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Genomic Strategies for Personalized Cancer Therapy

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1.1 Introduction

1.1.1 Definition of Precision Medicine in Oncology

In cancer therapy, the initial development effort was on empiric discovery of cancer therapeutics through a search of agents toxic against cancer cell lines. With a better understanding of cancer biology, there was identification of specific cancer targets, such as hormone receptors (HR) (estrogen, progesterone, androgen), human epidermal growth factor family receptors (HER2, EGFR), vascular endothelial growth factor (VEGF) receptors, and others. Perhaps the history of development of epidermal growth factor receptor (EGFR)-targeted therapy best exemplifies challenges on how to maximize the effects of targeted therapy. Initially, EGFR-targeted agents, such as gefitinib and erlotinib, were used as therapy for all non-small cell lung cancer (NSCLC) patients, resulting in only a small proportion of patients benefiting from that therapy. Several years later was there a realization that only individuals with lung cancer tumors with mutated EGFR protein had remarkable responses.

The goal of precision oncology is to understand the molecular mechanisms of cancer formation, and an individual's unique characteristics, in order to maximize therapeutic response and minimize treatment side effects. This can be accomplished by discovery of driver oncogenic pathways in the tumor of an individual, with a set of genetic and protein expression assays, and then directing a specific cancer agent against these pathways to maximize therapeutic response. In addition, there is a parallel effort to understand features about the host to understand an individual's unique drug metabolism or immune status to optimize anticancer effect and minimize toxicity.

The following sections describe methods used for precision oncology, recent advances in genome-guided treatment of selected malignancies, challenges in development of

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biomarkers, precision oncology clinical trial design, and ethical and technological issues ahead in this era of precision medicine in oncology.

1.1.2 DNA and RNA Sequencing Techniques

Over the past decade, molecular biology methods and techniques have become increasingly sophisticated, well-validated, and reliable, as well as more affordable and widespread. The application of precision genomics is still relatively early to the field of medical oncology, and is very much evolving. The ultimate goal is to achieve a true ability to tailor treatments for each individual patient, in real time, as their tumor(s) evolve during the course of drug treatment, as compared to the traditional paradigm of treating classes of patients based on the tissue site of origin of their malignancies [1]. Here, we will provide a general overview of the current landscape of assays and techniques in routine clinical use.

Many platforms have been and remain under investigation, and even those that have been widely adopted continue to be refined to improve accuracy and speed of output. Genomic platforms for precision oncology include tumor-based and blood-based assays, and techniques that target assessment of DNA (deoxyribonucleic acid) and/or RNA (ribonucleic acid) at the genetic level. Sequencing of DNA focuses on identification of potential actionable, or drug targetable, mutations, translocations, and amplifications or gains (involving heterozygous or homozygous deletions) [1]. The methods used include targeted next-generation sequencing (NGS) as well as traditional Sanger sequencing to identify pertinent mutations. Polymerase chain reaction (PCR) is used to determine gene dosage that can vary in amplified regions of DNA [1]. For sites of DNA translocation, while immunohistochemistry (IHC) has been the more traditional standard of identification and validation, use of fluorescence in situ hybridization (FISH) is used more commonly to improve accuracy of detection. A technique that has increased in usage over the past decade is comparative genomic hybridization (CGH), which has especially gained favor for assessing copy number alterations [2]. CGH is used in large-scale, genomically driven basket clinical trials in Europe (e.g. molecularly targeted therapy based on tumor molecular profiling versus conventional therapy for advanced cancer [SHIVA] [2]) and in the United States. Most prominently, the National Cancer Institute's Molecular Analysis for Therapy Choice (NCI-MATCH) trial [3], has used this method in completed portions or ongoing parts of these trial studies. As an example of accuracy of assessment, in NCI-MATCH, the use of NGS achieved a high sensitivity (~96.7%) and specificity (99.9%) [3]. This ability was also found to be highly reproducible across multiple sites (four centers, in the referenced analysis), providing strong support to the notion that basket trials can be done reliably on a wide-scale basis with confidence based on accurate NGS reporting. However, what will be critical in the years to come is the determination of whether smaller to mid-level centers will have the resources to invest in building NGS platforms that can live up to this standard, or whether larger centers will become central clearinghouses for testing of samples sent from other institutions. There has been a concurrent steep rise in the number and quality of commercial entities performing extensive NGS as well, and the turnaround times – and associated costs – of testing have improved in recent years. In fact, the more recent iteration of the NCI-MATCH trial was adapted in 2017 to focus on NGS performed by a limited number of labs, including initially those of Foundation Medicine, Caris Life Sciences, and

Memorial Sloan-Kettering Cancer (via the MSK-IMPACT platform developed at that institution). In 2018, the number of commercial laboratories designated by the NCI has more than doubled, with simultaneous wider expansion of academic laboratories designated for the same purpose (https://ecog-acrin.org/nci-match-eay131-designated-labs).

In the case of RNA, the focus of this sequencing strategy includes mutations and also overexpression of transcribed RNA, as well as translocations representing fusion transcripts [1]. The methods used include traditional Sanger sequencing, but have increasingly incorporated quantitative reverse transcription polymerase chain reaction (qPCR). Assessment of the RNA "transcriptome" has been touted as a preferable approach in some cases, especially due to the fact that transcribed RNA represents expressed forms of the underlying genetic code, rather than the comprehensive amount represented by DNA that may not be expressed at all in the tumor setting [4]. This is especially important as the driver or passenger mutations can become a "moving target" in the course of tumoral evolution during longitudinal treatment of patients with chemotherapeutic or cancer-selective drugs. Both coding and non-coding RNAs are detected through NGS of the transcriptome, and the coding portion is especially promising, as it includes nucleic acid forms of great translational interest (microRNAs, long non-coding RNAs, etc.) that can significantly affect cell metabolism, proliferation, and have other regulatory functions that have further impact on drug efficacy [4]. There is also an additional branch of examination that includes proteomics and even metabolomics; both fields remain under investigation and are being included as correlative biomarkers in clinical trials. How those strategies and methods will measure up to NGS of DNA and RNA will become more evident over time.

It is worth mentioning that at the current time, the most reliable and accurate forms of genomic sequencing of tumors involve assessment of tumor biopsy or surgical specimens. However, with the rise of technologies aimed at improving accuracy of cell-free DNA (cfDNA) and increasing numbers of commercial companies performing this form of assay, there is a growing movement to examine this avenue for several reasons [5]. First, and perhaps most importantly, the ability to assess genomic profiles using blood rather than tumor-based tissue is less invasive, and could potentially speed up the process. Second, with increased recognition of evolution of tumor heterogeneity – and consequent development of drug resistance over time – there is an increased desire to assess molecular changes in order to determine better treatment pathways. The ability to perform this analysis using cfDNA provides the opportunity to do this more feasibly than subjecting patients to repeat procedures and biopsies.

1.2 Precision Medicine in Specific Tumors

1.2.1 Lung Cancer

Lung cancer is the leading cancer in terms of incidence, with 2.09 million cases per year worldwide. It is also the most common cause of cancer death worldwide, with 1.79 million deaths per year [6]. There have been major advances in the management and treatment options for patients with lung cancer in the last two decades with advancement of genomic understanding, and targeted as well as immunotherapy discoveries.

1.2.1.1 Adenocarcinoma

The Cancer Genome Atlas (TCGA) research network published the results of molecular profiling of 230 resected lung adenocarcinoma cases in 2014 [7]. These results showed the presence of the following mutations: *TP53* (46%), *KRAS* (33%), *EGFR* (14%), and v-Raf murine sarcoma viral oncogene homolog B (BRAF) (10%). In addition, mutations in tumor suppressor genes *STK11* (17%), *KEAP1* (17%), *NF1* (11%), *RB1* (4%), and *CDKN2A* (4%) were noted. Eight percent of the samples had mutations in the *MGA* gene, which encodes a protein in the MYC pathway. Aberrations in the RTK/RAS/RAF pathway (76%) and PI3K-mTOR pathway (25%) were noted to be recurrent. Additionally, the presence of *EGFR*, *KRAS*, *BRAF*, and *NF1* were found to be mutually exclusive. *ALK* (1%), *RET* (<1%), *ROS1* (2%), and *ERBB2* (2%) fusions were noted in low numbers. Many of these mutations are considered to have a significant impact on the biology of the cancer, and targeted therapeutic agents to these mutations are being developed. These targetable mutations provide a significantly improved response rate (RR) and progression-free survival (PFS) compared to chemotherapy [8].

1.2.1.2 Squamous Cell Carcinoma

TCGA network noted high mutation rate in lung squamous cell carcinoma in the 178 samples of previously untreated stage I–IV lung squamous cell carcinoma cases in 2012 [9]. This showed high prevalence of *TP53* mutations (81%), and alterations in the CDKN2A/RB1 (72%), NFE2L2/KEAP1/CUL3 (34%), PI3K/AKT (47%), and SOX2/TP63/NOTCH1 (44%) pathways, and *BRAF* mutations (4%). Only 3% of the samples had *KRAS* mutations and *EGFR* mutations were found in 9% of the cases. FGFR (fibroblast growth factor receptor) mutations were found in 12% of the cases. In addition, inactivating mutations were found in *HLA-A* gene. This profile highlights the similarities and differences in the pathogenic gene expression of squamous cell lung cancer and lung adenocarcinoma (noted above) [9].

1.2.1.3 Small-Cell Lung Carcinoma (SCLC)

Just as in lung adenocarcinoma and lung squamous cell carcinoma, TCGA network evaluated 110 small-cell lung cancer cases in 2015. Interestingly, *TP53* and *RB1* had bi-allelic losses in 100 and 93% of the cases, respectively and were thought to be obligatory for the pathogenesis of small cell lung carcinoma (SCLC). In addition, *NOTCH* family genes were identified as tumor suppressors and regulators of neuroendocrine differentiation [10].

1.2.1.4 Epidermal Growth Factor Receptor (*EGFR*) Mutations

Testing for *EGFR* mutations is now standard practice for a patient diagnosed with adenocarcinoma in NSCLC. Discovery of *EGFR* mutations and their sensitivity to tyrosine kinase inhibitor (TKI) therapy has revolutionized the management of lung cancer. EGFR is a transmembrane protein that regulates cell growth via EGR ligands, which signal through one of these downstream pathways: arms-MAPK, PI3K/AKT, and JAK–STAT pathway [11]. Specifically, commonly found deletions in exon 19 and point mutations in exon 21 result in constitutive activation of EGFR, and these mutations have a profound response to TKI therapy. Less common exon 18 and exon 20 mutations have also been reported, and some exon 20 mutations have been associated with initial resistance to TKI therapy.

pThr790Met point mutation (*EGFR* T790M) located on exon 20 is detected in 50% or more of the patients who progress on TKI therapy [12]. T790M mutations are associated with acquired mutation to TKI therapy. Erlotinib and gefitinib were first-generation TKI therapies showing significantly improved RR and PFS in patients receiving these agents compared to chemotherapy [8]. Afatinib is a second-generation EGFR inhibitor, which has been tested in first, second, and third line therapy, and also in combination with chemotherapy in eight LUX-Lung trials, showing increased PFS and RR, as well as improved quality of life measures, in patients with *EGFR* mutations [13–17]. Patients with del 19 *EGFR* mutations and advanced disease had overall survival (OS) benefit with afatinib compared with chemotherapy [15]. Afatinib had poor activity in patients with T790M mutations [18]. In an open-label phase 3 trial, osimertinib, a third-generation irreversible EGFR inhibitor that inhibits both EGFR-TKI sensitizing and EGFR T790M mutations, showed improved PFS of 10.1 vs. 4.4 months, and improved RR of 71 vs. 31% compared to platinum doublet chemotherapy in the second line in patients who had progressed on first line EGFR-TKI therapy [19]. Osimertinib also showed improved PFS of 18.9 vs. 10.2 months for alternative EGFR inhibitor gefitinib or erlotinib, and improved duration of response of 17.2 vs. 8.5 months with standard EGFR-TKI in first-line setting. The data for survival from this trial at 25% maturity showed 18 months survival of 83% with osimertinib vs. 71% with standard EGFR-TKIs [12].

1.2.1.5 Anaplastic Lymphoma Kinase (ALK)

Discovery of fusion gene *EML4-ALK* due to inversion on chromosome arm 2p identified another targetable mutation in patients with lung cancer. Anaplastic lymphoma kinase (ALK) rearrangements are clinically identified by FISH of a dual-probe "break-apart" assay [20]. A first-generation ALK inhibitor, crizotinib, improved the PFS in patients with *ALK* fusion compared to platinum doublet therapy: 10.9 vs. 7 months [21]. Ceritinib, a next-generation ALK inhibitor, showed activity in patients who previously progressed on crizotinib as well as naïve to ALK-inhibitor therapy [22]. Alectinib, another next-generation ALK inhibitor, showed improved activity in crizotinib resistant as well as ALK inhibitor naïve patients with activity for intracranial disease [23, 24]. Recently, brigatinib, a next-generation ALK inhibitor, showed longer PFS (67% for brigatinib vs. 43% for crizotinib at one year), RR (71 and 60% for brigatinib and crizotinib, respectively) as well as intracranial response (78 and 29% for brigatinib vs crizotinib, respectively) in first-line setting [25].

1.2.1.6 BRAF, ROS1, MET

BRAF is a kinase in the RAS–RAF–MEK pathway. V600E activating mutation in *BRAF* seen in melanoma is also observed in about 50% of the *BRAF* mutated NSCLC. Dual inhibition with dabrafenib and trametinib has shown activity in a phase two, single arm study [26]. Due to structural homology between ALK and ROS1, crizotinib has been used in ROS1-rearranged cancers [22]. Similarly *MET* exon 14 mutations have been treated with MET inhibitors like crizotinib and cabozantinib [27]. Crizotinib is the therapy of choice in patients with *MET* exon 14 splicing variants as well as *ROS1* fusion in the large national cancer institute (NCI) sponsored clinical trial: Molecular analysis for therapy choice or MATCH (NCT02465060).

1.2.1.7 KRAS

KRAS is a GTPase that belongs to the RAS superfamily. It regulates cell growth and survival in response to mitogenic stimuli. *KRAS* mutations in lung cancer are activating mutations. To date, there is no effective direct inhibitor of KRAS, and no single agent activity was observed with multi-kinase sorafenib, selumetinib, trametinib (MEK inhibitors), nor ridafarolimus (mTOR inhibitor) in *KRAS* mutated lung cancers [11, 28]. However, novel combination therapies based on improved understanding of the biology of the disease have been proposed. Such targets include FGFR1 or MEK or programmed death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitor therapy [28, 29].

1.2.1.8 Other: RET, NTRK

RET proto-oncogene encodes the TKI receptor of growth factors and is a rare mutation in lung cancers. LOXO292 and BLU-667, selective inhibitors of RET kinase, showed significant clinical activity in patients with advance stage relapsed/refractory lung cancer, including central nervous system (CNS) activity [30, 31]. TRK inhibition in patients with tumor-harboring NTRK (neurotrophic tyrosine kinase gene encoding for tropomyosin-related kinase) fusions has been evaluated in phase I and phase II studies with TRK inhibitors such as larotrectinib and entrectinib in a tissue- and age-agnostic way, and these trials show early meaningful responses and improved PFS in patients, emphasizing that understanding of tumor genomics is key to improvements in this field [32, 33].

As noted above, with improving and new-generation targeted therapies, it is ever so important that tumors of all patients with lung cancer undergo routine next-generation testing in order to identify targetable mutations and to offer new therapies to the patients.

1.2.2 Head and Neck Cancers

Head and neck cancers (HNC) are the sixth-most frequent cancer worldwide [34]. In 2017, it was estimated that in the United States 64 690 people developed HNC and 13 740 died from these cancers (www.cancer.net). HNC are a group of anatomically defined cancers. Lip and oral cavity, pharynx consisting of oropharynx (OP), hypopharynx, and nasopharynx, larynx, nasal cavity, paranasal sinuses, salivary glands, thyroid cancers, skin squamous cell cancer in the head and neck area, mucosal melanoma, and cancer of unknown primary (CUP) in the head and neck area comprise the majority of the regions that are covered under this group of cancers. Historically, tobacco and/or alcohol consumption were thought to be the main risk factors for HNC, but in the last three decades the incidence of carcinogen-induced HNC has been declining, while human papillomavirus (HPV)-induced HNC, especially in the OP, has increased [35–37]. The presence or absence of HPV in the OP classifies two different diseases that differ in staging, prognosis, and clinical outcomes as well as in the molecular changes observed. For example, one of the first studies comparing HPV-positive and HPV-negative DNA showed that *WNT1*, *PDGFA* and *OGG1* aberrations were present in HPV-positive OP cancers [38]. Epstein–Barr virus (EBV) has been associated with the etiology of nasopharyngeal tumors. Generally, HNC have high mutational burden with *TP53* (60%) mutations, *CDKN2A* (25%) inactivation, *CCND1* (12%) amplifications, *EGFR* amplifications (9%), *FGFR1* amplification (5%), *FAT1* (7%),

and *NOTCH1* (4%) mutations being most common genomic alternations in patients with head and neck squamous cell carcinoma [39, 40].

1.2.2.1 HPV-Positive Cancers

HPV 16 is the most common type of HPV causing cancer in the OP cavity. HPV 16 is a DNA virus, the integration of which leads to production of oncoproteins E6 and E7. E7 protein causes a prolific environment for the cell by downregulating the tumor suppressor protein retinoblastoma (Rb). Under normal circumstances Rb is downregulated by p53. However, E6 oncoprotein encoded by the virus causes degradation of p53. It is important to note, however, that even though HPV virus degrades p53, the *TP53* gene in HPV-positive tumors is not typically mutated. In fact, HPV-positive HNC exhibits fewer DNA mutations compared to HPV-negative HNC [41, 42]. In 2015, TCGA reported 72% mutation rate in *TP53* and 22% mutation rate in *CDKN2A*, however it was noted that patients with HPV tumors lacked mutations in these genes [43]. HPV-positive tumors tended to have a higher level of *PIK3CA* mutations, loss of TNF factor-associated factor 3 (*TRAF3*), and *E2F1* transcription factor amplification [43]. In addition, HPV-positive tumors tended to lack *EGFR* mutations, and had unique mutations in *DDX3X*, *CYLD*, and *FGFR* [44]. How these differences in mutation may clinically be relevant is still being evaluated – they may explain the improved outcomes seen in HPV-positive cancers, or offer opportunity for new therapeutic targets. It has been noted that HPV-positive tumors have a much-improved prognosis compared to HPV-negative tumors with two year OS being 95% in HPV-positive patients vs. 62% in HPV-negative patients [45, 46]. These findings have led to the question of de-escalation of therapy in carefully selected patients with HPV-positive OP cancers. Currently phase 2 studies are underway. Mature results from one of the early generation de-intensification trials where both the dose of radiation and chemotherapy were decreased showed promising results of three year loco-regional control of 100% and three-year OS of 95%. In addition, there was better preservation of quality of life in patients who received de-intensified therapy compared to standard therapies [47]. Other studies are evaluating varying de-intensification methods including volume or dose de-escalation of radiation, chemotherapy de-escalation, etc. (www.clinicaltrials.gov study numbers: NCT02258659, NCT015309997, NCT01687413, NCT0188802). It is important to note that de-intensification, at this time, is under trials and should not be done outside of a clinical trial setting to minimize risk of harm in a disease that has excellent prognosis. Results of two studies reported at recent meetings showed that de-escalation of chemotherapy from cisplatin to cetuximab was associated with decreased survival in patients receiving cetuximab therapy. Results of RTOG1016 presented at the American Society of Radiation Oncology (ASTRO) meeting on October 22nd, 2018, showed five-year survival of 78 vs. 85% for cetuximab vs. cisplatin group with non-inferiority p-value of 0.51 (Trotti et al. NCT01302834, publication pending) [48]. Similarly, the results of De-ESCALaTE (Determination of Cetuximab versus Cisplatin Early and Late Toxicity Events) HPV trial showed two-year OS of 97.5 vs. 89.4% ($p = 0.001$) for cisplatin vs. cetixumab [49]. Hence, improved understanding of genomics and molecular mechanisms is important even in diseases with good prognosis because it may impact therapy and improve care of our patients.

1.2.2.2 HPV-Negative Cancers

Patients with HPV-negative HNC have tumors that develop either from pre-cancerous lesions like leukoplakia, or those that develop de novo. In 1953, Slaughter et al. introduced the concept of "field cancerization" which described large areas of dysplastic changes due to carcinogens – posing risk for relapse in those areas despite treatment of the tumor [50]. At the genetic level, the majority of HPV-negative cancers have mutations in the *TP53* gene located on chromosome 17p13, *CKN2A*, *CCND1*, and *PIK3CA* [41, 43, 51]. In addition, a high rate of *NOTCH1* inactivating mutations was also noted, suggesting its role as a tumor suppressor in HNC [51]. TCGA data also discovered immune pathway mutations in *HLA-A/B*, suggesting the role of immune recognition in HNC [52]. These studies highlighted important genomic drivers of HNC as well as molecular differences in HPV-negative and HPV-positive HNC; however, there are limitations and challenges, and further research is needed. The most significant limitations are lack of direct targets to these mutations that may be driving the tumor. Other than cetuximab and immunotherapy (nivolumab and pembrolizumab), at this time, we do not have any approved treatments that may target these mutations in HPV-negative disease – an area that needs further research and development.

1.2.2.3 Targeting the Epidermal Growth Factor Receptor (EGFR) Pathway

EGFR is overexpressed in more than 90% of HNC due to genetic amplifications [53]. EGFR is a transmembrane protein that signals through the PI3K-PTEN-AKT pathway and ras-MAPK pathway to promote cell growth, and can also act as a co-activator of JAK/STAT signaling leading to cell proliferation [52]. Focal amplifications in receptor tyrosine kinase predominate in HPV-negative tumors but are lacking in HPV-positive tumors [43]. Cetuximab, a chimeric IgG monoclonal antibody, is currently approved for use in HNC in both a locally advanced setting concurrent with radiation, as well as in metastatic setting as monotherapy and in combination with chemotherapy. However, noted above are results of two large trials showing worsening outcomes in patients with HPV-positive OP cancer when they received cetuximab [48, 49]. This may be due to decreased EGFR amplification in HPV-positive tumors or other reasons.

1.2.2.4 Thyroid Cancers

Evaluation of papillary thyroid cancers in the TCGA showed mutations in *BRAF*, *RAS*, *RET*, *TER*, *CHEK2*, *EIF1AX*, and *PPM1D* [40]. This has offered an opportunity to be treated by novel therapeutic options like BRAF inhibitors. Evaluation of anaplastic thyroid carcinoma showed increase in mutations in PI3KCA-PTEN-AKT-mTOR pathway, DNA mismatch repair (MMR) pathway, and histomethytransferases [52]. *NTRK* and *RET* fusions have specific therapeutic targets that are being evaluated in clinical trials, noted in Section 1.2.2.5. This knowledge offers an opportunity for evaluation of these pathways as therapeutic options.

1.2.2.5 Other Targets

Ultimately the goal of research is to advance care and outcomes for patients. Over the last decade or so the use of immunotherapy has revolutionized the field of oncology. The PD-1 : PD-L1 pathway which normally serves as a checkpoint to prevent excessive

inflammation is exploited in cancers, including HNC, where it leads to immunosuppression and a permissive environment for tumor growth. PD-1 inhibitors nivolumab and pembrolizumab were approved by the Food and Drug Administration (FDA) for metastatic or recurrent HNC in 2016 [54, 55]. Further investigations into the role of these drugs for locally advanced disease and as well as in combination with chemotherapy are ongoing.

Recently, LOXO292 a selective inhibitor of *RET* fusions, showed activity in thyroid and other solid cancers [31]. Similarly, larotrectinib and entrectinib, which are selective inhibitors of NTRK, showed promising early clinical activity in a "tissue-agnostic" way in solid tumors including thyroid cancers [32, 33, 56]. These results show promise of genomic-driven therapies in head, neck, and other cancers.

Overall, there is more knowledge and interest in genomic studies of HNC. More information about pathologic genes and pathways is available, especially with improving techniques. Further studies with targeted therapies either alone or in combination with other agents are being conducted in the quest to improve outcomes in HNC.

1.2.3 Hematological Malignancies

The concept of precision or personalized medicine has been in use for decades in blood transfusion, and more recently in diagnosis and treatment of solid tumors, but its use in hematological malignancies has been lagging behind. Hematological malignancies have traditionally been treated with surgical intervention, radiotherapy and cytotoxic chemotherapy. However, the era of precision medicine has ushered in novel methods for detecting, prognosticating, and treating these malignancies that is personalized to the person and disease, leading to superior outcomes. Here we review the implications of personalized medicine in lymphoma, leukemia, and MDS.

1.2.3.1 Lymphoma

Combination cytotoxic chemotherapy such as ABVD, Stanford V, and BEACOPP with or without the addition of radiotherapy remains the standard of care in Hodgkin lymphoma (HL) due to the sensitivity of the Reed-Sternberg cells to these modalities [57]. But gene profiling of the disease has led us to prognostic biomarkers as well novel target agents for this disease that affects both adults and adolescents [58]. E2496 intergroup trial put forth a 23-gene expression profiler that predicts outcome in advanced stage classical HL [59], giving us a tool to tailor the chemotherapy regimens. Recent molecular studies have recognized mutations in JAK–STAT and NF-kappa B pathways that are potential targets for therapy in relapsed refractory disease, but require further analysis for clinical use [60]. HL gene profiling studies also elucidated PD-L1, PD-L2 alterations as well as amplification of chromosome 9p24.1 as markers of advanced classical HL and immune evasion strategies employed by the tumor cells. This has led to the in-cooperation of checkpoint inhibitors in the treatment of advanced or refractory/relapsed HL in patients who harbor these mutations [61].

Non-Hodgkin lymphoma (NHL) is a heterogeneous disease that arises from B lymphocytes, T lymphocytes, or NK cells. NHL, like HL, is a new and exciting field for precision medicine use due to the presence of targetable genes and proteins that are being discovered at a rapid frequency. Gene expression profiling can be used to distinguish different types of

NHL as they often have overlapping features and morphological characteristics, making such distinction difficult without the identification of underlying genetic abnormalities [62]. It is important to accurately identify the subgroups, as the treatment, prognosis and outcomes are different for each and this exemplifies an optimal use of precision medicine to target better outcomes. Although cytotoxic therapy continues to be used in first-line therapy of most NHL, the addition of immune modulators is increasingly used after their presence is identified in the tumor as well as the microenvironment. Recent studies have shown that lymphoma microenvironments express PD-L1 and that PD-1 expression is high in intratumoral T-cells, making checkpoint inhibitors an essential tool in the fight against lymphoma [63].

Two studies using pidilizumab, a PD-1 humanized IgG1 mAb for the treatment of NHL, show great promise. The first is a phase II study that evaluated pidilizumab in patients with diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PBML), and transformed indolent B-cell lymphoma (BCL) after autologous hematopoietic stem cell transplantation (HSCT). The 16-month PFS and OS in 66 patients were 0.72 (90% CI, 0.60–0.82) and 0.85 (90% CI, 0.74–0.92), respectively. In a phase II study of 32 patients that utilized pidilizumab in combination with rituximab in relapsed follicular lymphoma, complete response (CR) was achieved in 52% of patients and partial response (PR) in 14% of them. Both studies indicated that there were changes to the tumor microenvironment as well as genetic profile post therapy, but larger studies are needed to confirm the findings [64]. Both nivolumab and pembrolizumab, anti-PD-1 Mabs, have exhibited promise in the treatment of lymphoma but chimeric antigen receptor T cells (CAR T Cells) is the ultimate personalized medicine model, where the patient's own T cells are enhanced to attack the tumor cells [65, 66]. Axicabtagene ciloleucel, an autologous anti CD-19 chimeric antigen receptor, was recently approved for treatment of relapsed/refractory large BCL, thus giving us a tool to treat refractory malignancies with precision medicine [67]. Although cytotoxic chemotherapy is the mainstay of treatment currently, precision medicine is slowly but surely making its way into the realm of lymphoma.

1.2.3.2 Leukemia

Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are both lethal malignancies that have been treated with cytotoxic therapies with moderate success, but the heterogeneity of these leukemias make precision medicine a necessity for more successful outcomes. Gene sequencing studies have elucidated multiple gene mutations and gene arrangements that are present in de novo as well as therapy-related AML [68]. Some of these genes are targetable and hence produce superior outcomes when added to the standard 7 + 3 regimen. FMS-like tyrosine kinase (FLT3) mutations are common in AML and portend poorer outcome with just intensive chemotherapy. The addition of an FLT 3 inhibitor, midostaurin, to standard therapy was shown to improve OS in patients with this mutation [69]. Similarly gemtuzmab ozogamicin, an anti-CD33 ab, added to standard therapy for patients with CD33+ AML was shown to have superior OS and reduced risk of relapse compared to 7 + 3 regimen alone [70]. TKIs such as imatinib and dasatinib are agents which target Bcr-Abl tyrosine kinase, the abnormal gene product of Philadelphia chromosome, and have been successfully used in Ph + ALL and chronic myeloid leukemia (CML) [71].

1.2.3.3 Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) is a constellation of conditions that manifest as chronic cytopenias. The treatment of MDS varies from symptomatic management with transfusions to hypomethylating agents and stem cell transplants depending on the risk profile. While great advances have not been made in targeted treatment modalities in MDS, it has been used in risk stratification to direct therapy. Karyotype, FISH, or gene mutation analysis help identify abnormalities that may predict progression to AML or mortality risk. Gene mutations TP53, EZH2, ETV6, RUNX1, TET2, DNMT3A, and ASXL1 [70], and cytogenetic abnormalities del(7q), del(5q), del(12p), del(20q), -7, inv(3)/t(3q)/del(3q), and complex cytogenetics are associated with progression to AML, thereby requiring early and aggressive therapy [72]. Identification of isolated Del(5q) has therapeutic implications as it responds well to lenalidomide [73]. Precision medicine plays an important role in risk stratification of MDS and directing therapy, thereby increasing survival and decreasing therapy-related morbidity in patients who would not benefit from therapy per the risk profile.

1.2.4 Gynecologic Malignancies

The realm of precision medicine in areas of gynecologic malignancies, including cervical, uterine, and ovarian cancers, is sparse [74, 75]. From its infancy in the Human Genome Project, genome-wide association studies illuminated genetic variants found more commonly in diseased versus healthy patients [76–78]. In 2006, TCGA Project was created to identify large genome sequences that play a role specifically in cancer [79]. One of its pilot studies examined 316 high-grade serous ovarian tumors which shed light on novel markers like p53, and somatic mutations like BRCA1 and BRCA2, that later revolutionized the field of targeted gene therapy [79, 80]. Current guidelines for management of gynecologic cancer involve primarily surgery, cytotoxic chemotherapy, and radiation, depending on the cancer stage. Here, we highlight some of the budding areas of precision medicine that could revolutionize how we approach gynecologic malignancy therapy.

1.2.4.1 Cervical

The pathogenesis of cervical cancer has not yet been elucidated, but the oncogenic effect of HPV infection is well understood. Transcription factors like ESR1, growth factors like FGFR2, and oncogenes like BRCA1, PIK3CA, and KRAS have been previously linked to cervical cancer, with worse prognosis among the adenocarcinoma versus squamous cell subtypes [81–87]. A recent meta-analysis of five different transcriptome datasets showed that the downregulation of KAT2B, a transcriptional activator, and PCNA, a DNA repair enzyme, lead to an increased risk for cervical cancer [88, 89]. The use of these proteins as potential biomarkers for targeted therapy is conceivable.

1.2.4.2 Uterine

One area of interest in uterine cancer includes PD-1 and its ligand PD-L1, which are proteins that mute the T-cell inflammatory response and therefore allow cancer cells to evade apoptosis. In a study of 437 solid tumor samples, expression of PD-1 was seen in 80–90% of endometrial and ovarian cancers [90]. Pembrolizumab, an antibody blocking PD-1, has

shown some promise as a targeted therapy in a range of different MMR-deficient cancers, including melanoma and endometrial cancer [91–93]. Loss of function of a tumor suppressor gene, called phosphatase and tensin homolog (PTEN), has also been highlighted in the pathogenesis of endometrial cancer via its downregulation of the PI3K/AKT/mTOR signaling cascade [94]. Temsirolimus, an mTOR inhibitor, was studied in a phase II trial of 62 patients with recurrent or metastatic endometrial cancer and showed greater response in chemotherapy-naïve patients, than in chemotherapy-treated patients, independent of PTEN status [95]. However, in a phase 2, 42 patient cohort of platinum-refractory ovarian cancer and advanced endometrial cancer patients, temsirolimus failed to meet predefined levels of efficacy and the trial was prematurely stopped [96]. Many of these potential biomarkers (i.e. AKT, PD-L1) also play a role in ovarian cancer pathology, as listed in Section 1.2.4.3.

1.2.4.3 Ovarian

Due to its insidious spread and late stage of diagnosis, ovarian cancer is among the most lethal of gynecologic malignancies. The goal of early detection is paramount. CA-125 has historically been the biomarker used, but miRNAs have been investigated as potential early biomarkers, as well as a glycoprotein called Human Epididymis Protein 4 (HE4), which is expressed only in epithelial ovarian cancer and is absent in healthy ovaries [97–102]. By combining the diagnostic utility of HE4 and CA-125, Moore et al. created the Risk of Ovarian Malignancy Algorithm (ROMA) to stratify the risk of finding ovarian cancer in women with an adnexal mass [103]. The strength of these biomarkers, individually and when paired, could revolutionize the way we treat this commonly metastatic, terminal disease.

Poly ADP ribose polymerase (PARP) 1, an enzyme involved in DNA repair as well as tumor cell proliferation and differentiation, has also been linked to several malignancies, including uterine, ovarian, breast, lung, lymphomas, and leukemias [88, 104]. The FDA recently approved olaparib, a PARP inhibitor, in platinum-sensitive ovarian cancer as successful maintenance treatment after chemotherapy [93, 105, 106]. Other PARP inhibitors including niraparib and rucaparib have also shown promising results in BRCA mutation-associated advanced ovarian cancer patients with recurrence and those who have received two or more chemotherapies, respectively [107–109]. The overall utility of PARP inhibitors may vary across the subtypes of ovarian cancer (i.e. clear cell carcinoma vs. high-grade serous) as the levels of biomarker expression differ. For example, Li et al. showed that expression of HER2 and PD-L1 were higher in clear cell when compared with high-grade serous cancer [110]. Even among a single tumor there is gene heterogeneity, making a single molecular target limited in its effectiveness. Therefore, several investigators are researching combination therapies. Perifosine, an Akt inhibitor, is being studied in a phase I trial as an adjunct with docetaxel in platinum and taxane-resistant epithelial ovarian cancer [111, 112]. Similarly, another AKT inhibitor, AZD5363, is being paired with olaparib in BRCA1-mutant ovarian cancer [112, 113].

Pertuzumab, known for its role in blocking HER2 expression, is commonly used for breast cancer but is now being investigated for potential use in ovarian cancer as well [114]. Bevacizumab, a monoclonal antibody that prevents angiogenesis by targeting VEGF, has been shown to be effective against several types of cancers including ovarian [115–117].

Brivanib, which is a dual inhibitor of VEGFR2 and FGFR, and cedirinib are also undergoing Phase II trials [118, 119]. The Notch pathway, an important regulator of cell differentiation and communication, has also been implicated in ovarian cancer proliferation [120–122]. Gamma-secretase inhibitors block the NOTCH signaling pathway in ovarian cell lines and may have clinical benefit in ovarian cancer therapy [120]. As more single-agent targeted therapies emerge, we hope to find ways to treat those most in need, such as patients with ovarian cancer that are resistant/refractory to current platinum-based chemotherapy.

1.2.5 Melanoma

Melanoma is classified based on tissue of origin: cutaneous melanoma, mucosal melanoma, and uveal melanoma. Melanoma was recognized as a relatively radiation- and chemotherapy-resistant malignancy with limited therapeutic options, such as interferon and high dose interleukin-2. For a long time dacarbazine was the only chemotherapy available for metastatic disease, and the effort of combining biological therapy with interferon and interleukin-2 and chemotherapy failed to show superior survival versus high dose interferon in high risk melanoma [123]. Only recently, when the biological and genetic underpinnings of melanoma were discovered and specific druggable targets were identified, successful new therapies were and are being developed.

Initially in 2002, it was reported that *BRAF* mutations occur frequently (up to 66%) in cutaneous melanoma [124]. Subsequently, meta-analysis of 2521 patients showed prevalence of *BRAF* mutation in 41% of cutaneous melanoma [125]. *BRAF* encodes serine/threonine protein kinase that controls mitogen-activated protein kinase involved in proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis [126]. The most common *BRAF* mutation in melanoma is valine to glutamic acid substitution V600E (89%). Other less common mutations include V600K (2.7%), and other mutations in other positions have also been reported (mostly in acral and mucosal melanomas) with low frequencies (<1%) [127].

After initial failure of the non-specific BRAF inhibitor, sorafenib, to show efficacy in melanoma [128], more specific inhibitors were developed: vemurafenib [129] and dabrafenib [130] induced superior responses and OS in patients with metastatic melanoma over that produced by dacarbazine. One of the mechanisms of resistance to BRAF inhibition is paradoxical activation of MAPK [131]. This was addressed by the combining of BRAF and MEK inhibitors in clinical practice: vemurafenib and cobimetinib [132], dabrafenib and trametinib [133], encorafenib and binimetinib [134] (improving overall survival rates from single agent BRAF to combination from 17.4 to 22.3 months, 18.7 to 25.1 months, and 23.5 to 33.6 months, respectively).

Activity of BRAF and MEK inhibitors in metastatic cutaneous melanoma was confirmed in adjuvant setting in resected Stage III melanoma [135] with improvement of estimated cure rates from 37% in placebo arm to 54% in dabrafenib plus trametinib arm [136]. Because this combination can induce 70% PRs, 15% CRs [137], and superior median event free survival of 19.7 versus 2.9 months in group receiving surgery alone, it is now contemplated that this therapy could be used in high-risk stage III and IV resectable melanoma in neoadjuvant setting.

Because of the momentous impact of BRAF and MEK inhibitors on management of advanced melanoma, European and American oncological societies now recommend that each advanced cutaneous melanoma should be tested for V600 mutation. Most commonly, the Cobas® 4800 BRAF V600 mutation test is performed.

NRAS mutations, the second-most common in melanoma, are found in 18% of cutaneous melanomas, more commonly in nodular melanomas and melanomas arising in chronic sun-damaged skin melanoma [125]. *KRAS* mutations and *HRAS* mutations in melanomas are infrequent (2 and 1%, respectively). *NRAS* are also found in acral and mucosal melanomas. Patients with *NRAS* mutated melanoma tend to be older (than with *BRAF* mutated melanoma), and have a history of chronic UV exposure. Currently there is no effective targeted therapy for melanoma with this mutation, although MEK inhibitors are being studied [138, 139], and 33% partial RR has been observed when MEK inhibitor was combined with cyclin-dependent kinase 4/6 inhibitor [140].

A mutation in the gene encoding neurofibromin 1 (*NF1*) is found in 14% of cutaneous melanomas, and loss in function of neurofibromin 1 causes decreased inactivation of RAS, and thus drives oncogenesis through MAPK and PI3K pathway. Melanomas with mutated *NF1* occur in sun-exposed skin, and frequently have desmoplastic characteristics [141]. There are no targeted therapy options for these tumors, although MEK inhibitors showed some activity in NF1 mutated melanoma cell lines [142].

Germline mutations and deletions in cyclin-dependent kinase inhibitor 2A (CDKN2A) [143] have been implicated in hereditary melanoma on the background of multiple atypical nevi. Genetic aberrations in cyclin-dependent kinase 4 (CDK4), melanocortin 1 receptor (MC1R), breast cancer 1 (BRCA1), BRCA1-associated protein 1 (BAP1), and telomerase reverse transcriptase (TERT) are also associated with higher risk of development of melanoma [144].

Chronically sun-damaged skin, as well as acral and mucosal melanomas, commonly have *KIT* mutations (seen in 28, 36, and 39% of these melanomas, respectively) [145]. *KIT* encodes a stem cell factor transmembrane receptor tyrosine kinase that is involved in melanocyte development. Upon binding with stem cell factor, KIT dimerizes and activates PI3K/AKT/mTOR and MAPK/ERK pathways. Trials with KIT inhibitors imatinib [146], dasatinib [147], and nilotinib [148] resulted in clinical responses (29, 18.2, and 26.2%, respectively), with highest activity observed in melanomas with *KIT* mutation, but not amplification, nor with concomitant *NRAS* mutation [146]. Nilotinib was mostly effective in melanomas with exon 11 mutation, including L576P (present in 90% of responders) [146], but not in melanomas previously pretreated with imatinib [149]. Therefore, testing for KIT mutation in mucosal, acral, and sun-damaged skin is currently recommended, for possible benefit from imatinib or nilotinib therapy.

Mutually exclusive with *KIT* mutated melanomas, mutation in platelet-derived growth factor receptor A (PDGFRA) can be found in 7% of acral melanomas and 4% of mucosal melanomas, and can be targeted with imatinib and crenolanib [150].

Amplification of CCND1 can be found in 44.4% of acral melanomas, 10.5% of lentigo maligna melanomas, and 5.6% of superficial spreading melanoma [151], and can cause resistance to BRAF inhibitors [152], but may be associated with responses to treatment with combination of carboplatin, paclitaxel, and sorafenib [153].

Uveal melanoma that arises from melanocytes within the uveal tract, with more than 90% of cases involving the choroid and the remainder affecting the iris and ciliary body, has a biology that is very different from cutaneous melanoma and has different molecular underpinning. There is no standard therapy for metastatic uveal melanoma and no adjuvant therapy improved chances for recurrence free survival after enucleation or plaque radiotherapy of early disease.

Current therapies, including targeted and immune checkpoint inhibitor treatment, for cutaneous melanoma have not been effective in uveal melanomas.

Aberrations in G(q) pathway (mostly either mutations in *GNAQ* or *GNA11* gene, but also in cysteinyl leukotriene receptor 2 [CYSLTR2], and phospholipase C ß4 [PLCB4] encoding genes) [154] can be found in found in all uveal melanomas. Currently there are several ongoing trials exploring activity of protein kinase C inhibitors, AKT inhibitors, and MET and MEK inhibitors, downstream from G protein receptor [155].

Selumetinib, a selective MEK1/2 inhibitor, demonstrated single agent activity in a randomized phase 2 study with doubling of PFS versus temozolomide or dacarbazine and RR of 14% [156], but when tested in a randomized double-blind clinical trial in combination with dacarbazine versus placebo and dacarbazine, it produced only 3% RR, and PFS rate was not different from dacarbazine alone [157].

Mutations in *BAP1* can be present in up to 20–40% of uveal melanoma [158], associated with monosomy 3, and with class 2 gene expression profile (GEP) [159] that predicts high risk of melanoma recurrence (25.9%), and are present in the majority of metastases from uveal melanoma. Unfortunately, there is no therapy that showed activity in BAP1 mutated tumors. There is a suggestion that BAP1 mutated cancers may be addicted to enhancer of zeste homolog 2/polycomb repressive complex 2 (EZH2/PRC2) pathway [160], and this could lead to investigating the use of EZH2 inhibitors [161] in treating uveal melanoma, but this view has been challenged [162]. Since histone deacetylase inhibition can reverse biochemical effects of BAP1 mutation, vorinostat was tested in uveal melanoma. No results of this study to date have been published.

Mutations in *EIF1AX* gene encoding eukaryotic translation initiation factor 1A, X-chromosome linked, and *SF3B1* encoding splicing factor 3B subunit 1 are found in 12 and 23% of uveal melanomas [158] and are associated with class 1A and B GEP, respectively [155]. These mutations are not actionable at present.

Analysis of gene expression in 57 patients with metastatic melanoma identified four subclasses [163]: (i) by presence of immune response; (ii) pigmentation differentiation; (iii) proliferation; or (iv) stromal composition genes. Individuals with proliferative subtype or low immune response had the worst prognosis.

A prospective study of 459 patients with uveal melanoma identified to prognostic subgroups; class 1 (1.1%) with low metastatic potential, and class 2 with high metastatic risk (25.9%) at median follow up of 17.4 months [159]. In this analysis loss of chromosome 3 did not add any additional prognostic information. This 15 gene GEP done on fine needle aspiration biopsy or surgical specimen outperformed TNM (tumor node metastasis) classification in uveal melanoma, and became standard for prognostication of uveal melanoma [164].

A similar role of GEP in cutaneous melanoma was evaluated in prospective study of 322 patients with cutaneous melanoma in EXPAND and INTEGRATE registries in which 88%

percent of patient had stage I or II melanoma. Breslow thickness, mitotic rate, and GEP classification predicted recurrence [165]. This was confirmed in a study of 523 patients with stage I or II cutaneous melanoma demonstrating significant difference in five-year relapse free survival rate; for class 1 of 88% and class 2 of only 52% [166]. Perhaps more frequent evaluation of patients with class 2 melanoma will impact earlier discovery on metastatic disease, but at present there is no data suggesting that more frequent follow-up of early stage, class 2 melanoma patients will result in improvement of survival.

Tumor mutational burden (TMB) varies between different types of melanoma, with the highest seen in cutaneous melanomas, especially with *NF1* mutations [167], and the lowest in ocular melanomas. Because of this dichotomy, there is no clinical role in testing for TMB in melanoma, because it will not provide additional guidance.

PD-L1 expression has been evaluated as a predictive biomarker for responses to immune checkpoint inhibitors targeting PD-1 and PD-L1 in multiple malignancies. In melanoma, PD-L1 expression correlates with immune activity signatures in melanoma [168]. While checking for expression of PD-L1 in melanoma is not a common practice, it could be performed to help with decisions regarding whether to use single immune checkpoint inhibitor therapy, nivolumab, or combination of nivolumab with ipilimumab, a cytotoxic T-lymphocyte-associated protein 4 targeting antibody. In patients with PD-L1-positive tumors, median PFS was 14.0 months in both the nivolumab plus ipilimumab and nivolumab alone groups, but in patients with PD-L1-negative tumors, PFS was longer with the combination as compared with nivolumab alone, 11.2 versus 5.3 months respectively [169]. Combination therapy is associated with a much higher rate of immune-related toxicities [169]. Recently, soluble PD-L1 was described as a potential biomarker for malignant melanoma treated with checkpoint inhibitors. High pretreatment levels of soluble PD-L1 were associated with increased likelihood of progressive disease on immune checkpoint inhibitor therapy [170].

In conclusion, over the last several years multiple biomarkers have been developed in melanoma to help with assessment of melanoma prognosis, and to guide therapeutic decisions regarding the use of targeted or immune treatment of this disease. It is expected that in the near future we will have even higher resolution of understanding of melanoma biology and that will lead to improved survival of individuals with this disease.

1.2.6 Gastrointestinal Malignancies

Currently, the role and utility of genomic profiling of gastrointestinal (GI) cancers is variable, depending on exact site of malignant origin, and moreover under continuous assessment with advance in profiling techniques as well as results of clinical trials using targeted agents. There is greater appreciation for the molecular heterogeneity even within the same form of GI cancers, aside from the heterogeneity between different sites of origin, despite the fact that most GI cancers are epithelial in origin. While the utility of genomic assessment is not nearly as high and as impactful as of yet compared to other forms of cancer – for example, NSCLC – ongoing trials and studies are squarely aimed at better defining molecular subclasses of each form of GI cancers to improve therapeutic targeting strategies. As of yet, the utility of molecular profiling of pancreatic cancers and hepatocellular carcinomas is limited. Thus, for this section, the principal focus will be on GI cancer types that have

most benefited from clinically testable markers that have resulted in meaningful improvements in clinical decision-making.

1.2.6.1 Gastroesophageal Cancers

Cancers of the esophagus and stomach have traditionally been grouped together for the purpose of clinical trial enrollments, with the supposition that they are biologically indistinct. In the past few decades, however, it has become more apparent that not only are cancers stemming from these sites quite different biologically, but also that histologic, and now molecular, subtypes provide insight into distinct biologic drivers and features of each type. In recent years, adenocarcinoma has overtaken squamous cell carcinomas as the most common form of esophageal carcinoma; this same histologic subtype has always been by far the most common cell type of origin for gastric cancers. For the latter, which until recently was mostly subdivided into categories based on histopathologic differences, there is now a better set of molecular subclasses that help define gastric cancer at the molecular level.

The most prominent molecular marker in clinical use in gastric cancer is HER2. Amplification of *HER2* (*ERBB2*, the *ERb-B2* receptor tyrosine kinase, also known as human EGFR 2) can be seen in as many as 35% of gastric cancer patients. The ToGA trial, which examined response of patients with metastatic gastric cancers to HER2-targeted therapy with the monoclonal antibody trastuzumab in combination with cisplatin and 5-fluorouracil, reported 22% incidence of HER2 amplification in this population [171]. *HER2* amplification is associated with a more biologically aggressive disease and the addition of trastuzumab significantly increased OS in patients whose tumors harbored this amplification [172, 173].

1.2.6.2 Colorectal Cancers

Mutant isoforms of *RAS* are relatively common in GI cancers but are most notably prevalent and clinically relevant to metastatic colorectal cancers (CRC), which display a 35–45% incidence of mutated KRAS and mutant N-RAS in an additional 5–10%. *RAS* mutations are nearly always mutually exclusive with BRAF mutations, and indicate a lack of responsiveness to drugs targeting the EGFR [174, 175]. This is most relevant for the CRC due to proven efficacy of EGFR inhibition (via cetuximab or panitumumab, either in combination with 5-FU based chemotherapies or as single agents) in this cancer. Cytotoxic chemotherapy using a 5-Fluorouracil with either irinotecan (FOLFIRI regimen) or oxaliplatin (FOLFOX) remain the principal treatment options in the first- and second-line settings for metastatic CRC. Both combinations can be administered together with biologic agents, including those targeting angiogenesis (bevacizumab, aflibercept) as well as targeted therapy using monoclonal antibodies directed toward EFGRs (cetuximab, panitumumab); the latter is restricted to use in patients with wild type RAS tumors.

Recent excitement from the use of checkpoint inhibitors, with stark improvements in survival in melanoma and NSCLC in particular, has led to many investigations of this class of drugs in GI cancers. However, recent successes in those cancers have not yet translated to large scale success in GI tumors, with the possible exception of those harboring high amounts of microsatellite instability (MSI-high). Presence of MSI is the only potentially effective potential marker of response to checkpoint inhibition in GI cancer at this point.

MSI comprises a relatively low number of total CRC cases (5–15%) [176]. While MSI has traditionally been associated with better prognosis compared to microsatellite stable (MSS) cases [177]; other studies have concluded that MSI in combination with *BRAF* mutation in right-sided colon cancers is associated with worse prognosis. Landmark clinical trials investigating checkpoint inhibitor immunotherapy found that CRCs, harboring MSI were most likely to respond to this treatment [174–176]; thus, MSI-high detection has become even more important as a predictive biomarker of response to treatment in chemo-refractory cases of CRC. Assessment of tumors for PD-L1 is under active investigation as correlative markers in recent and ongoing trials, but its clinical utility is speculative at best based on recent data.

The use of PD-1 blockade as a novel therapeutic strategy is a relatively new phenomenon within the past several years, albeit one that has very quickly gained ground in multiple forms of solid tumor malignancies. The roots of this immunotherapy explosion stem significantly from a Phase I trial that enrolled 296 patients with heavily pretreated malignancies; of these, only 19 patients had chemo-refractory CRC. A landmark Phase II trial investigating immunotherapeutic checkpoint inhibition enrolled a total of 33 such patients with chemo-refractory metastatic CRC. Objective responses were seen in patients with melanoma, NSCLC, and renal carcinoma, but no objective responses were observed in any of the patients with CRC [178]. It is important to note that these patients were heavily pretreated with 5-FU based chemotherapy, and also likely had treatment comprising standard-of-care combinations with platinum (oxaliplatin) and irinotecan. A separate Phase I study reported objective response in 1 of 14 cases of heavily pretreated CRC [179]. Based on this response (albeit just 1 of 33 total CRC cases), it was hypothesized that the patient whose tumors did respond likely had MMR deficiency with MSI, and that this tumoral genetic trait induced a higher susceptibility to checkpoint blockade. Thus, a Phase II trial moved forward, investigating use of PD-1 inhibition with pembrolizumab in 41 patients with heavily pretreated carcinomas, 32 of whom had cancers of the colon or rectum. Among these 32 cases, 11 harbored MSI; the other 21 were MSS. The two MMR deficient cohorts included patients with inherited germline MMR deficiency (Lynch syndrome) as well as patients with sporadic MMR-deficient tumors. Forty percent of the MSI cases showed a PR, and none of the 21 MSS tumors showed objective response [176]. Subsequent to the reports of data of this trial, the US FDA approved the use of pembrolizumab in patients with heavily pretreated forms of MSI-metastatic carcinoma; this approval was notable for the first-of-its-kind designation as being "agnostic" to the site of tumor origin, but rather focusing on the genetic status per MSI testing. Separate studies have delineated differences in prognosis and molecular drivers and passenger mutations based on "sidedness" of colon cancers; at the current time, data support the notion that right-sided colon cancers are more likely than left-sided colon cancers to metastasize and they are also associated with worse prognosis [180].

Approximately 5–10% of CRC cases harbor *HER2* amplification. The use of HER2-targeting agents in *HER2*-mutant and amplified CRC remains an area of active investigation. The HERACLES Phase II trial aimed to demonstrate efficacy of dual HER2 blockade using trastuzumab and lapatinib in CRC patients with chemo-refractory HER2-positive, KRAS wild-type disease [181]. In this trial, 5% of all screened patients (48/914) had HER2-positive tumors; 27 patients were eligible for the trial. Overall, it was felt that this drug

combination was well tolerated, and importantly 30% of treated patients achieved an objective response to this cancer-directed treatment, with an additional 44% obtaining stable disease. Further investigations are ongoing, including use of next-generation HER2-targeted agents. A recent case report described the use of the HER2 targeting drug trastuzumab-DM1 (in clinical use for HER2+ breast cancer) in a patient with chemo-refractory *HER2*-amplified metastatic CRC [182]. The authors reported a sustained response and overall improvement in quality of life in this patient following multiple prior lines of therapy, but prospective clinical trials will be required to elucidate the potential role of this drug on a more widespread scale.

1.2.7 Breast Cancer

Breast cancer can be classified into four intrinsic subtypes or molecular classes based on gene expression profiling. These four classes include basal-like, luminal A, luminal B, and HER2 positive. As we generally do not have the information of molecular subtypes at initial clinical presentation, clinical surrogates of triple negative for basal-like, hormone positive/HER2 negative for luminal A/B, and HER2 positive are generally used, though they are not always perfect correlations.

1.2.7.1 Basal-Like, or Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) is defined by being estrogen receptor (ER) negative, progesterone receptor (PR) negative, and Her2 negative, but beyond that TNBC is quite heterogeneous at the molecular level. TNBC generally shows high expression of basal cytokeratins, and growth factors like EGFR and CKIT, are highly proliferative, and often p53 mutant. They also demonstrate genomic instability [183]. There is a high association with BRCA mutations, with most *BRCA1* carrier breast cancers being TNBC, though the converse is not true. About 10–20% of TNBCs will have *BRCA1/2* mutations [184]. Most common mutations and activated pathways in TNBC include *P53* at 84%, *PIK3CA* 7%, *PTEN* loss 35%, *INPP4B* at 30%, with the latter two being negative regulators of the PI3 kinase pathway. *RB* mutations, *MYC* gain of function, and global hypomethylation as well as aneuploidy and genomic instability are also seen [185]. Androgen receptor (AR) expression can also be seen in 23–38% of TNBC [186, 187].

Attempts to further classify TNBC to determine predictive markers have been performed with limited success. Information gained from GEPs, TMB, homologous repair deficiency, MSI and tumor infiltrating lymphocytes (TILs) have failed to produce changes in treatment paradigms so far. I-SPY 2 (Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer. www.ClinicalTrials.gov Identifier: NCT01042379) and other clinical trials are attempting to clarify this question. Several molecular pathways and mutations that are currently being studied with targeted treatments in TNBC include AR (NCT02353988), EGFR (NCT02362230) as well as immunotherapy for tumors with increased MSI or PD-L1 expression (NCT02555657). New antibody drug conjugates targeting gpNMB (NCT01997333) or Trop-2 (NCT02574455) are also being investigated. The PI3K/AKT/mTOR pathway is activated in 10% TNBC either from mutations in *PI3KCA* or *AKT1* genes, or loss of *PTEN* [185] and offer a potential targeted treatment for many patients.

1.2.7.2 Luminal A/B, or Hormone Positive

Approximately 74% of breast cancers are HR positive (including 11% that are also HER2 positive) [188]; these breast cancers are generally more differentiated and lower grade compared to TNBC. Given the overexpression of the ER, all HR-positive breast cancers use endocrine therapy as the backbone of treatment. The current trend in HR-positive breast cancer is using molecular testing to discover patients that can avoid chemotherapy and can be treated with targeted endocrine therapy alone. The testing performed, including OncotypeDx, MammaPrint, and PAM50, do not predict response to any certain type of chemotherapy, but prognosticate and predict overall response to chemotherapy.

OncotypeDx is a reverse transcriptase PCR assay of 21 genes, 16 cancer related and 5 reference genes. Cancer-related genes include proliferation genes (Ki67) and invasion genes (stromolysin 3), as well as estrogen and Her-2 genes [189]. Validated by the TAILORx (Trial Assigning IndividuaLized Options for Treatment [Rx])prospective study in patients with HR-positive, lymph node (LN)-negative disease on tamoxifen, OncotypeDX gives a recurrence score ranging from 0 to 100, with lower numbers placing patients into low-risk categories that can safely avoid chemotherapy [190]. The updated TAILORx study found that women with score <26 have a low risk of recurrence without chemotherapy and little benefit gained with chemotherapy [191]. OncotypeDx was also retrospectively validated by SWOG 8814 in patients with up to three nodes positive and results from a prospective LN positive trial are highly anticipated (RESPONDRx [Rx for Positive Node, Endocrine Responsive Breast Cancer] trial). MammaPrint is a 70 GEP, used to determine a signature of a patient's tumor, with a high genomic risk signature having worse survival. Evaluated genes include known tumorigenesis-related genes such as *TP53*, *RB1*, *MYC*, *JUN*, and *CDKN2A* [192]. MammaPrint was validated in both HR-positive and negative patients, as well as LN-positive. The MINDACT (Microarray In Node negative Disease may Avoid ChemoTherapy) study looked at discordant cases (clinical high risk but genomic low risk and the reverse) and found that clinical high risk, but genomic low risk, could safely avoid chemotherapy. The study also found that clinical low and genomic high could avoid chemotherapy [193]. Further investigation is needed. PAM50 is also supported by the National Comprehensive Cancer Network (NCCN) and looks at GEP, proliferation, and tumor size. Further genomic platforms (i.e. Breast Cancer Index) are being studied to evaluate the benefit of prolonged endocrine therapy.

1.2.7.3 HER2 Positive Breast Cancer

Discovery of HER2 (human EGFR-2) expression in breast cancer has led to significant use of targeted treatment options. HER2 directed therapy with therapeutic antibodies, and now TKIs, has changed the landscape of this higher-risk breast cancer population, improving both disease-free and overall survival [194–196]. Testing for HER2 is performed initially by IHC and reflexed to FISH at most centers for intermediate scores on IHC [197].

1.2.7.4 Immunotherapy

No current biomarker for predicting response to immunotherapy in breast cancer exists. In general, TNBCs have higher mutational burdens with more genetic complexity. Evaluating TILs and PD-L1 expression by IHC are being studied, however these evaluations carry sensitivities and specificities with no set guidelines for cut-offs to indicate positivity. In general,

TNBC show higher PD-L1 expression and TILs [198, 199]. Studies looking at immunotherapy with checkpoint inhibitors (ICIs) in previously treated patients with metastatic TNBC show overall response rates (ORRs) in the 15–24% range, but with some patients having sustained response. The responses are increased with the addition of chemotherapy to immunotherapy [200]. Finally, TMB as well as MSI (or MMR deficiency) can be an indicator of response to ICIs. Overall, further work is needed to better understand how to utilize these markers in breast cancer.

1.2.7.5 Germline Testing in Breast Cancer

Current expanded panels can test for BRCA1/2, as well as TP53, STK11, PTEN, CHEK2, PALB2, ATM, BARD1, BRIP1, RAD51, MLH1, MLH2, MSH6, and PMS2. *BRCA* mutation is a marker a genomic instability and indicates a defect in MMR, which suggests a response to platinum salts as well as PARP inhibitors. The OlympiAD (Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation) trial showed an increased RR to olaparib compared with chemotherapy, which led to its FDA approval [201]. Many of these other mutations have treatment implications as well as clinical trial options. Based on this biology, theoretical interventions are currently being studied, and www.clinicaltrials.gov lists many potential treatment options.

1.2.7.6 Conclusion

Breast cancer is a rapidly evolving field with many opportunities for further research and development. Understanding the intrinsic subtype and receptor status is an important initial approach to treating patients with breast cancer, with expanded gene profiles offering many potential targeted therapies. Enrolling patients in clinical trials to clarify the use of these targeted agents is of utmost importance. The Targeted Agents and Profiling Utilization Registry (TAPUR™) is a multicenter study that offers patients clinical trial opportunities based on their genomic alterations that have known drug targets or predicted sensitivities to a TAPUR study drug. Given the high incidence of breast cancer, there is an opportunity to expand knowledge from breast cancer to other, less common cancers and continued research and clinical trial participation is encouraged.

1.2.8 Genitourinary Malignancies

Large-scale genome sequencing projects like TCGA has led to a better understanding of the genomic, epigenetic, and transcriptional landscape of genitourinary cancers (GU) [202–206]. These advances have identified pathogenic germline and somatic alterations that have opened exciting avenues for testing new targeted therapies. Here we present important advances in prostate, renal cell, and urothelial cancers in the context of precision medicine.

1.2.8.1 Prostate Cancer

Androgen suppression by androgen deprivation therapy (ADT) has been the backbone of metastatic prostate cancer (mPC) treatment for several decades [207]. Recently, several new therapies approved for mPC such as docetaxel, enzalutamide, abiraterone, sipuleucel-T, cabazitaxel, and radium-223 have shown survival benefit [208–213].

Genomic profiling in prostate cancer has identified several potentially actionable mutations and pathways. Somatic mutations in *AR* ligand-binding domain, namely H874Y, F876L, T877A, and W741L/C, have been correlated to ADT resistance and disease progression 13–15 [214–216]. Apart from *AR* somatic mutations, DNA repair pathways, phosphoinositide 3-kinase (PI3K), WNT, and cell-cycle pathways are recurrently mutated in metastatic castrate-resistant prostate cancer (mCRPC) [202, 217–221]. Recent studies have emphasized the role of AR signaling in regulation of DNA damage response (DDR) in prostate cancer [222–224]. Studies have shown that about 30% of mCRPC patients have genetic alterations that interfere with DNA repair [225, 226]. Furthermore, an increasing proportion of patients with localized prostate cancer also harbor pathogenic mutations in DNA repair genes [227]. Some of these aberrations may confer sensitivity to PARP inhibitors like olaparib. Targeting PARP is a validated therapeutic strategy in CRPC patients with aberrations in DNA repair genes like BRCA2 and ATM [228]. In TOPARP-A trial, 50 patients with metastatic CRPC patients who progressed through multiple lines of treatment treated with olaparib, showed response rate of ~33% [229]. Among 16 responders, 7 patients harbored somatic and germline mutations *BRCA2* mutations and 4 patients had *ATM* aberrations. Furthermore, these mutations were detectable in cfDNA which may prove to be a useful biomarker in the future [229].

PI3K alterations through loss of *PTEN*, amplification of *PIK3CA/B*, and activating mutation of *PIK3CA/B* and *AKT1* were seen in 49%, and WNT pathway in 18% of mCRPC patients [217–220]. Mutations in cell cycle pathway include loss of *RB1* seen in 21% of mPC and less common alterations in *CDKN2A/B*, *CDKN1B*, and amplifications of *CCND1* [203, 218]. Several clinical trials are testing PI3K inhibitors and CDK4/6 inhibitors in mPC.

The two most prevalent isoforms of AR splice-site variant 7 or 9 (AR-V7 or AR-V9) can be found in circulating tumor cells. AR-V7 and AR-V9 splice variants have deleted ligand binding domain and constitutively activate AR [230, 231]. Studies have reported that patients harboring AR-V7 or AR-V9 have lower response rates, higher rates of biochemical recurrence, and lower survival following treatment with enzalutamide and abiraterone [232–236]. However, two recent studies have failed to replicate these findings questioning the clinical utility of AR-V7 expression [237, 238]. Nonetheless, AR-V7 and AR-V9 are promising biomarkers that need further validation. It is important to note that agents targeting AR-V7, including onalespib and niclosamide, are currently under investigation [239, 240].

Pembrolizumab has been approved in MSI-high tumors regardless of the tumor histology [176]. Small percentage of prostate tumors (~4–25%) demonstrate MSI42 [241], resulting in sensitivity to PD-1 blockers. Recently, a phase 2 study showed that the combination of durvalumab and olaparib is well-tolerated and exhibited clinical activity in mCRPC [242]. Interestingly, a recently published small phase 2 clinical trial demonstrated encouraging efficacy of ipilimumab and nivolumab combination in AR-V7-positive mPC patients with DDR mutation [243]. There are ongoing clinical trials exploring the combination of olaparib and anti-PD-1/PD-L1 therapies in mCRPC.

Several genetic tests (Decipher, Oncotype DX, Prolaris) that predict probability of metastasis, prostate cancer mortality, or 10-year survival following radical prostatectomy or prostate biopsy are available but the clinical utility of these biomarker assays requires validation in prospective randomized trials [244–246].

1.2.8.2 Renal Cell Cancer (RCC)

More than two decades ago, the role of *VHL* loss in hypoxia signaling mediated by HIF1, HIF2-alpha and renal cell cancer (RCC) development was discovered [247]. Since then, several other genes implicated in RCC (*PBRM1*, *SETD2*, *KDM5C*, *PTEN*, *BAP1*, *mTOR* and *TP53*) pathobiology have been uncovered [248–252]. Sequencing studies have highlighted the molecular heterogeneity of this cancer across other histologic subtypes [206, 253, 254]. Recently, 843 RCC tumors that included 488 clear cell RCC (ccRCC), 274 papillary RCC (pRCC), and 81 chromophobe RCC (chRCC) revealed distinct molecular alterations in each histologic-subtype. Somatic mutation in *BAP1*, *PBRM1*, and *PTEN* correlated with survival [205]. *CDKN2A* alteration and increased DNA hypermethylation correlated with poor survival within all major histologic subtypes and increased Th2 gene signature within each RCC subtype was associated with poor survival [205].

Drugs targeting c-MET, FGFR, VEGFR, mTORC1, cytokines, and anti-PD-1/PD-L1 encompass the spectrum of approved RCC treatments. Most of the improved survival outcomes seen in RCC are limited to ccRCC. There is empirical evidence to suggest some degree of efficacy with conventional therapies in pRCC and chRCC due to overlap of dysregulated pathways [255, 256]. Foretinib, a multikinase inhibitor of MET, AXL, appears to benefit more in patients with germline *MET* mutations than in pRCC patients [257]. Furthermore, a recent study of genomic profiling in Type 1 and Type 2 pRCC patients showed alterations in MET, CKDN2A/B, and SWI/SNF (SWItch/Sucrose Non-Fermentable) pathways that could be potentially targeted with novel agents that are currently under development [258].

The molecular complexity and heterogeneity of RCC appears to be the biggest challenge in implementing personalized therapies [253, 259]. Routine use of genomic profiling to identify actionable mutations after failure of standard therapies is still evolving. Recently, investigators used circulating tumor DNA (ctDNA) in relapsed metastatic RCC patients to detect genomic alterations in several canonical cancer genes *TP53* (35%), *VHL* (23%), *EGFR* (17%), *NF1* (16%), and *ARID1A* (12%) [260]. However, the gene panel excluded *PBRM1*, *SETD2*, *BAP1*, *KDM5C* genes that are known to be important in RCC [254]. As more evidence emerges on the clinical utility and validity of ctDNA it may eventually play a role in the future.

1.2.8.3 Urothelial Cancers

Metastatic bladder cancer is an aggressive disease with median OS of 14 months [261]. Immune checkpoint blockers have been recently approved in advanced bladder cancer [262, 263]. However, no predictive biomarkers of response exist to systematically select patients who can benefit from therapy. Using integrated genomic approach in a cohort of 412 muscle invasive bladder cancer (MIBC) patients, TCGA has identified recurrent somatic mutations in *KMT2C*, *ATM*, *FAT1*, *CREBBP*, *ERBB2*, *SPTAN1*, *KMT2A*, *CDKN2A*, *TP53*, and *RB1* [202]. The study also used RNA-seq to classify MIBC into molecular subtypes and suggested potential therapeutic recommendations that could be tested in prospective clinical trials.

- Luminal-papillary subtype: Enriched for *FGFR3* mutation, *FGFR3-TACC3* fusions and/or amplification/overexpression. FGFR3 TKIs represent a potential therapeutic agent.

- Luminal-infiltrated subtype: Characterized by high expression of immune markers such as PD-L1 and PD-1, this is a subtype that has been associated with chemoresistance and would likely benefit from atezolizumab [262, 264].
- Luminal subtype: High expression of luminal markers (uroplakins) (UPK2 and UPK1A) and urothelial differentiation markers (FOXA1, GATA3, PPARG).
- Basal-squamous subtype: Subtype with the strongest immune expression signature including TILs with potential benefit in the atezolizumab trials [262].
- Neuronal subtype: Subtype with poorest survival. Characterized by expression of both neuroendocrine and neuronal markers and mutations in *TP53* and *RB1*, a hallmark of small cell neuroendocrine cancer. Platinum-etoposide combination chemotherapy may be preferred option.

Despite these advances, none of them have been successfully used to guide routine clinical care. But gaining insights from these studies are critical to tailor future clinical trials with targeted agents and bring us closer to the promise of precision medicine in bladder cancer.

1.2.9 Pediatric Cancers

1.2.9.1 Introduction

Greater understanding of biologic mechanisms driving pediatric cancer has created new avenues for research into targeted therapy for oncogenic mutations and pathways. Though pediatric malignancies currently represent only 1% of new cancers, they are the second-leading cause of mortality in children ages 4–15 [265, 266]. Targeted therapy may improve outcomes and spare younger patients long-term complications frequently associated with current multimodal regimens. Germline mutations have been demonstrated in the most common pediatric cancers to varying degrees, including leukemias/lymphomas, CNS tumors, and neuroblastomas [267]. One recent genomic sequencing study identified predisposing germline mutations in 8.5% of pediatric subjects with cancer [267]. Certain known autosomal dominant germline mutations, such as *TP53, APC,* and *BRCA1/2* are associated with several different pediatric cancers [267–269]. Mutations in the *RAS* family, for example, have been associated with the development of neuroblastomas as well as rhabdomyosarcomas [270]. Acquired genetic changes are also an area of active research with numerous sequencing projects looking at actionable pathways that drive tumor survival and proliferation. Research into treatments directed at these genetic changes has provided novel therapy options but remains challenging for several reasons including heterogeneity and limited sample size [271].

1.2.9.2 Leukemia and Lymphoma

Leukemia is the most prevalent cancer type in children [265, 266]. The incidence of pediatric leukemia is increasing though mortality has been declining over the past four decades due to great strides in treatment [265]. This subset of cancers appears to be less associated with germline mutations than other tumor types [267]. Germline mutations in *TP53* are most common; however, germline changes in other tumor suppressor genes, including *APC* and *BRCA2*, as well as oncogenes, *KRAS* and *RUNX1*, have been found [267]. Somatic

oncogenic mutations in these cancers, on the other hand, are frequently identified [272]. The most common leukemia in pediatric patients, ALL, has been linked to changes in *PAX5*, *TCF3*, *IKZF1, IKZF2, IKZF3,* and *EBF1* as well as other genes controlling the differentiation and growth of B-cells [272, 273]. A subset of these leukemias, Ph + ALL, are driven by a translocation t(9;22) and BCR-ABL tyrosine kinase overactivity [273]. Additional rearrangements commonly seen are *ETV6-RUNX1, TCF3-PBX1, MLL-AF4* [272–274]. Though attempts have been made to risk stratify patients according to these mutations, the prognostic implications of most remain in flux [274]. Somatic mutations in Janus kinases *JAK1*, *JAK2*, and *JAK3* have also been implicated in pediatric ALL [275]. Pediatric AML is most frequently associated with somatic changes in *FLT3* [276]. Several different variants of this mutation have been described with attempts made to explore the prognostic significance of the most frequently encountered mutations [277]. Notably *FLT3* mutations have also been described in ALL [278]. Pediatric lymphomas are also common, exhibiting similar prevalence and mortality trends as leukemias [265, 266]. Burkitt lymphomas have been linked to sporadic mutations in *MYC* and *TP53* [279]. T-cell lymphomas have been linked to *FBXW7* and *NOTCH1* mutations, though the clinical significance and prognostic relevance of these mutations is also still being studied [274, 280]. These genomic associations have led to the exploration of TKIs in pediatric leukemias. Imatinib has now been approved for first line treatment of Ph + ALL in combination with chemotherapy by the FDA [271]. Reports evaluating the safety and efficacy of sorafenib for *FLT3* mutants have also shown a positive response [281, 282].

1.2.9.3 Central and Peripheral Nervous System Tumors

CNS tumors are the most common solid tumors in the pediatric population. Owing to markedly increased leukemia survival over the past 50 years, brain cancer has become the leading cause of death in children despite its lower overall lower incidence, accentuating the need for novel therapies in this cancer type [265, 266]. CNS tumors that originate from glial cells, called gliomas, can be classified according to grade. High-grade gliomas (HGG) have been linked to mutations in *PIK3CKA*, *TP53, ATRX,* and histone H3 genes among others [283, 284]. Mutations in *PTEN,* commonly found in adult HGG, are less commonly seen in pediatric disease [283]. Low-grade gliomas (LGG) have demonstrated gene rearrangements in *BRAF* [285, 286]. These appear to be distinct from the missense *BRAF* mutations seen in high-grade lesions [285]. *ATRX* and histone H3 mutations can be seen with LGG as well [287]. Medulloblastoma, a CNS embryonal tumor, has classically been associated with genetic changes in the Wnt pathway and Sonic hedgehog (Shh) pathway, though two additional (groups 3 and 4) have also been described [288]. For medulloblastoma of the Wnt subgroup, two commonly implicated genes are *APC* and *CTNNB1*, which activate the Wnt pathway [288, 289]. Germline mutations in *APC* are associated with Turcot syndrome and predispose to the development of medulloblastoma as well as CRC [288]. Somatic mutations in *CTNNB1*, which codes for the b-catenin protein, are frequently seen in cases of sporadic medulloblastoma [289]. The Shh subgroup is strongly associated with somatic and germline mutations of *PTCH* and *SUFU* [288, 289]. The gene *PTCH* codes for the Shh receptor and can also predispose to several other malignancies that compose Gorlin's syndrome [288]. Amplifications in *GLI1* and *GLI2,* which code for transcription factors of the Shh pathway, have also been described

in Shh group medulloblastomas [288]. Medulloblastomas of the remaining groups C and D have been linked to *OTX2* and *FOXG1B* mutations [290]. Overexpression in proto-oncogenes *MYC* and *MYCN* to varying degrees and changes in genes for *TP53* and *MLL* can be seen across all medulloblastoma groups [288–290]. The amplification of *MYCN* found in medulloblastomas is also seen in neuroblastomas, pediatric tumors of developing cells of the peripheral nervous systems [271]. Germline and sporadic mutations in the anaplastic lymphoma kinase gene *ALK* have been demonstrated to have a strong association with neuroblastoma development and have been hypothesized to be strong drivers in tumor growth [270, 291, 292]. Additional sporadic mutations in *BRAF, RAS,* and *MAP2K1* and heterozygous germline mutations in tumor suppressor genes *BRCA1* and *SMARCA4* have also been described [269, 270]. Mutations in transcriptional regulator gene, *ATRX*, have been linked to neuroblastoma in older children and worse prognosis [293]. Agents that act on these mutations and pathways have shown some promising results. Crizotinib, an *ALK* inhibitor, has shown been shown to produce a good response in susceptible neuroblastoma tumors [294]. Sonidegib and vismodegib, two agents that act on the Shh pathway, are currently undergoing Phase II trials for medulloblastoma treatment [295, 296].

1.2.9.4 Bone and Soft Tissue Sarcomas

Rhabdomyosarcoma (RMS) is soft-tissue tumor originating from developing skeletal muscle cells most often affecting young children under 10 years of age [265, 266]. Rhabdomyosarcomas can be subclassified by histology to embryonal RMS (eRMS) and alveolar RMS (aRMS) [297, 298]. The two can also be distinguished cytogenetically as aRMS is strongly associated with gene fusion of *PAX3* or *PAX7* with *FOXO1* to form oncogenic gene *PAX-FOXO1* [297–299]. Germline mutations are not common [267]. Somatic mutations in eRMS include *FGFR4*, *PIK3CA*, *CTNNB1*, *BRAF, HRAS, NRAS, and KRAS* [270, 298]. *KRAS* mutations are common to both eRMS and aRMS tumors [270]. Ewing sarcoma is a poorly differentiated tumor that may arise from soft tissue or bone. Previously characterized over 20 years ago by the translocation of 2 genes to form *EWS-FLI*, more recent genomic studies have also found rare sporadic changes in *BRAF, CTNNB1*, and *NRAS* [270, 300, 301]. Somatic mutations in *STAG2*, which codes for proteins involved in chromatid exchange during cell replication, and *CDKN2A* have also been reported [298]. Osteosarcomas are the most common pediatric tumors of the bone [266]. They are frequently seen with germline mutations in *APC, RB1* and *TP53* among others, as part of cancer syndromes, as secondary malignancies from prior radiation, or in isolation [265, 267]. Somatic mutations in *ATRX* and *DLG2*, which is involved in epithelial polarity during cell division, have been described in addition to mutations in established tumor suppressor genes, *RB1* and *TP53* [302]. Osteosarcoma has a higher rate of mutation than other sequenced pediatric tumors with great heterogeneity even in intratumor findings [302, 303].

1.2.9.5 Other Embryonal Tumors

Retinoblastoma, a malignancy of immature retinal cells, is classically associated with mutations in *RB1* [265, 304]. Risk of developing the tumor in those with a germline mutation in *RB1* is approximated to be 9 out of 10 [304]. Nephroblastoma, also known as Wilms' tumor, is a tumor of the kidney associated with germline mutations in *WT1* and *WT2* [305].

Mutations in *WT1*, a tumor suppressor gene essential to the development of the urogenital system, are associated with Denys–Drash syndrome and WAGR (Wilms tumor, Aniridia, Genitourinary anomalies, and mental Retardation) syndrome [265]. Sporadic mutations found in this group include *CTNNB1*, *MYCN, WTX,* and *FBXW7* [305, 306]. Currently radiation and anthracyclines play an important role in treatment. However, their overall morbidity including infertility, scoliosis, and risk of secondary malignancy make the possibility of genomic targeted therapy an attractive future alternative [265].

1.2.9.6 Conclusion

A greater push to understand the prevalence and role of germline mutations in pediatric cancers is underway and is mirrored by an ongoing effort to identify more somatic changes that drive the survival and proliferation of rare tumors [307]. Precision medicine in the form of targeted therapies aim to continue the decline in pediatric cancer mortality.

1.2.10 Cancers of Unknown Primary Origin

Cancer of unknown primary origin (CUP) is defined as histologically confirmed cancer whose primary cannot be identified following standard diagnostic evaluation. CUP accounts for 3–5% of all invasive cancers [308]. Tumors from many primary and varying biologies are represented in CUP [309]. Median survival is 8–12 months. A subset of patients with CUP who have a favorable outcome have median OS in the range of 12–36 months [310].

Initial clinical evaluation should include complete history and physical examination, complete blood counts, serum chemistries, urinalysis, CT scans of the chest abdomen and pelvis, mammogram in women, and serum prostate specific antigen in men [311, 312]. Identification of primary site in the initial evaluation of CUP is thought to enable improved accuracy of cancer therapy and likely benefit patient survival [313].

1.2.10.1 Diagnosis

1.2.10.1.1 Light Microscopy Histologic evaluation by light microscopy allows classification of CUP into five subtypes: (i) Well or moderately differentiated adenocarcinoma (60%); (ii) poorly differentiated adenocarcinoma or undifferentiated carcinoma (29%); (iii) Squamous cell carcinoma (5%); (iv) poorly differentiated neoplasms (5%); and (v) neuroendocrine tumors (1%) [314].

1.2.10.1.2 Immunohistochemistry IHC staining has become indispensable for the pathologic evaluation of CUP. IHC uses antibodies usually linked to an enzyme or a fluorescent dye. When antibodies bind to the antigen in the tissue sample, an enzyme or dye is activated and the antigen can be visualized under a microscope [315]. Significant improvement in expansion of additional tissue-specific biomarkers in recent years has increased the diagnostic accuracy in identifying the primary site of CUP [316, 317]. A stepwise approach using IHC is recommended to establish tissue of origin. The first step would be to use lineage-restricted markers to determine tissue lineage, for example markers typical of carcinoma, sarcoma, lymphoma, or melanoma. Organ-specific markers can be used as a second step to establish the potential primary site [316, 318, 319].

1.2.10.2 Gene Expression Profiling

Gene expression profiling assays are done based on the idea that metastatic tissue has the similar molecular profile compared to primary site. Gene expression profiling measures the activity of genes by determining the pattern of genes expressed [320]. This can be done with either reverse transcriptase PCR or gene microarray techniques [321–323]. Gene expression profiling is shown to yield diagnoses at higher rates especially in the uncommon group of patients with poorly differentiated neoplasm of unknown origin [324, 325].

1.2.10.3 Mutational Testing with Next-Generation Sequencing (NGS)

NGS can be used to detect potentially actionable mutations and rearrangements. In some cases, testing biomarkers such as MMR, RAS, HER2 or ALK rearrangements may be tested [314]. Clinical benefit of targeted treatment in CUP based on molecular studies remains controversial [326–328]. Clinicians and pathologists must collaborate on the discreet use of these modalities on a case-by-case basis until more robust outcomes and comparative effectiveness data are published.

1.2.10.4 Treatment

Conventionally, empiric combination chemotherapy has been the standard first-line of treatment for CUP [327, 328]. Usually combinations using a platinum agent along with one of the newer cytotoxic agents such as taxanes, gemcitabine, and irinotecan are used as empiric combination chemotherapy [328–330]. Standard regimens have been known to have modest response rates in CUP. Less than 40% of the patients respond to the treatment with median survival of less than 11 months and 2 year survival less than 20% [327, 328]. With recent advances in gene expression profiling, a tissue of origin can be precisely predicted in the majority of patients although anatomic primary site cannot be identified. If patients do not fit into a specific treatable subset based on light microscopy and IHC, data is more convincing to support site-specific treatment based on prediction of site of origin by gene expression profiling. This is especially beneficial in patients predicted to have treatment-responsive tumors. Gene expression profiling followed by site-specific therapy should now be a part of the standard management of CUP patients who do not fit into a specific subset. Empiric chemotherapy should be considered for patients whose tumors are unclassifiable by gene expression profiling.

There has been increasing use of NGS which has enabled us to use targeted agents in a wide variety of advanced cancers. At present we do not have results of prospective trials to evaluate the efficacy of targeted treatment in CUP. The ongoing NCI-MATCH trial will give us additional information with precision medicine in many advanced cancer types [331]. In the future, randomized controlled trials with use of targeted agents in CUP will help guide us with treatment choices.

1.3 Biomarkers for Immunotherapy of Cancer

Immuno-oncology (IO) therapy, which harnesses the body's ability to mount an immune response against cancer cells, has revolutionized the treatment of multiple human cancers in the last two decades [332]. IO therapy augments T-cell activity by blocking cytotoxic T

lymphocyte antigen-4 (CTLA-4), PD-1 and PD-L1 [333]. However, only a fraction of the patients receiving IO therapy have a response. The role for biomarkers is to help identify a subset of patients who will respond to the therapy. What makes one tumor more responsive to IO therapy compared to others? In order for IO therapy to work, the tumor cells have to have the ability to present and release immunogenic neo-antigens, and a permissive environment for the T-cells to infiltrate the tumor's microenvironment to permit the invasion of T-cells so that activated tumor-specific T-cells can identify tumor cells and induce tumor lysis [334]. There is a great deal of ongoing research in this area of biomarkers to IO therapy to help predict patient's response. Generally speaking, biomarkers can be prognostic or predictive. Prognostic biomarkers provide information about individual risk classification based on the natural course of the disease. Predictive biomarkers provide information about treatment selection, i.e. will the patient respond to given treatment.

1.3.1 PD-L1

PD-1 is an immune checkpoint that prevents excessive immune response and autoimmunity. PD-1 protein expressed on the T cells, B cells, and natural killer cells (NK) binds to PD-L1 that is expressed on the tumor cells and the stromal cells [332]. This interaction of PD-1 on the immune cells and PD-L1 on the tumor cells leads to inhibition of the cytolytic effect of the immune cells [335]. By blocking PD-1 or PD-L1, IO therapeutic drugs tend to inhibit this inhibitory system and lead to activation of immune cells and eventually lysis of tumor cells. Mechanistically, if PD-L1 is overexpressed in tumor cells, then a higher response to IO therapy may be expected [336, 337]. In fact, in the NSCLC studies, higher PD-L1 expression was associated with improved ORR [338]. However, there are several challenges in using PD-L1 as a biomarker for IO therapy. First, presence of PD-L1 depends on the dynamic microenvironment of the tumor so it is a dynamic marker. Second, even some of the patients with low or no PD-L1 expression have had responses to IO therapy, indicating that PD-L1 may be sufficient but not necessary to predict benefit from ICIs [338]. Lastly, despite the boom in usage and approvals of IO therapy recently, no clear consensus exists as to the interpretations of the PD-L1 testing, i.e. different agents are used for the IHC testing and it is hard to draw conclusions across studies. In addition, the cut-off value for what is positive, what is high, and what is low PD-L1 expression also is not clearly defined. Currently, the approval for pembrolizumab and nivolumab is in conjunction with their particular PD-L1 IHC test [339].

1.3.2 Soluble PD-L1 (sPD-L1)

The clinical significance and mechanism of generation of sPD-L1 is not well understood, however sPD-L1 has been associated with aggressive renal cell carcinoma and shorter survival in multiple myeloma and DLBCL. To understand this better, Zhou et al. studied serum from patients with melanoma as well as healthy donors [170]. They noted that pretreatment levels of sPD-L1 were increased in patients with progressive disease, and that at five months of treatment with CTLA-4 or PD-1 blockade, patients with elevated sPD-L1 had a higher likelihood of response, indicating the potential to study sPD-L1 as a biomarker so that a subgroup where it is highly elevated may be identified and response assessed. Given

that it can be tested in peripheral blood adds to the value because of the ease and ability to be able to see dynamic changes during course of the illness.

1.3.3 Combined Positive Score (CPS)

In order to characterize better expression of PD-L1 in the tumor tissue, the tumor proportion score (TPS) was proposed (ratio of tumor cells positive for PD-L1 expression over the total number of tumor cells). However, not only the PD-L1 expression on the tumor cells may matter, but also the PD-L1 expression on the immune cells that are in the tumor, and impact on the effectiveness of IO therapy. Combined positive score (CPS) is the ratio of the number of all PD-L1 expressing cells (i.e. tumor cells, lymphocytes, macrophages; not just the PD-L1 expressing tumor cells) to the number of all tumor cells. CPS has been reported to be a robust and reproducible PD-L1 scoring method to predict response to IO therapy [340].

1.3.4 Tumor Microenvironment

Tumors not only have cancer cells, but they harbor a rich microenvironment of neutrophils, blood vessels, antigen presenting cells (APCs), tumor associated macrophages, extracellular matrix, cytokines and growth factors, all of which influence the anti-tumor responses. TILs influence treatment outcomes in a way that TIL is thought to confer a favorable response to IO [341]. In fact, the notion of categorizing tumor into "hot" or "cold" based on their immune microenvironment is being evaluated. A tumor may be inflamed due to CD8+ T cells and other immune cells that are present at the margins and within the parenchyma of the tumor or immune excluded due to presence of the immune cells in the margins but not in the parenchyma. Additionally, a tumor may be an "immune dessert" due to absence of immune cells [335]. Furthermore, treatment with chemotherapy or radiation causing immune infiltration, and thus converting a cold tumor to a hot tumor is an area of active research.

Not just the immediate microenvironment of the tumor, but the host's peripheral blood, which serves as the connector and messenger throughout our bodies, has been evaluated for biomarkers. Due to ease of accessing blood, this is a particularly attractive methodology. Significant differences in subsets of peripheral blood mononuclear cells (PBMC), and CD4+/CD8+ T cells in melanoma patients who did or did not respond to IO therapy were found using mass spectrometry. Similarly, subsets of NK were found to be different between IO responders versus non-responders [342].

1.3.5 Tumor Mutational Burden (TMB)

TMB refers to number of non-synonymous mutations found in a patient's tumor. It is measured by NGS and compares DNA sequences from patient's healthy cells versus tumor cells, followed by a complex algorithm the result of which is provided as number of mutations per megabase. TMB can be thought of as a surrogate marker of the number of immunogenic peptides that are generated by mutated genes, leading to an immune response in the tumor's microenvironment [343]. Phase III clinical trials in lung cancer and other cancers have shown that patients with high TMB respond better to immunotherapy than to

chemotherapy, even when PD-L1 levels were low [344, 345]. There are also sparse but early reports of mutational load and T-cell immune signatures being interdependent predictors of efficacy of concurrent chemotherapy and radiation therapy in patients with HNC [346]. These are clinically important findings because they have the potential to help select patients who may respond better to a given therapy, hence improving the scope of individualized precision medicine and providing an opportunity to the patients to benefit from potentially less-toxic treatments.

1.3.6 Microsatellite Instability (MSI)

Patients with MSI-high tumors are thought to have a subset of cancer type that is responsive to immunotherapy. It is thought that tumors that show MSI-high are hyper-mutated and hence express abundant neoantigens, and may have ample TILs, and hence show response to immunotherapy [347]. In May 2017, the FDA granted accelerated approval to pembrolizumab for pediatric and adult patients with MSI-high or MMR deficiency in solid tumors, agnostic of tissue type. This was based on results of five trials that showed that patients with MSI-high or MMR deficiency had ORR of 39.6% with 11 patients having CR, and 78% of the responders having sustained response of six months or greater [348].

1.3.7 MMR Deficiency

The MMR system consists of a family of proteins (MLH1, MSH2, MSH6 and PMS2) that detect and correct errors that occur during DNA replication. Mutations in MMR genes lead to defects in function of this system and causes MSI. Hence MMR deficiency causes MSI-high tumors. MMR-deficient tumors are thought to have more TILs (tumor-infiltrating lymphocytes) due to high mutational load [349].

1.3.8 Peripheral Blood Absolute Neutrophil Count/Absolute Lymphocyte Count

Peripheral blood biomarkers are easily obtainable and hence have an appeal for further development for clinical practice. Studies have evaluated trends of various blood-based biomarkers, but none have been approved for clinical use yet [350]. Increased baseline absolute neutrophil count (ANC) of more than $7.5*10^9$/l, baseline ANC : ALC (absolute lymphocyte count) ratio of 5.9 or more, increased myeloid : lymphoid (M : L) ratio and elevated monocytes were associated with worse prognosis [351, 352]. In addition, baseline ALC was positively associated with improved OS [351]. Given that these data show that peripheral blood indices are correlated with outcomes in patients treated with IO therapy, further investigation is warranted especially given the ease of sample collection and ability to monitor any dynamic changes during treatment.

1.3.9 Microbiome

It has been shown by metagenomic studies that there are different microbiomes in the gut of metastatic melanoma patients who responded to IO therapy versus those who did not

respond [353]. Not only the gut, but it is thought that the lungs also have a microbiome (caused in lungs by the disruption of normal mucosa by smoked tobacco) and that this unique microbiome may play a role in carcinogenesis of lung cancer [354]. Although these are early data and need validation, they are thought-provoking and indicate the need for a holistic approach to treating patients with IO therapy.

1.4 Clinical Trial Design in the Era of Precision Oncology

In an era of precision oncology when therapies are targeting specific oncogenic pathways, and recent FDA approvals of new therapies (pembrolizumab for treatment of MSI tumors and larotrectinib for tumors with tropomyosin receptor kinase A fusion) are based on the presence of a biomarker and not necessarily malignancy of a specific organ, there is a need for restructuring of design of cancer clinical trials.

For most of the history of development of cancer therapeutics, systemic cancer therapy depended on modestly higher toxicity against cancer cells versus normal cells. Therefore, even today, most of first in-human clinical trials are designed to maximize therapy dose until the maximum tolerated dose is reached. With development of targeted therapy and genomics to identify tumors with specific molecular vulnerability, it became apparent that an effect on target may be achieved at lower doses, or escalation even to very high doses of drugs that does not cause unacceptable toxicity.

In this context, there is an effort to seek rational dose escalation based on target saturation [355], or by use of clinical efficacy endpoint in early phase studies as a guide to expand cohorts, sufficiently powered for evaluation of activity [356]. In this latter strategy, investigators can use data about antitumor activity even from patients in phase 1 portion of study in application for drug approval.

In addition, one of the challenges for drug development for specific oncogenic target is that a number of individuals with tumor with specific genetic aberration is relatively smaller. For example, *RET* rearrangements in lung cancer are only seen in 1% of lung adenocarcinomas [7]. With this small patient population even an international Phase 3 study with hundreds of study sites is not feasible to demonstrate superiority over standard therapy. In recognition of this, and that precision-driven therapy can generate superior responses in Phase 1 and Phase 2 studies as compared to historical controls (alectinib produced 55% responses in RET rearranged tumors) [357], regulatory agencies introduced breakthrough designation [358] and commercial drug approval based only on safety and tumor responses, and not necessarily on demonstration of survival benefit.

In order to address the needs of precision oncology in the last several years, new designs of clinical trials have been proposed:

1) Umbrella studies to test multiple treatments and multiple biomarkers within the same protocol with a goal to have one study protocol appropriate for subtype of malignancy with low prevalence, and yet to allow for randomized comparisons between cohorts that can be added or dropped depending on outcomes in each study arm. The examples of these designs were studies for lung cancer, i.e. ALCHEMIST, BATTLE, Lung-MAP, National Lung MATRIX trial, and studies for colon (FOCUS 4) and breast (I-SPY) cancer.

2) Basket studies to test the effect of a single drug on a single mutation in a variety of cancer types, combining the traditional clinical trial design with evolving genomic data. The example of basket study was Molecular Analysis for Therapy Choice (NCI-MATCH) that was designed to assign patient with malignancies based on molecular abnormalities, not site of tumor origin, to targeted therapies. This study was available in more than 2400 oncology practice sites throughout the United States and was activated in August 2015, using validated and standardized 143 target gene sequencing at four study sites, using Ion Torrent PGM™ custom panel, and creating a regulatory umbrella for drugs from more than 20 companies [331].
3) Other trial designs include enrichment or targeted, marker-by-treatment interaction, modified strategy, and Bayesian biomarker-adaptive designs [359].

An example of collaboration between regulatory agencies and drug companies is recent approval of larotrectinib by the US FDA for the treatment of adult and pediatric patients with solid tumors that contain a neurotrophic receptor tyrosine kinase gene fusion. This approval was based on 75% ORR in total of 55 pediatric and adult patients from three clinical studies; a Phase 1 study involving adults, a Phase 1/2 study involving children, and a Phase 2 study involving adolescents and adults [33].

With several regulatory programs, such as orphan drug designation [360] and accelerated approval process that includes fast track, breakthrough designation [358], and priority review [361], FDA has provided guidance to pharmaceutical industry and sped up access to effective therapies for small populations of cancer subtypes identified through precision oncology diagnostics.

1.5 Ethical, Legal, and Social Issues of Precision Oncology

The ethical, legal, and social implications of precision oncology are vast and complex as the field continues to evolve. Here we address some of the factors that are challenging both to patients and healthcare providers alike.

1.5.1 Ethical Issues

A physician is tasked with treating patients within the jurisdiction of well-established medical guidelines, with the notion of *help but do no harm*. Precision medicine is redefining how we approach our cancer patients, in a realm that lacks conventional boundaries, in which therapies are not always backed by reproducible science, and in which selecting a treatment or withholding one may be a lethal choice. Looking to genes as the roadmap to therapy seems like a simple solution; however, we are barely scratching the surface of the iceberg-sized pile of information we uncover by decoding the human genome.

Large genome sequencing produces an exorbitant amount of data, which inherently creates a category of poorly understood, repetitive genetic information termed “variants of unknown significance (VUS)” [362, 363]. As McGraw insightfully phrased it: are these genes “drivers” of disease, enabling tumor growth and mutation, or are they merely “passengers” without clinical value? [364]. Do we re-contact patients when a VUS is later

identified by better technology as a potential biomarker or risk factor for disease? [362, 365]. Are we morally obligated to divulge all the genetic material that we find? There are not simple answers to these challenging questions. Ideally, a qualified geneticist should convey the data concisely, in the hopes of facilitating decision-making and avoiding psychological, social, or financial stress for the patient [366]. We know, however, that disclosing information is time-consuming and costly, with many hospitals lacking proper infrastructure or personnel to do it well [366].

Current practice is to regard "secondary findings" as information about genetic variants that are unrelated to the primary purpose of the test; other findings are considered "incidental" [362, 367, 368]. The American College of Medical Genetics and Genomics' (ACMG) recent stance is to allow patients to opt out of receiving secondary findings before testing. Many genetic providers feel that patient preference should dictate the way we return incidental results [362, 369, 370].

Objectively, polls show that patients fear discrimination based on this type of genetic data, and that physicians fear litigation from the uncertainty of the genetic results they find. To assuage some of these fears, the 2008 Genetic Non-Discrimination Act (GINA) was created, which forbids discrimination based on genetic results or family history in both health insurance coverage and occupation [362, 371]. Tumor boards have emerged to determine on a "gene by gene" basis how data should be exchanged between physician and patient [364]; one group's experience was to return germline findings only if there were pathogenic and somatic variations if they had a conceivable link to disease. Another consortium with individual patient and physician panels felt that the patient should receive two pamphlets prior to testing: one focusing on the genetic alterations with potential medical consequence for the patient's tumor, and the second explaining how the data collected could possibly be used in the future [372].

How do we obtain informed consent on genetic material that we do not know the clinical significance of? It is hard to describe the uncertainty of the data obtained, especially to patients with a wide spectrum of health literacy, variable interpretations of risks and benefits, and unique family dynamics [366, 373]. Many organizations rely on a concept called broad consent. The patient gives permission for his genetic data to be used in a wide array of appropriate but not yet defined research projects, as it is too difficult to anticipate the benefits or risks in advance. Although this approach is advantageous for global scientific research because "re-consent" is not required, some argue that the patient's autonomy is put at jeopardy by blindly making a decision without understanding all the ramifications at the start [366, 374, 375]. Nevertheless, precision medicine aims to guide both physicians and patients toward a better understanding of the cancer disease process, for the benefit of the individual patient and the oncology community as a whole.

1.5.2 Legal Issues

Accessing investigational drugs has become exceedingly difficult, with stringent FDA regulations on trial participants and safety restrictions. A college student with advanced head and neck cancer sued the FDA on grounds that they were violating her constitutional rights by barring her access to cetuximab treatment in the trial known as Abigail Alliance for Better Access to Developmental Drugs vs. von Eschenbach [376–378]. To help accelerate

time to drug approval, the US Congress passed the "Right to Try" bill, which allows terminally ill patients access to investigational drugs before they reach the approval phase [379]. This law protects the physicians who prescribe and the drug companies who produce the trial drugs from claims of negligence, and from litigation from harm that may be caused from use. The ramifications are unclear, as the scale may teeter between costly health risks, and in other cases, some form of benefit.

The concept of negligence in court depends on the experiences of many previous cases "as a collective" that are applied to the singular "incident" at hand. How can we make such assumptions when our number, "n," is so small? For example, how can we use novel targeted drug therapy in a larger group when the literature is published on the foundation of a limited number of case series or case reports? [373]. The SHIVA (A Randomized Phase II Trial Comparing Therapy Based on Tumor Molecular Profiling Versus Conventional Therapy in Patients With Refractory Cancer) trial, for example, was an open-label project in which 99 patients with solid tumors were randomized into "pathway-directed therapy" (i.e. precision oncology) using drugs like imatinib, sorafenib, and dasatinib, to name a few, versus physician-selected standard therapy (control). The primary endpoint was PFS, which was nearly identical in both arms: two to three months in the precision oncology group and approximately one to two months in the control group [2]. The investigators concluded that off-protocol use of unapproved targeted drugs might not be beneficial, and that we should use rigorous randomized trials despite their lengthy road toward completion [373].

Similarly, it is hard to develop guidelines and laws for the study and use of these investigational drugs spanning between different states (across the US), let alone internationally. Each state has its own governing body, with a unique understanding on what is considered legal and what is not. The challenge of creating a body of law that will cross geographical and cultural boundaries, while also maintaining the same stringent rules for all patients involved in these drug trials, is mammoth. Some organizations, like the Public Population Project in Genomics and Society (P3G) and GA4GH, are trying to streamline the legislative and policy-making process [380, 381]. However, much work is needed to set a foundation for which both doctors and their patients are covered by health care policy justly and equally.

1.5.3 Social Issues

Precision medicine challenges many social themes, including the balance between individual care versus public health needs, patient privacy versus public knowledge, as well as equity in treatment across different races and socioeconomic backgrounds [382].

For example, using expensive genome-wide association studies (GWAS) to attack a tumor proliferation gene in a small subset of patients is thought to be cost-effective, by stratifying treatment or identifying non-responders, possibly avoiding detrimental drug side effects. But, critically assessing how much time is gained by metrics like PFS or treatment complications is yet to be seen from an economic standpoint as these targeted therapies are inordinately expensive [383]. What happens to those oncology patients that are "orphaned" by genetics, whose tumors are not well understood by modern sequencing techniques? Is it ethical to offer them therapies that based on our limited knowledge (i.e. small case series) may yield little benefit, in the face of a recurrent or resistant cancer? [384]. As Fleck so

eloquently stated, how can we compare interventions like dialysis and kidney transplants, which provide modest improvements to quality of life, to targeted cancer therapies whose results may be marginal with a much higher economic cost [385]. He argues that if we provide these "last chance" therapies to cancer patients, where do we draw the line for other chronically ill patients who suffer from various progressive and debilitating diseases? To promise unlimited healthcare, despite cost or manpower required, is a promise no resource-limited medical system can keep.

Another dichotomous relationship lies between patient privacy and global knowledge sharing [382]. Many centers try to maintain the anonymity of their patients, both from family members who may be involved in their care and from a patient's workplace or neighborhood. But if we are to expand our knowledge base and hope to cure patients across continents, there needs to be a collective effort to share results, including social characteristics. To date, there is not one well-curated database that currently contains all these pathogenic variants [371]. TCGA and the Internal Cancer Genome Consortium (ICGC) are trying to connect researchers to raw data sequencing, but they do not systematically gather clinical or demographic information [380]. Similarly, the literature relies heavily on white Caucasians as research participants, somewhat skewing the GWAS studies we base our medical decisions on [382, 386, 387]. How do we address barriers such as race, gender, and socioeconomic status that may limit a patient's involvement in these targeted therapy trials? An analysis of 31 FDA-approved drugs found that although the number of eligible patients grew from 5 to 8%, the general benefit was witnessed in only a fraction (0.7–4.9%) of the patients [388]. It is hard to treat a wide breadth of socially diverse patients with our new fine-tuned, complex, and individualized drugs. That is the balance that we must aim to maintain – help one patient in the hope of helping them all.

1.6 Databases, Data Sharing, and Challenges of Precision Oncology

Oncogenic process frequently caused by genomic damage of cell can stem from molecular alteration of any downstream cellular processes. In addition, cancer tissue microenvironment, host metabolic pathways, and immunity contributes to tumorigenesis. Recognition of this multilevel system has sparked interest in gathering data from genome, transcriptome, protein expression, cytokines, tumor immune status, comorbidities, and history of tumor responses to prior therapy. This in turn has created a need for large databases and data sharing.

There are several cancer genomic repositories and databases such as The Cancer Genomics Hub (formed from data from Cancer Genome Atlas, Cancer Cell Line Encyclopedia, and Therapeutically Applicable Research to Generate Effective Treatments) [389], European Genome-phenome Archive [390], ICGC [391], The Catalog of Somatic Mutations in Cancer [392], cBioPortal [393], and others.

In addition, there are several publicly accessible tools for clinical researchers for data integration and interpretation such as canEvolve [394], UCSC Cancer Genomics Browser [395], Cancer Program Resource Gateway – Broad Institute (CPRG) [396], and others.

The low prevalence of many driver mutations requires interinstitutional collaboration and inclusion of community oncology practices. This in turn, through data sharing, can generate widespread knowledge, and help with guidance in care of an individual patient.

There are, however, multiple issues related to data sharing, starting with the informed consent form, compliance with privacy and confidentiality under Health Insurance Portability and Accountability Act (HIPAA), data lineage and provenance, data quality, compatibility of databases, scalability, and others.

These challenges can be overcome as epitomized by The Oncology Research Information Exchange Network (ORIEN), a research partnership between Moffitt Cancer Center, The Ohio State University Comprehensive Cancer Center, City of Hope Comprehensive Cancer Center, University of Virginia Health System Cancer Center, University of Colorado Cancer Center, University of New Mexico Comprehensive Cancer Center, Morehouse School of Medicine, USC Norris Comprehensive Cancer Center, John P. Murtha Cancer Center, Huntsman Cancer Institute, Dartmouth-Hitchcock Norris Cotton Cancer Center, Winship Cancer Institute of Emory University, Stephenson Cancer Center, Holden Comprehensive Cancer Center, Roswell Park Comprehensive Cancer Center, Markey Cancer Center, and Indiana University Melvin and Bren Simon Cancer Center [397].

TAPUR Study [398], sponsored by the American Society of Clinical Oncology with participating 113 clinical sites, is another example of a precision oncology network. This non-randomized trial catalogs different genomic profiling tests used by oncologists, learns about prescribing practices, collects data on clinical outcomes (efficacy and toxicity), and educates oncologists on how to use targeted drugs.

Some non-profit and commercial companies that are involved in analysis of the cancer genomic profile of an individual patient frequently provide additional information about similar genomic profiles based on their own databases and available therapies or clinical trials that target a specific genomic alteration. However, such references often lack individual clinical context. Therefore, molecular tumor boards, usually set up in academic institutions, started to assist clinicians with recommendations regarding management of tumors based on gene sequencing, GEPs, and protein expression assays. In addition, they can provide recommendations regarding screening family members for familial cancer syndromes when there is presence of germline mutations.

Molecular tumor boards however have limited throughput and are not accessible to most oncologists and their patients. There is a need, therefore, for development of technologies that can assist in real time with making clinical decisions that address the evolution of individual clinical scenarios.

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