for cancer patients. For instance, early-stage endometrioid tumors are often treated with adjuvant radiotherapy, while similarly staged serous tumors are treated with chemotherapy. Based on these genetic similarities, clinicians might now consider treating copy-number-altered endometrioid patients with chemotherapy rather than adjuvant radiotherapy and formally test such hypotheses in prospective clinical trials.

TCGA has paved new roads to understanding the origin and causes of cancer, as well as suggesting the driver genes in the cancers analyzed (Blum et al., 2018). Additionally, it has altered the diagnostic landscape by translating evidence-based classification of tumors into molecularly defined classes that may enable treatment of patients with significantly more precision than previously. These changes may ultimately permit the development of clinical trials with higher probability of success by allowing stratification of patient treatment. TCGA has been the face of the US involvement in cancer genomics and has served as a model for similar national and international programs, such as the International Cancer Genome Consortium (ICGC). Finally, TCGA offers a website that is easy to access and explore (NCI Genomics Data Commons [GDC], https://gdc.cancer.gov), where researchers can retrieve relevant data and access cloud-based analysis tools (Grossman et al., 2016). Despite these steps forward, a connectivity gap has remained in our ability to identify genomic variants, associate them with regulatory elements, and understand how those molecular abnormalities affect human cancers (connecting genomic variations to human phenotypes).

PRECISION ONCOLOGY: GAPS IN GENOMICS (CHALLENGES OF APPLICATION AND CLINICAL TRANSLATION)

While mutational profiling of tumors to define cancer patient populations with an improved likelihood of benefitting from specific treatments is a major tool of most precision oncology efforts (Flaherty et al., 2020), molecularly targeted therapeutic strategies are not readily available for most mutations. Some analyses have also demonstrated the limits of therapeutic decision-making strategies that are based on mutational profiling alone (Le Tourneau et al., 2015; Saad et al., 2017). Although the addition of transcriptomic profiling to the assessment of mutational status has been suggested to improve molecular tumor characterization for selection of specific cancer treatments (Rodon et al., 2019), neither reliably predicts changes in the levels or functional status of their corresponding proteins, which the majority of anticancer drugs target. Furthermore, most common tumors have many mutations of unknown significance, making it difficult to establish which of these abnormalities are etiologically important oncologic drivers.

As previously indicated, even when a targeted drug is a good match for a specific mutation, it is not always effective. For instance, although a wide range of kinase inhibitors are approved by the FDA for treating various forms of cancer, patient-to-patient differences in response remain, including when patients have the same genomic abnormality and cancer type. One example of such a difference is in patients with melanoma or colorectal cancer where both tumor types harbor the same BRAF mutation; however, a targeted therapy that works in melanoma might not help patients with colorectal cancer (Hyman et al., 2015; Sclafani et al., 2013). In the absence of being able to meaningfully interpret the contribution of genomic features to a specific patient's tumor biology, the cancer community finds itself limited in their armamentarium of available therapies for cancer patients. These gaps place precision oncology at a crossroads.

The complex nature of cancer means that relying exclusively on genomics for diagnosis and treatment is insufficient, as the needs of many cancer patients remain unresolved. This is because while genomics allows us to understand a cancer's input codes (genes), their output codes (proteins) are needed to fully capture the informational state of a tumor and provide a more complete and precise picture of how to understand and treat the underlying molecular pathology. This is because proteins and their post-translational modifications (PTMs) add a new layer of biology that is hidden from genomics and is the site of action of most therapeutic interventions. A cancer proteome (direct measurement of the quantity of proteins and PTMs) is essential to narrow the gap between cancer genotype and cancer phenotype.

Nevertheless, while proteomics (examining the ensemble of proteins in tumor cells) has the potential to open new frontiers in the diagnosis and treatment of cancer, and adds a new dimension of molecular insight into an individual patient's disease that cannot be readily gleaned from genomic analysis alone, its complementary utility is maximized when combined with genomics (proteogenomics) within and across samples. Technically, mass spectrometry (MS)-based proteomics data are typically

matched against existing mapped peptides from a reference protein database. A downside to this approach is the possibility that the protein in question from a patient may be novel and thus not referenced in the database. In contrast to proteomics data analysis that relies on reference protein databases alone, integrating genome, transcriptome, and proteome data within and across samples helps to circumvent this limitation and represents an equal partnership in which each component contributes and each component benefits. Biologically, integrating genome, transcriptome, and proteome data within and across samples can help explain proteomic activity patterns directly or indirectly driven by altered DNA copy number, chromosomal amplification and deletion events, epigenetic silencing, and changes in microRNA expression, thus bridging and representing a more complete picture of the biology of a cancer. Furthermore, analysis of protein PTMs, particularly phosphorylation, enables the detection of signaling network adaptations driven by genomic changes.

Advances in the technology for quantifying thousands of proteins and their PTMs in a single tissue, including proper handling of biopsy specimens, were developed more recently than genomics and are still undergoing refinement. Yet, proteomic analysis can today rigorously and reproducibly determine steady-state protein levels as well as a variety of PTMs, such as phosphorylations and acetylations. Several important conclusions can be drawn from recent cancer studies that combined comprehensive genomic and proteomic techniques involving tumors from cancer patients. Proteogenomics shows that RNA expression levels are often poor predictors of actual protein levels; enables a more whole characterization of signaling and regulatory pathways that can provide insight into which pathways are activated and inactivated in a given tumor; enables customized proteomics database search to identify novel proteins and prioritize putative neoantigens; and helps to prioritize genomic aberrations that potentially act as oncogenic drivers, such as copy-number drivers (Dhingra et al., 2005; Mertins et al., 2016; Rogers et al., 2008; Wen et al., 2020; Zhang et al., 2014, 2016). This combined molecular information can provide insight into the patient's response to therapy and the mechanisms underlying resistance or toxicity. Once the genomic and proteomic interactions are understood, this groundwork can provide the possibility to extend the use of targeted inhibitors to signaling pathways that have been epigenetically activated to predict outcomes from therapies and to design new drug combinations.

PRECISION ONCOLOGY: PROTEOGENOMICS AND CPTAC

Adding this new dimension of translational biology to the TCGA program, connecting genotype to phenotype, CPTAC was launched in 2006 to develop analytical rigor and reproducibility of proteomic measurements (untargeted and targeted MS-based proteomics) in coordination with the FDA and the American Association for Clinical Chemistry (AACC) (Addona et al., 2009; Carr et al., 2014; Kennedy et al., 2014; Paulovich et al., 2010; Regnier et al., 2010; Rodriguez et al., 2010; Tabb et al., 2010). Having addressed the pre-analytical and analytical barriers to the field of proteomics—protein identification and quantification; data acquisition, analysis, and reporting; experimental design; biospecimen handling; and lack of standardized reagents—CPTAC applied its standardized workflows in 2011 to three previously genomically characterized tumor types from TCGA (colorectal, ovarian, and breast) (Mertins et al., 2016; Zhang et al., 2014, 2016). Although TCGA produced a comprehensive catalog of somatic mutations found in cancer, the effects of many of those mutations on cellular functions or patients' outcomes remain incompletely understood. Therefore, the goal of these tumor analyses was to characterize the relationships between proteins and PTMs derived from altered genes and related biological processes, to determine whether the additional layers of molecular information would help better understand the molecular basis of cancers in ways not possible through genomics alone. Key insights from these initial CPTAC studies included the importance of *trans*-acting genomic aberrations on protein expression (Zhang et al., 2014, 2016), the ability of proteomics to re-classify molecular subtypes (Mertins et al., 2016; Zhang et al., 2014), and the power of phosphoproteomics to identify entire pathways associated with clinical outcomes (Mertins et al., 2016; Zhang et al., 2016).

CPTAC's pioneering approach to the clinical characterization of tumors using proteogenomics demonstrated that combining several omics approaches can produce a more complete understanding of cancer biology and identify possible new therapeutic interventions that can be monitored by proteogenomic targeted testing into the clinical laboratory. The success of these activities led the NCI in 2016 to expand CPTAC's comprehensive tumor characterization program and to partner for the first time with NCI-sponsored clinical trials, to support projects that elucidate biological mechanisms of response or toxicity. Using prospectively collected human tumors optimized for proteomic- and genomic-based measurements (Mertins et al., 2014), CPTAC investigators (and their collaborators) in the past four years have comprehensively characterized the proteogenomics landscape of 13 additional cancer types, with several flagship studies published (Chen et al., 2017, 2020; Clark et al., 2019; Dou et al., 2020; Gao et al., 2019; Gillette et al., 2020; McDermott et al., 2020; Mun et al., 2019; Vasaikar et al., 2019). Selected highlights include kidney cancer, lung cancer, head and neck cancer, and pediatric brain cancer.

Biological insights from proteogenomics

CPTAC investigators demonstrated that for the three previously characterized tumor types from TCGA (colorectal, ovarian, and breast), combining proteomic and genomic resources uncovered transcript levels that by themselves are not sufficient to predict protein levels in many scenarios and that PTMs, such as protein phosphorylations, provide critical data on functional regulation (Mertins et al., 2016; Zhang et al., 2014, 2016). For instance, in the colorectal study (Zhang et al., 2014), it was shown that RNA abundance did not reliably predict protein abundance; most DNA focal amplifications did not result in corresponding elevations in protein level; a subset of genes were identified that might be targeted for therapy in future studies; and five proteomic subtypes were discovered, with one subtype being associated with highly aggressive tumors with poor clinical outcome. In the breast cancer study (Mertins et al., 2016), new

protein markers and signaling pathways were uncovered for breast cancer subtypes carrying frequent mutations such as PIK3CA and TP53 mutations. This study also identified 10 genes with CNAs that could be functionally connected to CNA gain-and-loss *trans*-protein-level effects. Interestingly, E3 ligase *SKP1* and the ribonucleoprotein export factor *CETN3* on chromosome arm 5q, which is frequently deleted in basal-like breast cancer, were found to be potential regulators for the expression of *EGFR* and *SRC* kinase. In addition, clustering tumors based on phosphorylation pathways revealed a G-protein-coupled receptor subgroup that is not readily identified at the RNA level.

For kidney cancer (clear cell renal cell carcinoma) (Clark et al., 2019), there was upregulation of protein signatures linked to immune responses, epithelial mesenchymal transition (EMT), and multiple signaling pathways (e.g., hypoxia, glycolysis, and mammalian target of rapamycin [mTOR]). However, proteins involved in the tricarboxylic acid (TCA) cycle, fatty acid metabolism, and oxidative phosphorylation were downregulated. The disconnect between mRNA and protein levels was particularly evident for genes involved in oxidative phosphorylation. This study also revealed that phosphorylated cyclin-dependent kinase 1 (CDK1) and mitogen-activated protein kinase 1 (MAPK1; also known as ERK2) were upregulated at the protein level. In addition, clustering proteomic and genomic data identified four immune-based subtypes based on distinct immune cell subpopulations in the stroma, which might have the potential to predict patients' response to treatment and overall survival. The four immune-based subtypes are CD8-positive inflamed tumors, CD8-negative inflamed tumors, vascular endothelial growth factor immune desert tumors, and metabolic immune desert tumors.

For lung adenocarcinoma (Gillette et al., 2020), it was observed that a key protein, PTPN11 (also called SHP2), was frequently and highly activated in *ALK*-fusion- and *EGFR*-mutation-driven tumors. PTPN11 is a known therapeutic target, and these data suggest that patients whose tumors harbor *ALK* fusions or *EGFR* or *KRAS* mutations might benefit from therapies that reduce PTPN11 activity. The study also revealed features that help explain aspects of the immune system's response to tumors as well as flagging potential targets for immunotherapy. For instance, it was noted that immunologically "cold" tumors harboring mutations in the gene *STK11* also showed a strong signal, detectable only through proteomics, for neutrophil degranulation, a process that may affect the anti-tumor immune response. Additionally, the study found evidence that some immunologically "hot" tumors had in fact mounted their own defenses, populating themselves with immune-inhibitory cells. Integrated proteogenomics further identified a number of potential therapeutic vulnerabilities, including anti-CTLA4 therapy and IDO1 inhibition, in immune-hot tumors. And by comparing tumors and normal tissues across patients with and without a history of smoking, multiple pathways that were expressed differently between smoker's and never-smoker's tumors were identified. This study was done in coordination with an independent lung study in Taiwan that further identified new interactions between lung tumors and the immune system, pointing to opportunities for new therapies and a deeper understanding of lung cancer biology that was not revealed using only genomic data.

For HPV-negative head and neck squamous cell carcinoma (Huang et al., 2021), three therapeutically actionable molecular subtypes were identified. The first subtype, high chromosome instability (CIN), showed the worst prognosis. It was associated with the larynx, a strong history of smoking, and high instability of chromosomes. Because this subtype was associated with frequent aberrations of the CCND1 and CDKN2A genes, and high activity of the enzymes CDK4 and CDK6, this type of cancer may respond to anti-cancer drugs called CDK4/6 inhibitors. The second subtype, basal, showed protein elevations of several basal factors, the most basic set of proteins needed to activate gene transcription. It was characterized by high epidermal growth factor receptor (EGFR) ligand expression (e.g., AREG and TGFA) and high EGFR pathway activity, suggesting potential response to EGFR monoclonal antibody (mAb). The third subtype, immune, was found among tumors in people who did not smoke and showed high expression of multiple immune checkpoint proteins. Researchers hypothesize that these tumors may respond to anti-cancer drugs called immune checkpoint inhibitors. Overall, 32% of the CIN tumors, 62% of the basal tumors, and 83% of the immune tumors had potential for treatment with CDK inhibitors, EGFR mAb, and immunotherapy, respectively. Furthermore, proteogenomic comparison of the mutually exclusive relationship between *FAT1*-truncating mutations and 11q13.3 amplifications revealed dysregulated actin dynamics, elucidating the association between these genomic aberrations and poor prognosis.

In pediatric brain cancer (Petralia et al., 2020), comprehensive proteogenomic analysis was performed on tumors across seven histologic types of childhood brain tumors (low-grade glioma, ependymoma, high-grade glioma, medulloblastoma, ganglioglioma, craniopharyngioma, and atypical teratoid rhabdoid tumor). Proteomics identified two new subgroups of pediatric craniopharyngioma, with one subgroup having similar proteomic/phosphoproteomic features to pediatric low-grade $BRAF^{V600E}$ glioma. Genomically, pediatric craniopharyngiomas harbor beta-catenin gene mutations, while adult craniopharyngiomas have $BRAF^{V600E}$ mutations, revealing that BRAF inhibitors are not appropriate for these pediatric tumors. However, this study showed that a subgroup of pediatric craniopharyngioma share proteomic features with pediatric low-grade $BRAF^{V600E}$ glioma, despite being wild type for *BRAF*. The clinically actionable implication of this observation is that based on protein expression similarity, the oncogenic signaling networks in pediatric craniopharyngioma and $BRAF^{V600E}$ tumors are apparently related and could potentially be treated with targeted therapies such as MEK/MAPK inhibitors that have been successfully used for low-grade $BRAF^{V600E}$ glioma. Furthermore, proteogenomic analysis revealed an association between downregulation of IDH1/2 protein abundances and poor survival in pediatric high-grade gliomas, thereby expanding the prognostic molecular markers for pediatric brain tumors beyond the presence/absence of histone mutations, a pathognomonic feature of the disease.

Challenges and opportunities to implementing proteogenomics in clinical trials and drug development

Proteogenomics is emerging as a new discipline in the clinical setting because of its potential to fill clinical gaps that exist

with single-omics approaches. Although the individual methodologies used to perform proteogenomics are well validated in research settings, they face several challenges which are being addressed by CPTAC. Prominent among these challenges is the need for biopsy-scale small sample size to conduct proteogenomic characterization, being able to meet clinical laboratory requirements and regulatory considerations for proteomic measurements, and the need for effective collaboration and data sharing involving large datasets while maintaining public access to these datasets.

Biopsy-scale small sample size

In regard to challenges of using biopsy specimens for proteogenomic characterization, micro-scale techniques for comprehensive proteogenomic analysis from single-core needle biopsies have been developed recently (Satpathy et al., 2020). Until now, joint analysis of proteins and genetic changes required too much tissue to be of immediate clinical utility. In the micro-scale study, needle core biopsies obtained from HER2-positive breast cancer patients before and after HER2-targeted therapy underwent comprehensive untargeted proteogenomics analysis. Results showed a reduction of HER2 protein phosphorylation after HER2-targeted therapy in tumors from the patients who responded to treatment, and no corresponding reduction in tumors from those who did not respond. In addition, tumors that did not respond to treatment demonstrated diverse resistance mechanisms to HER2-targeted therapeutics that might be addressed with alternative therapeutic approaches. This method brings the potential of clinical proteogenomic approaches to the precision oncology clinical trial setting, to stratify patients diagnosed with breast cancer and identify the appropriate systemic therapy.

Clinical laboratory requirements and regulatory considerations

Meeting clinical laboratory requirements and regulatory considerations for MS-based proteomic measurements, analytical parallels can be made to the field of genomics. Untargeted MS-based proteomics has evolved as a powerful technology that enables the proteome-wide detection and quantification of proteins in complex samples (Zhang et al., 2019). And while next-generation sequencing (NGS) in genomics provides a powerful tool for both untargeted mutation discovery and targeted clinical assays designed to detect preselected mutations, MS-based metrology has followed a similar path. However, in contrast to unbiased MS-based proteomics, targeted MS-based proteomics (single-plex or multi-plex) is selective in the proteins to be measured, eliminating several clinical limitations of conventional untargeted MS-based proteomics, such as reliance on reference protein databases due to internal physical reference standards (Lange et al., 2008; Pan et al., 2009), and limited sensitivity for low-abundance analytes by focusing the full analytical capacity of the instrument on a discrete number of analytes (Gillette and Carr, 2013; Picotti et al., 2013; Wang et al., 2009). It should be mentioned that MS-based proteomic techniques (untargeted and targeted) can be used with a wide range of biological samples, including serum or plasma, frozen tissue, and archived specimens (formalin fixed and paraffin embedded) (Zhang et al., 2019). This preclinical work is now enabling the implementation of targeted MS-based assays in clinical laboratories and drug development, especially as targeted MS-based fit-for-purpose assays are becoming available for the quantification of panels of cancer-associated proteins (Whiteaker et al., 2014).

For instance, CPTAC has collaborated with clinical reference laboratories to develop and implement a targeted MS-based proteomic assay that circumvents the interference of autoantibodies. This CLIA complaint assay, which represents one of the earliest translations of a targeted MS-based proteomic assay to the clinical environment, is for the accurate measurement of serum thyroglobulin levels in patients with known or suspected antithyroglobulin autoantibodies or heterophile antibodies (Hoofnagle et al., 2008; Hoofnagle and Roth, 2013). Thyroglobulin is an important cancer biomarker used in monitoring patients receiving treatment for differentiated thyroid carcinoma. This MS-based assay has replaced traditional immunoassays used for the quantification of thyroglobulin levels in patient samples and is now offered by six clinical reference laboratories in North America (Clarke et al., 2012; Kushnir et al., 2013; Netzel et al., 2014; Shuford et al., 2017). While targeted MS-based proteomic assays used in clinical labs are laboratory-developed tests, the FDA and CPTAC have held public workshops addressing analytical validation of protein and peptide MS-based assays (Regnier et al., 2010; Rodriguez et al., 2010).

In a recent pharmacodynamic biomarker study, CPTAC also demonstrated the use of a targeted MS-based assay panel to quantify proteins and their protein networks for a pharmacodynamic and proof-of-mechanism study to confirm the inhibitory mechanisms underlying new therapeutic compounds and to inform drug dose and scheduling in clinical trials. In this study, researchers used targeted MS-based proteomics to identify Ser635-phosphorylated RAD50 protein as a novel pharmacodynamic biomarker for DNA damage checkpoint signaling kinase inhibitors, such as those against ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related), which are being tested in clinical trials in a variety of cancers. This study contains the first demonstration of targeted MS-based proteomics being used to measure a pharmacodynamic biomarker for clinical deployment (drug development) and highlights the potential to implement this approach in clinical settings, to identify new pharmacodynamic biomarkers and measure them accurately (Jones et al., 2018).

Collaboration with public sharing of data and resources

Collaboration has been an integral part of cancer research for a long time, but more recently, the nature of collaboration is evolving from the conduct of research within institutions to team science approaches that involve partnerships across academic, governmental, and for-profit institutions in order to respond to the complex challenge of cancer. Although this type of interdisciplinary multi-site collaboration is needed to accelerate the pace of all research and encourage long-lasting partnerships involving researchers, clinicians, and patients (Börner et al., 2010; Jones et al., 2008), barriers still exist to sharing data, software, and research products among consortium partners and the scientific community. While challenging, multi-site collaborations result in increased research productivity as demonstrated in TCGA and CPTAC activities,

where investigators collaborate and share data across the consortium, with data and resources made available to the public.

As a testament to the power of collaboration, CPTAC's approach to proteogenomics cancer research and the use of standards among labs to ensure rigor and reproducibility have paved the way for the development of two Cancer-Moonshot-inspired initiatives in global and military health. The International Cancer Proteogenome Consortium (ICPC; https://icpc.cancer.gov) (Rodriguez and Pennington, 2018) encourages collaboration and public data sharing (Data Sharing Pledge) in proteogenomic cancer research among research institutions around the world that represent the diversity of people and of cancers around the world, as cancer knows no borders. This pledge to share data among partnering institutions and the public has recently been honored in the first studies by the consortium. Studies include oral squamous cell carcinoma (Chen et al., 2017), early-onset gastric cancer (Mun et al., 2019), hepatitis B virus (HBV)-related hepatocellular carcinoma (Gao et al., 2019), and lung adenocarcinoma in Taiwanese never-smokers population (Chen et al., 2020). At present, ICPC consists of research institutions that span 14 countries (Australia, Canada, Germany, China, India, Japan, South Korea, Spain, Sweden, Switzerland, Taiwan, the Netherlands, United Kingdom, and the United States) with projects focused on a wide range of cancer types. The Applied Proteogenomics Organizational Learning and Outcomes (APOLLO; https://apollo.cancer.gov) (Fiore et al., 2017) program encourages collaboration and public data sharing among the NCI, the Department of Defense, and the Department of Veterans Affairs, to incorporate proteogenomics into patient care on active-duty military service members in the Department of Defense and veterans.

The advent of such initiatives and the high-throughput technologies they use will only increase the sheer amount of data being generated. Unfortunately, accessing usable data from public repositories is challenging due to intersecting issues of data location, characterization, quality assessment, use approval, and compliance, resulting in delays and unreasonable resource expenditures. Addressing this challenge, the NCI is leading the charge in data and resource sharing through several public repositories. For example, CPTAC has contributed to the development of some of the largest and highly curated public repositories (a community data warehouse) of proteogenomics datasets, assays, and reagents in cancer research. These NCI community resources include genomics data at the NCI GDC, proteomics data at the NCI Proteomic Data Commons (PDC; https://pdc.cancer.gov), imaging data at the NCI The Cancer Imaging Archive (TCIA; https://www.cancerimagingarchive.net) and the NCI Imaging Data Commons (IDC; https://portal.imaging.datacommons.cancer.gov), proteomic-targeted assays at the NCI Assay Portal (https://assays.cancer.gov) (Whiteaker et al., 2014), and antibodies at the NCI Antibody Portal (https://antibodies.cancer.gov). Many of these components are within the broader NCI Cancer Research Data Commons (https://datacommons.cancer.gov), which is a cloud-based data science infrastructure connecting datasets with analytic tools to allow users to share, integrate, analyze, and visualize cancer research data to drive scientific discovery.

FUTURE DIRECTIONS: THE NEXT HORIZON IN PRECISION ONCOLOGY

Ever since the announcement of the Precision Medicine Initiative (Collins and Varmus, 2015), there have been progressively increasing efforts to identify and understand the basis of cancer using high-throughput technologies and the development of specialized treatments for specific subtypes of cancer, based on molecular evidence.

Looking forward, opportunities in precision oncology involve the integration of (multi)omics data to better understand the molecular mechanisms, processes, and pathways discriminating health and disease to provide further insights into cancer treatment and diagnosis. It seems reasonable to expect that proteogenomic data will illuminate mechanisms of drug sensitivity and resistance as well as identify new targets for drug development. Accomplishing these goals will involve a natural progression from increasingly detailed maps of pathogenic processes (a basic science goal) to clinically actionable assays based on that increased molecular understanding (a clinical goal).

The future of precision oncology will, in this scenario, depend on technological advances including the continued improvement of tissue acquisition techniques (Ferry-Galow et al., 2018) along with advances in MS and information technologies, which will permit more rapid, quantitative comprehensive proteomic examination of clinical cancer biopsy specimens (Satpathy et al., 2020). It will also be important to improve the capability of measuring a variety of PTMs in tumor biopsies before and on treatment. Sequential proteogenomic data obtained during treatment promises to reveal mechanisms of drug action and cellular response/resistance to treatment that would be difficult to obtain in any other way. Quantitative assessment of these treatment-related molecular alterations also has the potential to accelerate the development combination cancer therapeutics. Another area of importance will be the ability to quantitatively analyze protein networks at single-cell resolution to help understand the molecular heterogeneity prevailing in tumors (Budnik et al., 2018; Tsai et al., 2020) as well as the special interactions between distinct cell types within tumors and their microenvironments. Furthermore, connecting imaging data (histopathology and radiology) with molecular data (genomics and proteomics) through artificial intelligence (AI) and machine learning (ML) techniques to rapidly analyze large datasets will play an important role in precision oncology. This includes identifying potential biomarkers that could determine the presence and/or type of cancer (diagnostic), providing insight into the patient's overall outcome with or without standard treatment (prognostic), or identifying which treatment the patient is most likely to benefit from (predictive).

As proteogenomics refines the subclassification of histological subtypes of cancer, cancer biologists will focus their attention on ways to modulate the essential drivers of malignancy in each molecular subtype. As a drug is developed, its mechanism of action should be more clearly understood in the context of a particular molecular subtype. For instance, non-small cell lung cancer, which has long been subdivided histologically into adenocarcinoma and squamous carcinoma, has been

reclassified as a collection of more rare subtypes, defined by key genetic changes involving ALK, EGFR, MET, ROS1, BRAF, NTRK, and many other alterations. However, to take full advantage of these advances and harness the potential of precision oncology, we need to disseminate the implementation of molecular tumor characterization into clinical trials. This approach to advancing patient care was emphasized in March 2019, when the FDA released a guidance document for industry entitled, "Enrichment strategies for clinical trials to support demonstration of effectiveness of human drugs and biological products" (FDA, 2019b), which provides approaches where treatments are targeted at groups of patients based on clinical laboratory tests and genomic or proteomic factors. The guidance forms part of the FDA's effort to help expand the use of these approaches and facilitate development of innovative enrichment strategies in tandem with advancing science (FDA, 2019a).

Advances in technology have played a key role in our ability to quantitatively measure proteins on a large scale, resulting in better unification with genomics over the past decade. The clinical implementation of these technologies and the shift toward a deeper understanding of disease based on molecular biology will require a high level of expertise across multiple medical and scientific disciplines, including basic science, bioinformatics (multiomics data and ML), medical and surgical oncology, pathology, genetic counseling, tumor and population genetics, and clinical trials.

CONCLUSIONS

Precision oncology will continue to evolve during the next decade. Extensive analysis of cancer genomics data from thousands of tumors from all major cancer types has facilitated the molecular classification of cancer in modern medicine, to guide precision oncology approaches for patients. Genomics should be, and is becoming, an essential component of precision medicine clinical trials and patient care, including genetic data from tumor biopsies and/or blood taken before treatment and at relapse, to assign the tumor to a molecular subtype of cancer and detect the outgrowth of tumor subclones. This approach to the longitudinal evaluation of the molecular characteristics of a patient's malignancy using tumor biopsies or blood samples will deepen our knowledge of genotype-phenotype correlations and mechanisms of therapeutic sensitivity and resistance. However, at present, genomic data alone do not always provide sufficient insights into patient prognosis or treatment. Therefore, other forms of molecular data must be considered in conjunction with genomics to further our understanding of cancer and improve patient care.

Proteogenomic approaches to learn more about the molecular makeup of patients and their tumors will help in identifying potentially actionable therapeutic molecular targets. It will then be possible to study the relationship between molecular findings and cancer treatment outcomes and to accelerate novel clinical trials with biomarkers of prognostic and predictive value. We anticipate that the integration of proteogenomics directly into the fabric of clinical trials and patient care, along with interdisciplinary collaboration, data sharing, and patient involvement will help in identifying cancer subtypes that respond well or poorly to available therapies, thus prompting a virtuous cycle in which new drugs and regimens are developed that target the vulnerabilities and mechanisms of resistance of the recalcitrant cancer subtypes. This new horizon in precision oncology will transform the way we think about patients and treatment—not just for the different types of cancer, but potentially for many other diseases as well.

ACKNOWLEDGMENTS

This work was performed by multidisciplinary collaborative teams of investigators from the U.S. National Cancer Institute's The Cancer Genome Atlas (TCGA) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC). CPTAC collaborates with domestic and international research institutions to accelerate the understanding of the molecular basis of cancer through the application of proteogenomics, standards development, and publicly available datasets. We are grateful to all of the researchers, clinicians, patients and families who contributed to these studies.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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