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REVIEW ARTICLE

Comprehensive genomic profiling for oncological advancements by precision medicine

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Abstract

Considerable advancements in next generation sequencing (NGS) techniques have sparked the use of comprehensive genomic profiling (CGP) as a guiding tool for precision-centered oncological treatments. The past two decades have seen the completion of the human genome project, and the consequential invention of NGS. High-throughput sequencing technologies support the discovery and commonplace use of individualized cancer treatments, specifically immune-centered checkpoint inhibitor therapies, and oncogene and tumor suppressor gene targeted therapies. Nevertheless, CGP is not commonly used in all clinical settings. This review investigates the clinically relevant applications of CGP. Studies published between the years 2000–2023 have shown substantial evidence of the benefits of integrating CGP into routine care practice, while also making important comparisons to current-standard oncological treatment strategies. Findings of a comprehensive genomic profile includes predictive, prognostic, and diagnostic biomarkers, together with somatic mutation identification which can indicate the efficacy of immunotherapies and molecularly guided therapies. This review highlights the importance of CGP in identifying driver mutations in tumors that subsequently can be effectively targeted with molecular therapeutics and lead to drug discovery, allowing for increased precision in treating tumors selectively based on their specific genetic mutations, thereby improving patient outcomes.

Keywords Comprehensive genomic profiling · Precision medicine · Oncology · Immunotherapy · Targeted therapy

Introduction

The identification of molecular aberrations through the practice of comprehensive genomic profiling (CGP) is a fundamental pillar in the development of precision medicine [1]. Numerous studies which were published between the years 2000–2023 indicate evidence of the strong benefits of integrating CGP into routine care procedures, in comparison to current oncological standard-of-care. Evolution of sequencing technologies led to the development of next generation sequencing (NGS) and have provided medical professionals with the ability to mass sequence genes from distinct cancer types in a concurrent manner [2]. At the end of the nineteenth century, cancer was classified as a disease of cells, and that theory has been the governing principle in oncology ever since [3]. The fundamental premise in cancer development is that a cancer forms when a mutated cell divides and proliferates uncontrollably [4]. Many cancers can be attributed to somatic mutations, which cannot be identified through germline genetic testing [5]. A comprehensive analysis of a tumor sample can reveal major

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genomic variant classes such as single nucleotide variants, insertions/deletions (indels), copy number variants, fusions, and splice variants [6, 7]. In current clinical practice, cancer specific genes are commonly analyzed, such as epidermal growth factor receptor (EGFR) in patients with lung cancer [8]. CGP provides a more extensive overview and can identify targetable alterations not commonly associated with specific cancer types [9].

Precision medicine encompasses the theory that individualized treatment plans can be curated for patients based on their characteristic genomic profiles [10]. Each individual cancer and tumor have unique aspects that can be applied to matched-therapies, which have been proven to be more effective in cancer treatment than generalized therapies [11]. The generic pipeline that leads to the use of precision medicine is as follows: clinical research facilitates the identification of novel biomarkers with potential diagnostic or curative effects, which can be applied to the selection of a precise treatment plan for an individual [9]. Due to rising technological advancements, a substantial number of biomarkers have been recently recognized. The identification of novel proteomic, transcriptomic, genomic, epigenetic, and immunological biomarkers opens a larger pathway for informed directives related to oncology treatment. Such biomarkers have been identified by certain biological and molecular characteristics, and include circulating tumor DNA (ctDNA), DNA methylation, unique genomic and molecular testing results, as well as other results offered by companion diagnostics [12]. The rapidly expanding landscape of currently available oncologic biomarkers represents an area for potential exponential growth in the sphere of cancer treatment. Figure 1 depicts how CGP for patients with one cancer type may gain access to targeted therapies indicated for another cancer type due to the presence of mutations in genes that would not otherwise be investigated. CGP may also yield biomarkers that bring eligibility for clinical trials and access for more therapeutics, including immunotherapies. In addition to predictive, prognostic, and diagnostic potential, widening the array of biomarkers may result in drug discovery. Each year regulatory approval is given to more molecularly guided therapies that identify actionable biomarkers [13]. Implementation of these biomarker-guided treatments is an ongoing process, with the goal of becoming standard of care [13]. As knowledge about genomic profiles of cancer patients expands, it is becoming increasingly prominent that the shift toward precision-centered therapies will be more effective in cancer care than site-specific tumor testing [11].

CGP by NGS is a powerful genomic characterization technique that can work off the principles of basic DNA and RNA biology to provide oncologists with an important guide for therapeutic decision-making [14]. By concurrently sequencing and examining cancer related genes, information collected by this test can identify common biomarkers between various cancers, as well as possible pharmaceutical therapies, connections to databases, and clinical trials which may not have previously been available to the patients [15].

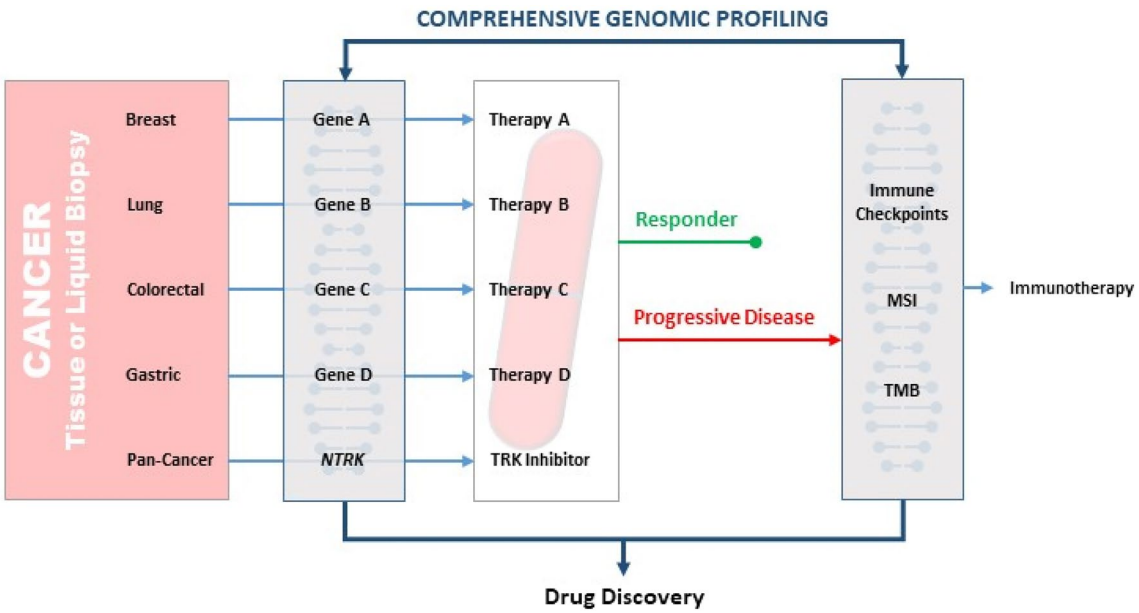

Fig. 1 Graphical overview of how Comprehensive Genomic Profiling leads to oncological advancements by precision medicine. *MSI* microsatellite instability, *TMB* tumor mutational burden

Oncological treatment strategies

Due to the complex nature of cancer and its corresponding subtypes, stratifying cancer care into standard procedural groups is challenging. In a general sense, common treatment strategies that span most cancers include systemic therapy (e.g., chemotherapy, monoclonal antibodies, kinase inhibitors), radiation therapy, and surgery [16]. These treatment options can be used simultaneously with one another, and vary mostly on an individual's age, cancer subtype, and stage of progression. The rise in molecular genomic research, particularly in the area of tumor-agnostic profiling has led to the development of new cancer treatment paradigms, namely targeted therapies related to immune driven oncological treatments, and targeted therapies for oncogene and tumor suppressor gene driven cancers [17]. Tumor-agnostic treatment can target genomic aberrations regardless of the origin of a malignancy. Although this novel treatment paradigm carries with it many promising results, there are certain limitations associated with type of oncological treatment strategy [18]. Pan-cancer treatments are often discovered using the "basket trial" technique, testing treatments tailored to specific genomic abnormalities on the same tumor type, regardless of tumor histology. A limitation of this technique, however, is that one genomic alteration may have different impacts on various tumor types. Thus, the efficacy of tumor-agnostic treatment may vary by disease site [19].

Oncogene and tumor suppressor gene driven cancers

The two main genes that play a role in cancer formation can be classified as oncogenes and tumor suppressor genes. Identification of such genes through CGP can uncover information about each tumor and related targeted therapies.

Oncogene driven cancers refer to those cancers that are caused by the unregulated and accelerated cell division from mutated proto-oncogenes. When a gain-of function mutation occurs, this essentially converts the proto-oncogene into an oncogene. Proto-oncogenes function to aid in cell growth and division, but when the proto-oncogene is mutated into an oncogene, this can lead to uncontrolled division and growth—causing tumorigenesis [20].

Tumor suppressor genes are a type of gene that help to prevent irregular cell growth, maintain DNA repair, and induce apoptosis of cells. When a loss-of-function mutation occurs in a tumor suppressor gene, the abilities of the tumor suppressor gene are inhibited, and uncontrolled cell division can occur [21].

Identification of novel oncogenes and tumor suppressor genes through CGP

CGP has the ability to identify four main types of genomic alterations. These include base substitutions, insertions and deletions, copy number alterations, and rearrangements/fusions [14]. An important step in the interpretation of these alterations is determining their driver status. If a driver mutation is present, this means that the cancer has the potential to continue to mutate and spread [22]. Passenger mutations are the nonsense mutations in the background that should be disregarded [22]. By using low-throughput sequencing technologies, some commonly recurring somatic mutations in proto-oncogenes have been identified and targeted in specific cancer types [22]. For example, in non-small cell lung cancer a recommended list of somatic mutations has been released with associated targeted therapies, as shown in Table 1 [23].

Known as the 'guardian of the genome,' the wild-type p53 tumor suppressor gene functions to protect DNA against damage that could be caused by cells with an impaired genome [24]. Through tactics of cell cycle arrest or apoptosis, this gene can protect the human genome from further damage due to proliferation of DNA damaged cells [25].

TP53 is one of the most prevalent mutated tumor suppressor genes, found in about 30–50% of all cancers, and is due to the occurrence of loss-of function mutations [26]. More specifically, *TP53* is caused by missense mutations of the p53 gene, resulting in the loss of tumor suppressor functions of wild-type p53 [27]. Additionally, mutated p53 genes are more susceptible of acquiring gain-of-function mutations, leading to oncogene formation [27].

Table 1 Commonly recognized oncogenes in non-small cell lung cancer and associated targeted therapies [35, 36]

EGFR	*ALK*	*ROS1*	*BRAF*	*MET*	*RET*	*KRAS G12C*	*NTRK*
Erlotinib	Crizotinib	Crizotinib	Dabrafenib	Capmatinib	Selpercatinib	Lumakras	Larotrectinib
Afatinib	Ceritinib	Ceritinib	Trametinib	Tepotinib	Pralsetinib		Entrectinib
Gefitnib	Alectinib	Lorlatinib	Ecorafenib				
Osimertinib	Brigatinib	Entrectinib					
Dacomitinib	Lorlatinib						

When a p53 mutation occurs, either inherited or sporadic, tumor suppressor abilities of the p53 gene are lost. If an inherited p53 mutation exists alongside a new sporadic p53 mutation, then the germline p53 mutation Li-Fraumeni syndrome occurs, where p53 is completely inactivated leading to a cascade of different tumors [24]. Individuals diagnosed with Li-Fraumeni syndrome gain predisposition to cancer, most commonly in adrenocortical carcinomas, breast cancer, central nervous system tumors, osteosarcomas, and soft-tissue sarcomas [28, 29].

Due to the complex nature of a tumor suppressor gene, therapeutic treatment strategies are complex and still being developed. In order to target the mutated *TP53* gene of the p53 apoptotic pathway, three categories exist: targeting the molecules that have regulatory and inhibitory effects on p53 for degradation, re-introducing wild-type p53 function, or selectively killing mutated p53 cells [27]. Various strategies of implementation have been explored and are summarized in Table 2.

The identification of germline p53 mutations is an example highlighting the importance of identifying germline genetic mutations. Another example includes germline BRCA mutations, as a gBRCA mutation leads to targeted treatment choices with PARP inhibitors, risk stratification for breast and ovarian cancers Through the practice of genetic counseling, germline findings can be identified, giving patients a better idea about their risk for development of certain cancers as well as provide a better understanding about the behavior of their cancer. These findings may also reveal specialized treatments, not generally considered for use in standard treatment of certain cancers. Thus, a comprehensive understanding of germline genetic mutations is also a powerful tool in oncology treatment.

Although there is substantial information covering the oncogenes of cancers such as NSCLC, other cancer classes do not have commonly identified somatic mutations that can be targeted with precision therapies. The application of NGS techniques can lead to the identification of previously unknown oncogenes and tumor suppressor genes [30].

CGP can test hundreds of cancer related genes for the four main classes of genomic alterations in order to determine what therapies can be applied to the patients and gain a broader understanding of the efficacy of these therapies on an individualized basis. Currently some companies that offer CGP testing include Foundation Medicine, Illumina, Roche Diagnostics; Thermo Fisher Scientific, LabCorp, QIAGEN; and Exact Sciences, among others. Each company offers various styles of CGP with different panel sizes and assays [31].

From a technical standpoint, CGP is a bioinformatic approach using aspects of computational biology to cumulatively test for genomic mutations rather than single-gene testing as done in routine practice. Multi-gene assays provide higher insight into cancer genomic makeup and thus can more accurately guide oncologists and members of the treatment team prior to initiation of various therapeutics.

Recently, CGP by NGS has seen major refinements, leading to advancements that can provide clearer information about epigenetic modifications, as well as genomic structure and variations. Epigenetic modifications include some biomarker activity discussed above, such as DNA methylation, histone modification, and noncoding RNA action [32]. These traits can act as oncological markers or reveal information about cancer driver activity. Appearing at early stages of cancer development, epigenetic markers are diagnostically applicable in oncology, describing non-DNA related transcriptional repression of gene activity [33].

This review will take Illumina's TruSight Oncology 500 (TSO500) assay as an example of a commercial CGP by NGS platform. The Illumina TruSight Oncology 500 assay is designed for CGP of solid tumor samples to identify variants and key biomarkers. This pan-cancer assay tests 523 DNA & RNA genes simultaneously, to identify genomic alterations and biomarkers such as tumor mutational burden (TMB) and microsatellite instability (MSI). After profiling, results must be interpreted using bioinformatics analysis of clinical actionability through the PierianDX software [34]. Several studies to date have used the TSO500 assay to identify genomic alterations and biomarkers in tumor specimens (Table 3).

Immunotherapy

Immunotherapy is a rapidly growing field of research that has had promising results in the cohort of oncological treatment. By harnessing the body's immune system, immunotherapy aims to use natural immune responses to treat cancers [43]. One particularly progressive branch will be highlighted in this review, known as immune checkpoint inhibitors (ICI).

ICIs have become the main line of defense for patients of solid and liquid tumors. ICIs function by reducing the

Table 2 Exploratory therapies for the targeting of the TP53 pathway [27, 37, 38]

	Re-introduce wild-type *p53* function	Degradation of mutant *p53*	Selectively killing mutated *p53* cells
Potential therapy	APR-246 monotherapy COTI-2	Ganetespib SAHA	CRISPR/Cas9 & RNAi

Table 3 Summary of various studies using the TSO500 assay

Study name	Synopsis
Use of an Integrated Pan-Cancer Oncology Enrichment Next-Generation Sequencing Assay to Measure Tumour Mutational Burden and Detect Clinically Actionable Variants	108 formalin fixed, paraffin embedded tissue samples from colorectal, lung, esophageal, and control samples were sequenced using the Illumina NextSeq instrument utilizing the TSO500 assay. A comparison of TSO500 and whole genome sequencing yielded similar results of TMB, MSI, single-nucleotide variants, indels, and copy-number variants, indicating less than 5% variability between repeated controls [39]
Microsatellite instability testing and Lynch Syndrome screening for colorectal cancer patients through tumor sequencing	233 colorectal cancer patients were tested for Lynch Syndrome by analysis of MSI status by commercial MSI-PCR assay. 63 of these patients were also screened using the Illumina TSO500 panel. The overall percent agreement of the two testing methods was 100%, indicating accurate results from both [40]
P2.04-76 Tumor Mutational Burden by TSO500 Next Generation Sequencing Panel and Clinical Outcome in Non-Small Cell Lung Cancer	62 NSCLC patient samples were analyzed by the TSO500 assay to determine TMB status. TMB status indicated higher efficacy to immunotherapy by pembrolizumab or nivolumab [41]
80 Evaluation of the TruSight oncology 500 assay for routine clinical testing of tumor mutational burden (TMB) and clinical utility for predicting response to pembrolizumab	TMB scores for 294 patients were evaluated using TSO500, with F1DCx, and whole exome sequencing as reference panels. This was a comparison test against the FDA approved FoundationOne®CDx test. TMB scores by TruSight oncology were found to be valid for 294/294 patients [42]

body's anti-tumor immune response. The two main immune checkpoints that will be discussed here are CTLA-4 and PD-1. Figure 2 shows the relationship of PD-1/PD-L1 and CTLA-4/B7 with their respective ICIs.

When immune checkpoints are identified through immunohistochemistry (IHC), and relevant biomarkers are identified through CGP, a clear picture of potential therapeutic benefit form a novel therapy such as immunotherapy can be understood. The conjunction of immune checkpoint identification through IHC and biomarker identification through CGP represent diagnostic improvements with

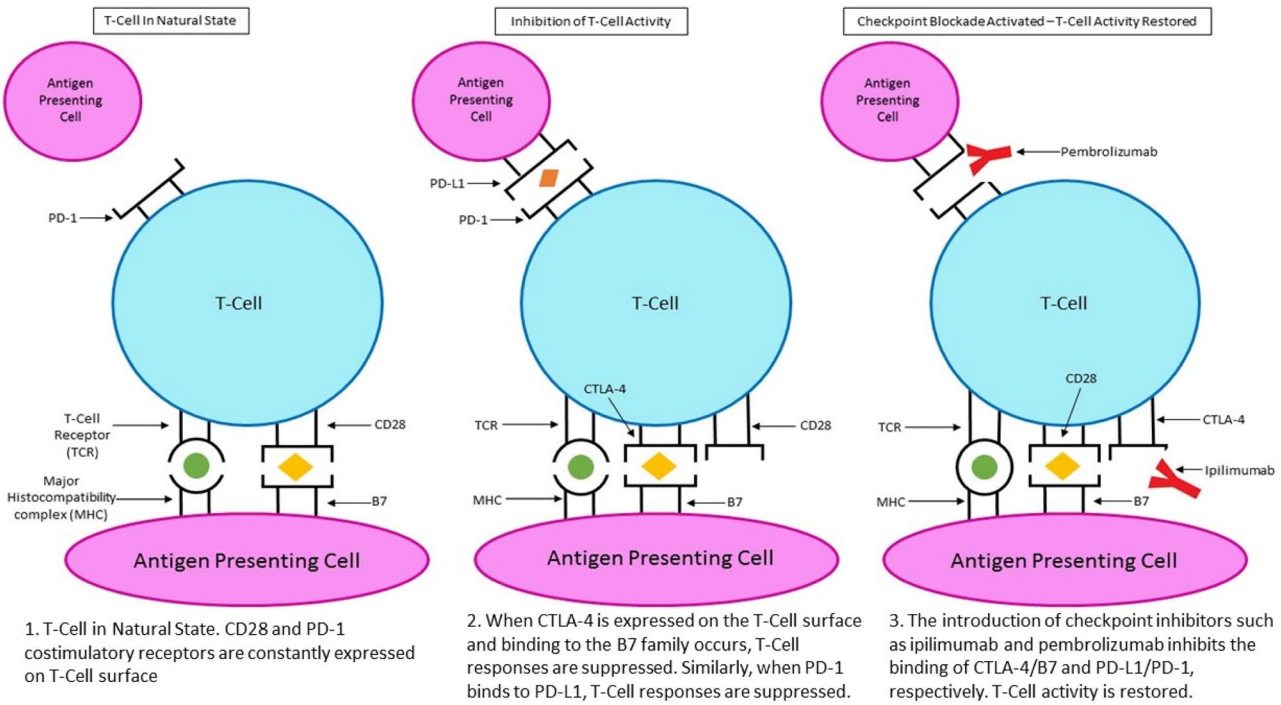

Fig. 2 Relationship between PD-1/PD-L1 and CTLA-4/B7 and immune checkpoint inhibitor therapy

direct actionable impact on cancer patients and improved outcomes from therapy.

CTLA-4

The cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is an immune regulatory agent that modulates the activities of T-cells, primarily in lymph nodes [44]. CD28 is a co-stimulatory receptor that is constantly expressed on the surface of T-cells, aiding in their regular immune function [45]. While CD28 aids T-cells in regular function, CTLA-4 down regulates T-cells, blocking their ability to destroy dangerous cells and keep the immune system from harming healthy cells. Both CTLA-4 and CD28 function to regulate the activities of T-Cells. When CTLA-4 is bound to ligands from the B7 family such as B7.1 and B7.2, T-cell function is suppressed [46]. When ligands of the B7 family are found in the tumor microenvironment, this can indicate that the cancer has developed immune-evading capabilities, thus preventing T-cells from launching a potentially threatening attack on them [47]. Due to the nature of CTLA-4, this agent can act as a target for the ICI known as 'Anti-CTLA-4' therapy. In 2011, the monoclonal antibody known as ipilimumab, was approved by the FDA as a novel treatment for metastatic melanoma [48]. Ipilimumab is an inhibitor of CTLA-4, essentially allowing for a stronger T-cell response to be launched against the cancerous cells.

PD-1/PD-L1

Programmed cell death protein 1 (PD-1) is a receptor, similar to CD28, which has constant presence on the surface of select T-cells (follicular T-cells) and can be induced into expression on others (circulating T-cells) [49]. The stimulation of PD-1 expression can be achieved through exposure to cytokines and transforming growth factor [49]. PD-1 functions as an immune regulator, particularly during T-cell functions such as infection, T-cell tolerance, and homeostasis [50]. The main function of PD-1 is to prevent the immune system from launching an autoimmune response [51]. When PD-1 binds to PD-L1, a transmembrane protein, the PD-1 mechanism is inhibited, and T-cells cease to attack other cells in the body [52]. When a tumor develops the ability to utilize the PD-1/PD-L1 signaling pathway to avoid an immune response this is known as tumor immunosuppression [53]. Expression of the tumor evasion mechanism PD-L1 on the tumor surface, known as immune checkpoints, is one way tumor immunosuppression can occur [53]. Immune checkpoint inhibitor (ICI) therapy can be used to ensure that the binding of PD-1 and PD-L1 does not occur [52]. Pembrolizumab (Keytruda), is an ICI therapy that was approved by the FDA in 2017 and works by inhibiting the PD-1/PD-L1 signaling pathway [52, 54]. This therapy was specifically approved for use in patients with solid metastatic, microsatellite instability high (MSI-H), or mismatch repair deficient (dMMR) tumors [54]. Nivolumab (Opdivo) is another type of ICI therapy approved to work as an anti PD-1 monoclonal antibody [55]. Nivolumab has been seen to have significant applications, as one study examined the effects of nivolumab on the length of survival for patients of squamous-cell carcinoma of the head and neck [56]. This study saw median survival of patients who received nivolumab to be 7.5 months, while those who received standard therapy survived a median length of 5.1 months [56].

Biomarkers can indicate how well checkpoint inhibitors may work

ICIs have revolutionized oncological treatment strategies, with cancer immunotherapy being designated 'breakthrough of the year' by *Science* in 2013 [17, 57]. Immune checkpoint inhibitors, however, do not work for everyone in the same way. Research has revealed that the number of neoantigens that may be present in a tumor can correlate with the efficacy of a checkpoint inhibitor therapy, known as tumor neoantigen burden (TNB) [58, 59]. Additionally, microsatellite instability (MSI), and tumor mutational burden (TMB) are large contributing factors that can predict responsiveness to checkpoint inhibitor therapy [60]. Essentially, the more alterations that a tumor contains, the more targetable that tumor is. Using comprehensive genomic profiling to test for specific biomarkers in a tumor sample can indicate the likelihood of success through the use of a checkpoint inhibitor therapy [60].

Microsatellite instability

Microsatellites are areas within the genome where DNA replication errors occur, specifically when there is a variance in length of the DNA fragments, usually of 1–6 base pairs long [61, 62]. Microsatellite instability (MSI) is a genomic marker that occurs due to mutated mismatch repair proteins (MMR) known as mismatch repair deficiency (dMMR) [63]. For years MSI has been most commonly tested in colorectal cancer and has specifically been known to be a hallmark of patients with Lynch Syndrome [64]. Recently, however, testing for MSI has been correlated to increased efficacy of ICIs. Research has revealed that MSI can be an important indicator of ICIs for patients of various cancer types. In 2017 the FDA approved MSI as a pan-cancer biomarker [65]. MSI has the ability to predict the response an individual may have to ICIs [65]. By applying large-panel NGS testing to tumors of different types, MSI status can be determined, and can

conclude if ICIs would be a viable option for cancer treatment, regardless of tumor origin [65].

MSI is most generally made up of an abundant number of insertion and deletion mutations in the microsatellite areas of DNA [66]. MSI can be classified into high (MSI-H) and low (MSI-L) subgroups [67]. When analyzing MSI, the recommended reference panel to use is the Bethesda Reference Panel [67, 68]. In the Bethesda Panel, there are five microsatellites of focus, two being mononucleotide (BAT-25, BAT-26) and three dinucleotide (D2S123, D5S346, D17S250) [67, 68]. When there is instability in two or more of these loci, the tumor is classified as MSI-H, as recommended by the National Cancer Institute [68].

One study, Keynote-177, analyzed the efficacy and safety of the PD-1 blockade with pembrolizumab in comparison with chemotherapy for individuals with MSI-H (dMMR) metastatic colorectal cancer [69]. This first-line open label clinical trial demonstrated a statistically significant improvement in the co-primary endpoint of median progression-free survival of 16.5 months vs 8.2 months with standard chemotherapy alone. Furthermore, for those patients who received pembrolizumab, 55.3% were alive after 12 months and 48.3% after 24 months. Comparatively, for those patients who received standard chemotherapy, 37.7% were alive after 12 months and 18.6% after 24 months. These results indicate the superior effectiveness of treatment by pembrolizumab, thereby avoiding chemotherapy, in patients with MSI-H-dMMR metastatic colorectal cancers [69, 70].

Tumor mutational burden

Tumor mutational burden (TMB) is one of the most important predictive biomarkers that CGP tests for and is considered a pan-cancer biomarker that can be compared across multiple cancers on the same size panel. TMB is a measure of the amount of somatic mutations present in a megabase of a tumor's genome, which can provide important information leading to the use of specialized cancer immunotherapies [71, 72].

Tumor mutational burden is measured as a score, i.e., the more mutations per megabase present, the higher TMB score recorded. Score classification of TMB can vary. This review will choose one scale of comparison. Following a specific studies' TMB classification format, a score of 1–5 mutations/mb is classified as small, 6–19 mutations/mb is intermediate, and ≥ 20 mutations/mb is high [73]. The score can be indicative of how well an immunotherapy will perform in a patient. Studies have shown that an individual with a high TMB score is a good candidate for ICIs [74].

As a tumor evolves with mutations, a small subset of those mutations will be neoantigens. A neoantigen is able to be recognized by the immune system and be targeted for destruction [75]. The neoantigens in a tumor follow a flow-mechanism as follows: these immunogenic molecules form from somatic mutations in tumor DNA, travel for presentation to the major histocompatibility complex (MHC), become expressed on the cell surface, and trigger CD8+ and CD4+ T-cells to elicit an immune response [75, 76]. As neoantigens only account for a small subset of mutations that occur in tumor DNA, a TMB score can be indicative of the general amount of these neoantigens present. For example, as the TMB score increases, the immunogenic neoantigen presence in the tumor will also increase [76, 77]. Thus, following this logic, a higher TMB score can indicate a higher response rate to checkpoint inhibitor therapy.

Various studies have explored the causes of high TMB and have concluded that one example consists of a large cohort of patients with significant TMB are those who have NSCLC caused by various carcinogens and mutants, such as smoking [78]. One study used CGP by NGS to determine TMB score in patients with NSCLC [79]. The study aimed to determine if patients who were smokers had higher TMB scores and thus preferentially reacted to ICI therapy. Patient results indicated that those who were previous smokers had a higher association with TMB and would be more likely to respond to ICI therapy. High TMB score is associated with higher neoantigen presence, inducing stronger CD8+ T-cell immune activity. The study concluded that TMB has the potential to be a very effective biomarker for ICI therapy [79]. TMB score acts as a predictive biomarker for a variety of cancer types and is consistently seen to correlate high TMB score with a higher overall survival rate [80].

Companion diagnostic tests

Aside from the checkpoint inhibitors mentioned above, many other checkpoint inhibitors exist and have been approved for use in various cancers. Table 4 shows an overview of some of the checkpoint inhibitors that have been approved for use by the US FDA [81, 82].

A companion diagnostic test is an indicative test that can suggest the effectiveness of a checkpoint inhibitor by

Table 4 Overview of current approved checkpoint inhibitor therapies for the PD-1/PD-L1 and CTLA-4 blockades

	PD-1/PD-L1	CTLA-4
Checkpoint inhibitor	Pembrolizumab	Ipilimumab
	Nivolumab	
	Atezolizumab	
	Cemiplimab-rwlc	
	Avelumab	
	Durvalumab	

testing for the number of targeted antibodies that are being expressed on a cells surface. Testing of tumors for genomic alterations prior to the commencement of treatment helps to ensure that the most effective targeted treatment is chosen for a specific tumor, thus avoiding ineffectual treatments, and increasing efficiency and success of therapeutics [83]. Several companion diagnostic tests have been approved for use in the PD-1/PD-L1 and CTLA-4 pathways (Table 5).

Tumor tissue profiling

Tissues that are collected for a sequencing analysis are most commonly of the form of formalin fixed paraffin embedded (FFPE) blocks. FFPE indicates that a fresh tissue sample is preserved in formalin solution, and then embedded into a paraffin wax block before being processed for analysis. Once the tissue is secured in the FFPE block, the morphology and cellular details of that sample will be preserved for many years [85]. Although the utilization of fresh-frozen tissue represents the gold standard for tissue analysis, this is not feasible for many processes—thus requiring an alternative such as FFPE blocks [86]. Studies have been done to determine if the formalin induced mutation on DNA presents a large factor of error in sequencing results [85]. These studies have determined that these mutations are insignificant and sequencing readouts from an FFPE tissue can be observed accurately and with a high degree of confidence [85, 86].

Liquid biopsy

Although solid tissue testing in the form of fresh-frozen or FFPE block tissue yields accurate results, there are many limitations that are associated with this type of sequencing. Some of these include the reluctance of patients to sign up for clinical research studies, longer screening times, and potential for repeat tissue biopsy requirements [87]. To overcome these challenges, liquid biopsy is being explored as a more accessible, minimally invasive, and efficient testing medium [88, 89]. Liquid biopsy involves the testing of blood, or other fluids that contain elements such as cell free DNA (cfDNA), or more specifically to cancer, circulating tumor DNA (ctDNA) [90, 91]. During the normal cell cycle, cells break down and dislodge into the bloodstream, including cells from tumors [92]. The cells that dislodge from tumors and circulate in the bloodstream are what are known as ctDNA [92]. ctDNA has the capacity to reveal important information about a tumor, some of which cannot be accessed through a solid tissue biopsy [92]. Liquid biopsy provides the ability for physicians to track the progression of cancer in real time, supplying integral information about the metastatic progression of the disease and improving the likelihood for early cancer diagnosis [93]. The common applications of liquid biopsy are summarized in Fig. 3. Recently, ctDNA has been proven to accurately identify the likelihood of response to an immunotherapy, as well any potential resistances [94]. This novel testing strategy has been deemed a predictive tool for the implementation of immune-centered oncological treatments [94]. The use of ctDNA as a tool to detect mutations in plasma or serum of individuals diagnosed with cancer has also shown promising results in terms of early cancer detection, indication of tissue of origin, and response and resistance for potential treatment strategies [95].

Although the use of liquid biopsy has not yet become standard practice, the FDA has approved some single and multi-gene assays used to detect genomic alterations and connect them to specific targeted therapies [91]. The FDA approved the Guardant360 CDx assay as a CGP test to detect single nucleotide variants and indels in 55 cancer related genes, copy number alterations in two cancer related genes,

Table 5 Approved companion diagnostic tests relevant to the PD-1/PD-L1 blockade and/or the CTLA-4 pathways, according to the U.S. Food & Drug Administration, as of 2021 [84]

Diagnostic name and manufacturer	Related checkpoint inhibitor	Associated cancer types
PD-L1 IHC 22C3 PharmDx by Dako North America, Inc	Pembrolizumab Cemiplimab—rwlc	NSCLC Gastric or gastroesophageal junction adenocarcinoma Cervical cancer Urothelial carcinoma Head and neck squamous cell carcinoma Esophageal squamous cell carcinoma Triple negative breast cancer
FoundationOne CDx by Foundation Medicine, Inc	Pembrolizumab	Solid tumors greater than 10 mutations/mb
Ventana PD-L1 (SP142) Assay by Ventana Medical Systems, Inc	Atezolizumab	Urothelial carcinoma Triple Negative Breast carcinoma NSCLC
PD-L1 IHC 28-8 PharmDx by Dako North America, Inc	Nivolumab in combination with Ipilimumab	NSCLC

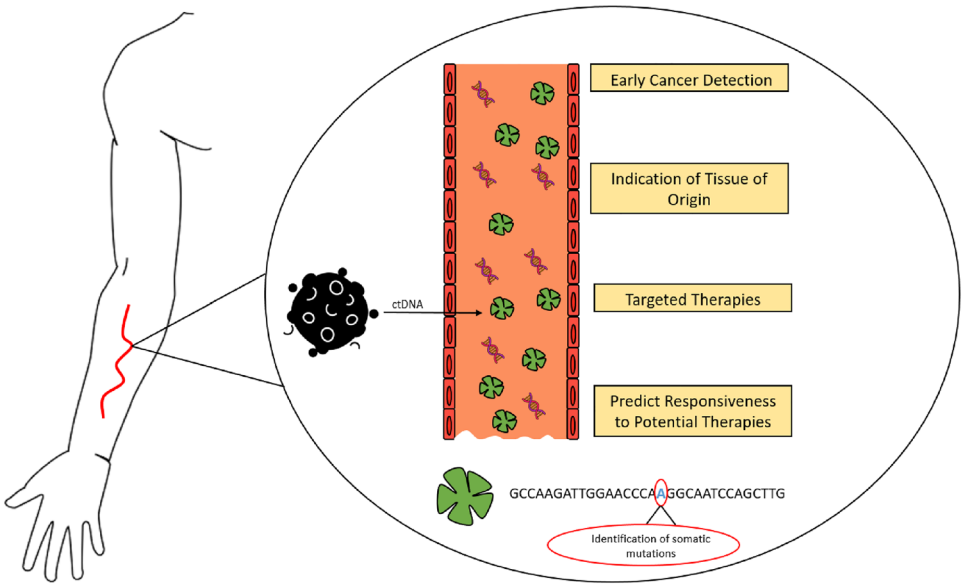

Fig. 3 Overview of the main applications of liquid biopsy testing

and fusions in four cancer related genes in plasma cfDNA [91]. Additionally, F1 Liquid CDx is an FDA approved liquid biopsy CGP test that can analyze 324 genes and identify substitutions and indels in 311 of those genes, rearrangements in four genes, and copy number alterations in three genes with plasma cfDNA [91]. The utility of ctDNA is growing rapidly, but one important limitation is detection. If there is minimal tumor shedding or lysis, the ctDNA fraction may be too low to be detected, thereby resorting to standard tissue sampling.

Future directions of CGP

It has been well established that the use of next generation sequencing in an oncological setting can help to determine the best treatment path by refining initial diagnostic accuracy, thereby identifying more relevant and effective treatments [96]. Having been proven to have standard outcomes regardless of race, sex, and ethnicity, results of a CGP test can help reduce patient exhaustion by starting a treatment regime based on genomic defects and surpassing generalized treatment plans [97, 98]. Presently, clinical utility of CGP in practice has not been thoroughly assessed, and even less so for non-common cancers [99]. To improve the breadth of clinical implementation of CGP, trials of CGP for treatment of non-common cancers are commencing. Primary endpoints being assessed include actionable and druggable genomic alterations, giving an idea of what types of treatments may be able to be implemented. The future of CGP will be largely determined by the present trials being conducted and the findings from these trials which will aid in the integration of this method into clinical practice.

Summary and conclusion

Due to the aggressive and invasive nature of cancer, many cancer types remain to be incurable, with limited available treatment strategies. Advances in targeted therapies have opened a new avenue of treatment options for patients and can be implemented to a variety of cancers depending on their genomic makeup. Comprehensive genomic profiling is a driving force in the accurate implementation of precision-centered therapies. Once a CGP test is completed, novel and unknown variants will be identified, alongside important predictive, prognostic, and diagnostic biomarkers. The results of the test are summarized in a report and can be used to educate and inform physicians about important elements of their patient's cancer. As more CGP assays are approved and released, the use of this sequencing technology is becoming increasingly common. CGP has the potential to induce long-term effects on overall oncological treatment strategies and will thoroughly aid in improving general prognostic outcomes for a vast majority of cancer patients. Knowledge is power, and the more knowledge a physician has about an individualized cancer case, the better the result for that patient will be. Additionally, new research opportunities, novel targeted therapeutics and other discoveries have the possibility of being uncovered through the use of this technology. CGP makes the dream of personalized cancer treatment become a reality.

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Declarations

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