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Targeted Molecular Therapy in Palliative Cancer Management

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Abstract

Acknowledging the correlation of response to therapy based on the "targeting the target" concept, FDA demonstrated confidence in precision therapy approaches by approving FoundationOne®CDx test in late 2017 as an indicated diagnostic for cancer patients. More than 100 precision therapies involving both solid and liquid malignancy have since been approved by FDA as indicated therapy in a variety of cancer types as related to correlated molecular target. We provide clinical justification of consideration for precision therapy guided by matched molecular target, specifically focusing on PI3K/mTOR/AKT, BRCA, CDK4/6, EGFR and BRAF V600E for advanced disease cancer patients who previously failed optimal NCCN guideline directed standard of care.

Keywords: Molecular target; Cancer patients; Precision medicine

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Introduction

The concept of precision medicine, which takes into account individual variability between patients vis-à-vis treatment strategies, dates back to our early understanding of the importance of blood typing. Since then however, the concept has been widely applied and dramatically improved with the development of the field of bio-informatics which further carried the personalization of therapy from the ABO blood types, to a completely different and more sophisticated levels of patients' characterization at the molecular level. Although the concept of precision medicine can virtually be applied to any disease, oncology has arguably been at the forefront of these advances, mostly given our not-so-recent understanding of cancer as a genomic disease. Powerful methods such as proteomics and genomics, along with the availability of robust computational tools for data analysis have opened the way to a whole new era: the era of precision medicine. These advances have given oncologists unprecedented predictive power in the diagnosis and prognosis of cancer, narrowing down relevant discrimination to the level of single genes, single transcripts, or even single proteins within a malignant cell. Beyond the demystification of the etiology of cancer, this further allowed the identification of molecular therapeutic targets specific to cancer cells, thus providing a framework whereby therapies can be specifically matched to corresponding molecular targets.

Traditionally, different solid tumor classification has grossly remained unchanged and is mostly based on histopathology. Consequently, patients have also been treated with an exclusive focus on the tissue of tumor origin- lung, liver or breast, for example. However, with the completion of the Human Genome Project and the development of solid databases and analytical softwares, the technology of next-generation sequencing (NGS) and comprehensive genomic profiling (CGP) have emerged as new and powerful tools to classify different cancers based on specific molecular signaling [1,2]. The identification of molecular changes underlying the well-known hallmarks of cancer – sustained proliferation, angiogenesis, evasion of tumor immunity, immortality, sustained growth signaling, invasion and metastasis

has led to the identification of 12 essential signaling pathways that contribute to these core processes [3,4]. Targeting these "hub" elements represents a promising approach to effectively halt cancer progression. In fact, several driver mutations have been identified and successfully targeted, showing survival and response benefit, regardless of cancer histology [5,6]. The translation of NGS and CGP into current oncology practice has thus taught us that although tumor histology matters, so too does-and perhaps to a more significant extent- its genomic landscape.

Along the traditional histology-based classification, therapies have historically focused on targeting the main apparent hallmark of cancer cells: Rapid proliferation. As such, chemotherapy which targets rapidly dividing cells has for long remained the mainstay of cancer therapy along with surgery, radiation therapy, and later hormonal therapy. Yet, for patients with advanced stage IV metastatic disease, survival rates remain below satisfactory, with 5-year relative survival rates generally ranging between 2% and 27% [7]. For lung cancer, the leading cause of cancer mortality across genders, survival rates are limited to 5% at 5 year, while colorectal and breast cancer 5-year relative survival rates are 14% and 27% respectively.

On the other hand, the significance of classifying tumors based on genomic alterations has been demonstrated across several tumor types. The development of tyrosine kinase inhibitors for example has been one of the early success stories for lung cancer patients harboring mutations in the epidermal growth factor receptor (EGFR) [8]. Many other studies have provided evidence in support of precision therapies directed at specific molecular alterations. A major 14-site study of metastatic lung adenocarcinoma patients harboring a variety of targetable alterations (KRAS, EGFR, ALK, HER2, BRAF, PIK3CA, MET, NRAS, and MEK1) compared overall survival (OS) in patients who received targeted therapies vs. those who received systemic non-targeted therapies: OS was significantly improved in those receiving targeted therapy (3.5 years) compared to non-targeted therapy (2.4 years, *P*=0.006) [6]. Similarly, a meta-analysis evaluating 570 phase II single-agent studies and 32,149 patients compared OS, progression-free survival (PFS), and response rate (RR) in patients receiving targeted therapy vs. those receiving non-targeted therapy. The results were consistently positive for targeted therapy: RR was 31% vs. 10.5% (*P*<0.001), prolonged median PFS was 5.9 months vs. 2.7 months (*P*<0.001), and OS was 13.7 months vs. 8.9 months (*P*<0.001). In another study of advanced NSCLC patients (n=4,064), 871 patients with mutations in EGFR, ALK, or ROS1 received either targeted or non-targeted therapies: OS was significantly higher in those treated with targeted therapies (18.6 vs. 11.4 months, *P*<0.001) [9]. Similar results have also been reported in NSCLC patients (n=17,644) whereby patients who received therapy matched to one of the screened genetic alterations (EGFR mutations, ALK rearrangements, HER2 (ERBB2), KRAS, BRAF, and PIK3CA mutations) had a significantly higher overall response to first-line treatment compared to non-matched therapy (37% vs. 33%; *P*=0.03) with a more significant effect in second-line treatments (17% vs. 9%, *P*<0.0001). This translated into longer PFS (10 vs. 7.1 months; P<0.001) and OS (16.5 vs. 11.8 months, *P*<0.0001) [10].

Literature Review

The positive effect of targeted therapies on PFS and OS which will be described in more detail with in this review, is however not the result of the novel therapies per se, but rather due to effective matching between the alteration and the targeted therapeutic agent. In fact, patients lacking a target mutation do not benefit as much as those harboring such alterations, when treated with the same targeted therapy: one meta-analysis of advanced NSCLC patients compared the effectiveness of an EGFR tyrosine kinase inhibitor in patients with or without an EGFR mutation. Those with an EGFR mutation had a significantly improved PFS (12 vs. 3.4 months, *P*<0.001). Furthermore, median OS time was nearly doubled in EGFR-mutant patients compared to EGFR-WT patients (23.3 vs. 12.1 months, respectively, *P*<0.001) [11]. Similar effects were observed with the use of immune checkpoint inhibitors in a large study across 275 sites involving 28,998 patients. OS was significantly higher in patients with high Tumor Mutational Burden (TMB-High) compared to patients without the indicated genetic change (TMB-Low) (18.6 vs. 11.4 months, *P*< 0.001) [12].

With the above evidence for survival advantage when matching therapies to specific alterations in mind, the era of precision oncology is expanding at a faster rate than ever. The real opportunity however lays in transforming what seems to be a new molecular classification and combining it with the emerging field of matched therapy, thus resulting in novel strategies that match therapies to molecular alterations irrespective of the tumor histology. For example, aberrant HER2 signaling is well established in breast cancer but is also an oncogenic driver in a small subset of lung cancers [13]. Similarly, although BRAF mutations are most often associated with melanoma, these can also be found in hairy cell leukemia, colon cancer, lung cancer, thyroid cancer, and brain tumors [14-19]. Thus, the genetic makeup of a newly diagnosed tumor may be just as important when exploring possible treatment strategies, and a thyroid tumor for example with a certain mutation may share more molecular vulnerabilities with a melanoma driven by the same molecular aberration/signaling pathway defect, than with another thyroid tumor without the same alteration.

Many examples of targets that have been successfully applied across different tumor histologies exist: gene fusions involving NRTK genes (tropomyosin receptor kinase) which lead to constitutively activated or over-expressed kinase function, have emerged as a novel target of cancer therapy across multiple tumor types, including colorectal cancer, sarcomas, thyroid cancer, glioblastomas, lung cancers, head and neck squamous cell carcinoma, gastrointestinal stromal cell cancer, appendiceal cancer, and ductal cancer of the breast [20]. Such observations and subsequent trials have culminated in the recent accelerated FDA approval of larotrectinib (VITRAKVI), a highly selective inhibitor of NRTKs, for adult and pediatric patients with solid tumors with a NRTK gene fusion that are either metastatic or who have no satisfactory alternative treatment [21-23]. This is

the second tissue-agnostic FDA approval for the treatment of cancer. Other examples include the microsatellite instability status (MSI-H) -a reflection of tumor cells' defective DNA repair, the tumor mutational burden (TMB) -a reflection of a tumor cells' antigenic potential, and thus an indicator of response to immune therapy, and the expression of the programmed death ligand 1 (PD-L1) on cancer cells surface [24-26].

Furthermore, some highly targetable mutations may be rare within certain histology and thus only discovered in the context of a negative trial. Everolimus for example, an inhibitor of the mammalian target of rapamycin (mTOR) was reported to induce significantly durable efficacy in two patients with bladder and thyroid cancer, respectively, despite a lack of efficacy in these two cancer types generally [27,28]. Interestingly these two responders shared specific mutations in the mTOR signaling pathway, rendering them both uniquely sensitive to everolimus. In light of all the above, the Food and Drug Administration (FDA) has recently adopted a new strategy for clinical trial design, termed "basket trials" which allow the incorporation of precision medicine into clinical trials. Such designs allow the simultaneous evaluation of multiple targeted therapies in patients grouped by molecular markers, along with tumor histology [29]. The design is predicated on the hypothesis that the presence of a molecular maker predicts response to targeted therapy, independent of tumor histology. Examples of such trials include the CUTOM trial (Molecular Profiling and Targeted Therapies in Advanced Thoracic Malignancies, the National Cancer Institute (NCI) MATCH trial (Molecular Analysis for Therapy Choice), and the NCI-IMPACT trial (Molecular Profiling-Based Assignment of Cancer Therapy (ClinicaTrials.gov identified NCT01827384) [30,31].

As a proof of principal, we identified five genetic signal targets that we felt are central to cancer molecular networks which can be matched with FDA approved therapeutic complements [32]. In this review, we describe these alterations and how they have been successfully targeted by molecular therapies within their FDA-indicated populations. We also discuss each alteration's respective pathway, and mechanisms of sensitivity and resistance to indicated therapy. We further gather, present, and discuss available efficacy data in support for potential expansion of indications outside the current histology-defined populations.

PI3K/mTOR/AKT pathway

The PI3K/mTOR/AKT pathway is one of the most significant pathways associated with physiological growth as well as the expansion and survival of cancer cells. This pathway's homeostatic importance is shown in its genetic conservation from the last eukaryotic common ancestor, and its oncogenic importance is seen in its high dysregulation rate across many morphologically and histologically distinct cancers [33-38]. Amplification, mutation, and deletion of numerous oncogenes and tumor suppressor genes drive the oncogeneticdysregulation of this pathway [39]. As of December 2019, there are five FDA-approved drugs that specifically target PI3K signaling mechanisms.

Here, we describe the current use of PI3K pathway inhibitors and discuss the opportunities for expanding these drugs to target various cancers with similar oncogenetic profiles. By expanding the utility of these drugs beyond their current indications and by genetically sequencing patient tumors, we may be able to identify new opportunities for targeted therapy based on molecular characterization. Similar PI3K pathway oncogenic signatures across a variety of cancers offer a unique opportunity to expand the use of precision treatments with proven efficacy to potentially susceptible cancers.

Signaling overview

Under normal conditions, the PI3K pathway is initially activated by growth factors and extracellular signals that upregulate receptor tyrosine kinases and G-protein coupled receptors to the cell surface. Upon dimerization of receptor proteins, PI3K, a family of proteins with catalytic (p110) and regulatory (p85) domains, are activated and propagate signaling by phosphorylating the membrane-bound secondary messenger PtdIns P2 (PIP2) to PtdIns P3 (PIP3). The activation of PIP3 is commonly recognized by a family of intermediary proteins called AKT (protein Kinase B), which can promote cellular growth by phosphorylating key cell cycle and metabolic proteins [37]. AKT additionally upregulates proteins such as the mammalian target of rapamycin (mTOR), which acts as a part of two complexes, mTORC1 and mTORC2, which function to drive and regulate growth, respectively [40-42]. Intracellular signal regulation of this pathway includes the phosphatase and tensin homolog (PTEN), which dephosphorylates PIP3, and p85, which acts as the PI3K regulatory protein, among others [39].

Relevant drugs and drug classes targeting PI3K

Despite the oncogenetic significance of PI3K pathway proteins being known for decades, there are still only five FDA approved drugs for targeting this pathway. These include two mTOR inhibitors (everolimus and temsirolimus), two isoform-specific PI3K inhibitors (alpelisib and idelalisib), and the pan-PI3K inhibitor copanlisib. As of yet, the vast majority of cancers with PI3K dysregulations are without approved targeted therapy, despite having similar genetic signatures to those with indications for treatment. Below, we discuss the development and applicability of drugs within these classes.

Pan-PI3K inhibitors have activity against each of the four PI3K catalytic isoforms (p110α, β, γ, and δ), in contrast with isoform-specific inhibitors designed to bind protein pockets in distinct domains, the flatter conformation of pan-PI3K inhibitors allows for deeper affinity to p110 binding pockets [38,43]. Targeting a broader spectrum, pan-PI3K inhibitors have potentially greater applicability, but often cause significant side effects (SE), and for that reason, have been limited in clinical efficacy [38]. The oral inhibitor buparlisib is considered a bridge between pan- and isoform-specific PI3K inhibitors, as it acts against all PI3K subunits but with significantly higher affinity for p110α. Buparlisib is being studied in three phase III trials in HR+, HER2- breast cancers (BrCs) (BELLE-2, 3, and 4), and is currently involved in numerous trials of a diverse set of cancers. In the 2012-2014 BELLE-2 trial, patients with PIK3CA (the gene that encodes p110α) mutated cancers had a median progression free survival (PFS) of 7.0

months with buparlisib/fulvestrant treatment as opposed to 3.2 months with placebo/fulvestrant treatment (P <0.001); there was no difference for patients with PIK3CA wild type (WT) treated with buparlisib or placebo [44]. In the 2013-2016 BELLE-3 trial designated for patients who had relapsed after endocrine and mTOR therapy, similar results were seen in mutated and WT PIK3CA cancers (PFS of 4.2 vs 1.6 months, respectively) [45]. SE of buparlisib treatment include mood changes, as it can cross the blood-brain barrier, although could potentially be useful for patients with PI3K-dysregulated brain metastases [38]. Copanlisib received accelerated approval in 2017 for refractory follicular lymphoma (FL) after its success in the CHRONOS-1 clinical trial, where monotherapy resulted in an overall response rate (ORR) above 59%, which was supported by two-year follow-up [46]. Copanlisib is currently being investigated in the treatment of many cancers, including phase III trials for non-Hodgkin's lymphomas (NHL). Further elucidation of the relationship between copanlisib and PI3K-dysregulated tumors will help determine future uses of this drug [38,47]. Major SE of copanlisib include infections, hyperglycemia, hypertension, among others. Additional pan-PI3K inhibitors currently in trials are pictilisib and SF1126.

Due to the clinical success and fewer off-target SE of isoform-specific PI3K inhibitors, several clinical trials are moving forward with this class of drugs. Alpelisib is a PI3Kα-specific inhibitor that gained FDA approval in 2019 to treat HR+, HER2- BrCs based on its success in the 2015-2017 phase III SOLAR-1 trial [48,49]. Here, patients treated with combination alpelisib/fulvestrant demonstrated increased PFS in PIK3CA-mutated cancers (11.0 vs. 5.7 months in WT) [49,50]. Because of the potent inhibition of alpelisib toward PIK3CA dysregulated tumors and the prevalence of this dysregulation, it is currently being tested in 25 different clinical trials [48,50]. Idelalisib is a PI3Kδ inhibitor that is approved to treat chronic lymphoid leukemia (CLL), follicular B-cell NHL, and small lymphocytic lymphoma (SLL), and is effective in treatment of these diseases due to the relatively specific expression of PI3Kδ in hematopoietic cells [38,51]. Idelalisib, in combination with rituximab, received FDA approval in 2014 for the treatment of CLL after the phase III trial NCT-1539512 demonstrated significantly increased ORR (81% to 13%, P<0.001) and overall survival at 12 months (92% vs 80%, P=0.02) in treatment groups [52]. Additionally, idelalisib received FDA approval for relapsed patients with NHL and SLL based on the 2011-2014 trial NCT01282424. Here, the ORR was increased to 54% in NHL and 58% in SLL, although both populations demonstrated noticeable SE [53]. Other isoform-specific inhibitors of PI3Kβ and PI3Kγ are currently in development [38].

Outside of breast cancer and lymphoma, PI3K inhibitors have been studied in numerous solid tumors. A preclinical study to determine the efficacy of PI3K pathway alterations in predicting sensitivity to PI3K inhibitors in lung cancer cell lines found PIK3CA mutation did predict sensitivity to PI3K inhibitors [54]. In a Phase I study of copanlisib in patients with advanced solid tumors or NHL, one patient with endometrial carcinoma which exhibited PIK3CA and PTEN mutation and loss achieved a CR [55]. Interestingly, in the first in human Phase I study of alpelisib in patients with PIK3CA mutated solid tumors, a CR was also observed in a patient with endometrial cancer. There were also seven PRs in patients with cervical, breast, endometrial, colon and rectal cancers and seventy patients (52.2%) maintained SD for greater than 24 weeks [56]. Another study investigated the response of advanced cancer patients with PIK3CA mutations to PI3K/AKT/mTOR inhibitors: in a sample of 217 patients (endometrial, ovarian, CRC, breast, cervical and head and neck), 11.5% (25/217) harbored the PIK3CA mutation. Of those, 17/25 received a PI3K/AKT/mTOR pathway inhibitor, 6/17 (35%) achieved PR (vs. 6% in WT PIK3CA), while 6/17 achieved SD (6-20 weeks). In another Phase I study of buparlisib, 7 out of 35 patients were treated for ≥ 8 months. However, PI3K alterations were not a requirement for study enrollment and only 5 of those patients had PIK3CA mutations therefore it is unknown if PIK3CA mutations are a biomarker of response.

Other pathway drugs

Additionally, drugs such as AKT inhibitors and mTOR inhibitors have been developed to inhibit other aspects of the PI3K pathway and would block potential resistance mechanisms to PI3K inhibition. Both Akt and mTOR inhibitors are generally well tolerated. AKT inhibitors downregulate PI3K pathway signaling and, of these, ipatasertib is currently in phase III trials for breast and prostate cancers [38]. mTOR inhibitors, including FDA approved drugs like temsirolimus and everolimus, have antitumor activity and have found success as monotherapies as well as a part of dual-PI3K/mTOR combination therapies [38].

SUMMARYof PI3K/mTOR/AKT

Clinical evidence supports relationship of antitumor activity involving targets of PI3K signal and signal pathway. Results also suggest evidence of agnostic activity involving a broad range of cancer histologies.

The PI3K pathway is dysregulated in a large number of cancers, but for most cases, there are no approved targeted therapies. While there are approved drugs that are effective against distinct oncogenic profiles, they are currently only designated in specific clinical circumstances (for example, alpelisib and HR+, HER2- BrCs). Current clinical trials are looking to expand the applicability of these drugs, as many of the dysregulations targeted by current approved therapy have are seen in other cancers. Identifying these connections can help to target the PI3K pathway with proven drugs in unproven, but similarly susceptible, cancers. Taken together, the data presented strongly suggests that targeting the PI3K pathway in PIK3CA mutated advanced cancer has shown benefit and requires further investigation.

BRCA 1/2 signal pathway

The *BRCA1* and *BRCA2* genes code for proteins that work in a common pathway of genome protection, but each protein acts at different stages in recognition and repair of DNA damage during cell replication. *BRCA1* functions as both a checkpoint activator and a mediator of DNA repair, compared to *BRCA2* which has a direct role in homologous recombination (HR). While the direct connection between these two proteins is not fully understood, it has been shown that mutations in these genes express similar

phenotypes. This supports the idea that the mechanism of HR requires both of these proteins to function and mutations anywhere along the pathway can result in similar aberrations to cell replication [57]. Women with germline *BRCA1* mutations have an increased risk of developing breast and ovarian cancer, and men have been shown to have a slightly higher risk of developing prostate cancer [58]. Less commonly germline mutations in *BRCA2* have also been associated with a higher risk for gall bladder, bile duct, stomach cancer and melanoma [59]. These mutations are typically frameshift mutations that lead to nonsense mutations [60]. Currently, more than 1800 mutations have been identified in *BRCA2* and nearly all of these events have led to a partial or complete loss of function of the tumor suppressor genes [61]. Cancer driven signaling is also enhanced by somatic *BRCA1* and *BRCA2* mutations. Thus opening a door for therapeutic management targeting *BRCA1*/2 signaling defects.

BRCA2's main function in HR is the recruitment of the recombinase protein RAD51 to the location of the DNA double-strand breaks. This recombinase protein then mediates the reaction of the HR and ultimately stops oncogenic transformation [62]. *BRCA2* contains multiple different domains that allow it to have its function in HR. These domains consist of a domain specific for DNA-binding of single-stranded DNA and double-stranded DNA in addition to eight BRC repeats that allow the binding of the recombinase protein, RAD51 [57]. While point mutations within any of these domains cause decreased function of HR, BRC repeat mutations most notably compromise the interactions of *BRCA2* and RAD51 by changing the binding affinity and altering the function. Loss of function results in deficient HR and the sequential mutations that lead to cancer formation [63]. These mutations are also found in individuals with HBOC syndrome [64].

The *BRCA1* gene is expressed in multiple different tissues, most notably breast and ovarian tissue. In addition to HR seen in *BRCA2*, *BRCA1* also plays a role in nonhomologous end joining and single-strand annealing [57,65,66]. *BRCA1* more specifically interacts with tumor suppressor proteins, DNA repair proteins, and cell cycle regulators through its various functional domains. The amino-terminal RING domain has E3 ubiquitin ligase and a BRCT domain [57,67]. These domains normally tag intracellular proteins for destruction and facilitate phospho-protein binding, respectively. However, protein destruction is not seen in response to this E3 ubiquitin ligase, but it is instead believed to play a role in signaling [68-71]. The mutations that have been identified to play a role in the *BRCA1* gene include a set of five different alterations. These consisted of an 11-base pair deletion, a 1-base pair insertion frameshift, a nonsense mutation, a missense substitution, and an inferred regulatory mutation [67,72]. In addition, a collaborative study of 372 patients with breast or ovarian cancer showed that nearly 22% of patients had a *BRCA1* mutation. After further investigation, these patients were shown to have 38 distinct mutations and up to 86% of them resulted in a truncated *BRCA1* protein [73]. Currently, more than 1600 different mutations have been identified and a majority of them follow the same trend of the previous study being that a majority are frameshifts resulting in nonsense mutation or non-functional protein [67].

Many *BRCA1* mutations are located within the RING and BRCT domains. This indicates that both domains are important in suppressing oncogenic transitioning of breast and ovarian cancer [73-75]. Another protein, the *BRCA1*-associated RING domain protein 1 (BARD1), also plays a major role in tumorigenesis with *BRCA1* mutations [70]. *BRCA1* E3 ubiquitin (Ub) ligase activity is enhanced when associated with BARD1 and this complex adds K6 linkages to cellular proteins. While the entire mechanism is not known, the mutations in the RING domain have been shown to cause uncontrolled cell replication and tumorigenesis through the loss of cell cycle checkpoints. It is proposed that the *BRCA1*–BARD1 complex is involved in the activation of cell cycle checkpoints, G1/S, S-phase and G2/M. The G1/S-checkpoint is activated through the phosphorylation of *BRCA1* by ATM or ATR. This activation facilitates the phosphorylation of p53. When p53 is phosphorylated, it induces the expression of the cyclin-dependent kinase (CDK) inhibitor p21 [76]. The overall depletion of the *BRCA1*–BARD1 complex compromises the induction of p21. It also causes a decrease in activation of the G1/S checkpoint and to a lesser extent the other cell cycle checkpoints [77]. Similarly, the BRCT binding domain allows *BRCA1* to interact with ATM phosphorylated proteins (include abraxas, BRIP1, and CtIP). The binding of these proteins makes up *BRCA1* protein complexes that have additional DNA damage pathway recognition [57]. How these multiple *BRCA1* complexes work in a coordinated manner is still unclear, but continued research is being done to elucidate the role of the domain and associated complexes in the oncogenic transformation.

Polyadenosinediphosphate-ribose polymerase inhibitors (PARPi)

While there are numerous *BRCA1*/2 mutations among different cancer types, the majority of treatments that target BRCA, outside of general chemotherapy, include polyadenosinediphosphate-ribose polymerase inhibitors (PARPi). Poly ADP-Ribose polymerases (PARPs) are a superfamily of proteins that play a role in post-translational modification. They specifically interact with histones and other nuclear proteins that are responsible for chromosomal stability [78]. The reason these proteins are of interest is that PARPs are responsible for the repair of single-stranded breaks within the DNA. They do so through the process of base excision repair. When single-strand breaks appear, they must be corrected through ligation prior to DNA undergoing replication. If this does not occur during replication single-stranded breaks may become double-stranded breaks [79,80]. While this may seem counter-intuitive to induce DNA strand breaks in people with deficient enzymes for HR, it is because this deficiency that makes these drugs have high efficacy in these specific cancer types [80,81]. In the event that PARP is unable to repair a single-stranded break, the BRCA proteins can compensate by repairing the double-stranded breaks that form [80,82]. This compensatory mechanism is missing in *BRCA1*/2 mutated cells, therefore they are unable to address the accumulating double-stranded breaks. Consequently, the double-stranded breaks will persist and accumulate until cells lose viability, ultimately halting cancerous cell proliferation. The two current treatments

that have each shown great efficacy when compared to single-agent chemotherapeutic agents in clinical trials are Olaparib and Talazoparib.

Response to PARPi In tumors harboring *BRCA 1/2* mutations

In both trials discussed below, treatment with PARP inhibitors resulted in increased progression-free survival (PFS), the primary endpoint, and therapeutic index when compared to chemotherapy in breast and ovarian cancer. These trials showed that PARPi were not only more effective, but they also had many fewer adverse side effects when compared to the conventional chemotherapy agents [83,84]. The first of the trials, the phase III OlympiAD trial, was performed to examine the efficacy of the drug Olaparib. This trial contained over 300 patients with metastatic HER2-negative, *BRCA 1/2* mutated breast cancer were randomly assigned to Olaparib or to the standard chemotherapy treatments [85]. After 14 months, Olaparib patients were shown to have an improved PFS relative to those treated with chemotherapy (7.0 versus 4.2 months). The patients also had a hazard ratio of 0.58 for disease progression or death (95% CI 0.43-0.80). However, the overall survival between the two groups was not significantly different (25 months). This trial also showed that PFS with Olaparib was greater in the triple-negative subgroup than in patients with hormone receptor-positive disease (HR: 0.43 vs 0.82, respectively) [83,85]. In addition, the number of adverse events was also lower with Olaparib than with chemotherapy. Serious adverse events were seen in 51 percent of patients on chemotherapy while only 37 percent had adverse effects of the Olaparib treatment [83,85].

A case report also examined the role that Olaparib could play in the treatment of pancreatic cancer with a BRCA mutation. After the patient received only traditional Chinese therapy, the mass was found to have grown substantially over the next two years and metastasized to the lung and brain. Following palliative brain radiotherapy and 6 cycles of gemcitabine with albumin-bound paclitaxel chemotherapy; he received maintenance Nivolumab. The lesion remained stable for 3 months. At that time next-generation sequencing was performed and showed multiple mutations, including *BRCA2* L1908Rfs*2 exon11 mutation. At this point, Olaparib was added to nivolumab and the serum CA-199 level descended from 750+ to 460.0 U/mL after 1 month. The patient remained stable until March 2018. In summary, the efficacy of Nivolumab and Olaparib treatment was evaluated for progressive disease (PD), and its progression-free survival (PFS) was 7.4 months [86].

Similar to Olaparib, in the phase III EMBRACA trial, over 400 patients with metastatic HER2-negative, BRCA-associated breast cancer were randomly assigned treatment with Talazoparib or standard chemotherapy [84]. After 14 months, Talazoparib patients were shown to have an improved PFS relative to those treated with chemotherapy (8.6 versus 5.6 months). The patients also had a hazard ratio of 0.54 (95% CI 0.41-0.71). However, the overall survival between the two groups was not significantly different, but more results are still being reviewed. This trial also showed that Talazoparib had similar findings of adverse events compared to the standard chemotherapy, but Talazoparib patients reported improvements in their quality of life which wasn't seen in chemotherapy treatments [87]. Overall, these findings in the clinical trials and case report show that both examined PARPi have equal if not better efficacy in all aspects of treatment when compared to the standard chemotherapy treatments. Other PARP inhibitors have subsequently been approved with similar results [88-91].

Prognostic value of *BRCA 1/2* mutations

Xu et al examined the association between the presence of a BRCA mutation and overall ovarian cancer survival (OS and PFS) (92). In their study, 18,396 ovarian cancer patients from 34 different studies were analyzed to find a trend regarding the prognosis of patients with *BRCA1*/2, *BRCA1*, and *BRCA2* defects. Overall, what they found is that carriers had significantly improved OS and PFS benefits in patients with ovarian cancer [92]. Further analysis revealed that this finding remained constant regardless of the tumor stages, study design, sample size, number of research centers, duration of follow-up, baseline characteristics adjusted and tumor histology. Their analysis showed that BRCA mutation carriers had a 33%, 27% and 43% reduction in all-cause mortality for *BRCA1*/2, *BRCA1*, and *BRCA2* mutants, respectively. They also found that progression-free mortality had a similar reduction of 38%, 32%, and 52%, respectively [92]. These findings have been supported by other studies (93-98), however, some discrepancies have been found [99-102]. While there have been significant findings regarding the prognostic value of *BRCA1*/2 mutations in ovarian cancers, multiple studies have failed to find similar findings in breast cancer [103,104]. Because of this, more research needs to be done in order to fully understand the prognostic value of *BRCA1*/2 mutations on breast and ovarian cancer.

Currently, there are no approved first-line treatments specifically targeting the BRCA genes in cancers such as prostate, lung, and pancreatic cancer. However, there are clinical trials being done with numerous different PARPi that show promise. An Open-Label Phase II Study showed that Olaparibmonotherapy in patients with advanced cancer with a germline *BRCA1*/2 mutation was performed to test the therapeutic value of PARPi in cancer. Two hundred and ninety-eight patients with advanced refractory solid tumors with a *BRCA1*/2 mutation were enrolled in this trial where they received 400mg of Olaparib twice daily. Twenty-three patients in this study had pancreatic cancer, 8 patients had prostate cancer, and the rest of the cancers had either breast or ovarian cancer. The results showed that patients with pancreatic cancer had disease progression after treatment with standard chemotherapy. In these 23 patients, the response rate was 21.7%. 36.4% had a PFS of 6 months and 41% were alive at 12 months. In the prostate cancer patients, they had a response rate of 50%. 62.5% had a PFS of 6 months and 50% were alive at 12 months. This small study showed the potential effectiveness of PARPi in BRCA mutated pancreatic and prostate cancer [105]. Similar studies have been shown in other trials examining the effects of other PARPi on pancreatic and prostate cancers with BRCA mutations [106,107]. There have also been case reports

with PARPi in NSCLC with BRCA mutations in other countries. In one patient it showed that there were positive results with a PFS of over 6 months [108]. The promising results of trials like these are what has led to the development of trials in the United States. Currently the NCT01788332 and NCT02679963 trials are active and results are soon to come.

Resistance to PARPi

Multiple studies have been performed with the hopes to determine the mechanism of resistance to PARPi [80,109,110]. In one study of *BRCA1*-deficient tumors, a large majority of the PARPi-resistant cells showed reduced mRNA expression of a select number of DNA damage associated proteins. The most notable of these proteins were SHLD1, SHLD2, and TP53BP1 [111]. Through different approaches to the same overall mechanisms, these proteins are believed to be able to induce resistance to PARPi. TP53BP1 and SHLD1/2 proteins do so by causing a loss of resection or bypassing the defective NHEJ of the BRCA proteins and participating in BRCA independent NHEJ [109,110]. While these cells maintain their BRCA defect and loss of HR, they do not induce the DNA double-stranded break from PARPi exposure, allowing the cell to remain viable. There has also been associated stability of the replication fork in response to these aberrations from the upregulation of the ATR/CHK1 pathway [80]. Another mechanism has been proposed as well. It is believed that tumor cells may develop a PARPiresistance by restoring the cell's ability to undergo HR. The leading mechanism of this is through a somatic reversion of a mutated *BRCA1*/2 allele [80]. These mechanisms, however, are not well understood and further studies are needed to fully understand them. This does show the importance of developing new drugs that are able to target these resistance mechanisms. Once developed, these drugs may be used in combination with PARPis for increased efficacy and an overall better response. Of note, these mechanisms are only proposed for *BRCA1* mutant tumors and less is known about the mechanisms of resistance seen in *BRCA2* mutated cancer cells.

Summary of *BRCA1/2*

The *BRCA1*/2 mutated gene signaling play a role in many different DNA repair cancer supportive pathways. The common theme of these pathways is to control cell replication and correct any DNA damage before replication occurs. However, when mutated or missing, it has been shown to lead uncontrolled replication and tumor development. Currently, chemotherapy is being used in multiple different types of cancers, but because these drugs are nonspecific for the *BRCA1*/2 mutations, their ability to have a direct effect on the cancer cells is limited. However, this limitation appears less with Olaparib and Talazoparib due to increased specificity for cells undergoing rapid proliferation with the *BRCA1*/2 mutations. Since this mechanism was elucidated, continuous testing has been done and this drug has shown promising results including an increase in PFS with a smaller hazard ratio of disease progression and death. Olaparib and Talazoparib have also revealed to have fewer side effects when compared to standard of care chemotherapies. Expression of *BRCA 1/2* mutations in non-breast/ovarian cancer has been observed and clinical evidence of response and benefit has been described.

CDK4/6 signal pathway

Cyclin-Dependent Kinases 4 and 6, in conjunction with their regulator Cyclin D1 (CCND1), control cell cycle progression by regulating the G1-S checkpoint. The cell cycle has four main consecutive phases: G1 is a phase of growth to prepare for cell synthesis, S phase is DNA synthesis, G2 is a second phase of growth to prepare for cell division, and M phase is cell division. There is also a G0 phase of arrested growth, or quiescent phase. There are checkpoints at G1/S, G2/M, and halfway through M phase to ensure that cells do not divide in unfavorable conditions, or with significant mutations. CDK4/6 and CCND1 overexpression or dysregulation can result in uncontrolled proliferation.

CDK4/6 inhibitors

Currently there are three FDA-approved CDK4/6 inhibitors: abemaciclib, palbociclib, and ribociclib. These drugs have found success and are frequently used to treat hormone responsive breast cancer (i.e. ER or PR positive) and play a role in mediating endocrine resistance. Abemaciclib is a CDK4/6 inhibitor that is approved as a monotherapy and in conjunction with fulvestrant, an endocrine-related therapy, in HR positive, HER2 negative advanced breast cancer that was previously treated with endocrine therapy. Three landmark trials defined abemaciclib's role in advanced breast cancer therapy. The first is MONARCH 1, a phase II study which demonstrated that abemaciclib had proven clinical activity as a single agent therapy in previously heavily treated patients with HR-positive, HER2-negative breast cancer that had developed resistance to previous endocrine therapy with a response rate of 19.7%, medial progression-free survival (PFS) of 6.0 months, and median overall survival (OS) of 17.7 months [112]. The second trial is MONARCH 2 which showed a significant 7.2 month improvement in progression free survival between abemaciclib plus fulvestrant compared to placebo plus fulvestrant [113]. MONARCH 3 was a double blind, randomized phase III trial comparing abemaciclib plus a nonsteroidal aromatase inhibitor to placebo plus a nonsteroidal aromatase inhibitor in post-menopausal women with no previous systemic therapy. The trial concluded that abemaciclib arm showed significant improvement in objective response rate (ORR) when compared to the placebo arm: 48.2% vs. 34.5% [114].

Ribociclib is an inhibitor of CDK4/6 and works by inhibiting the phosphorylation of the RB protein (retinoblastoma protein) which induces G1 phase arrest and halts cell-cycle progression. Similar to abemaciclib, it is approved for HR positive, HER2 negative breast cancer. The 2016 randomized double-blind, placebo- controlled phase 3 study compared the efficacy and safety of ribociclib + retrozole to placebo + letrozole in hormone-responsive breast cancer patients who had not received previous systemic therapy, endocrine-based or otherwise, for advanced breast cancer. PFS in ribociclib+letrozole was 63% compared to 42.2% in the placebo group, and the ORR in patients with measurable disease at baseline was 52.7% and 37.1% respectively [115]. More recently, *in vitro* studies have combined CDK4/6 inhibitors with PD-L1 inhibitors and have seen a synergistic effect [116].

Palbociclib has also been approved for the treatment of HR-positive and human epidermal growth factor receptor 2 (HER2) negative locally advanced or metastatic breast cancer, either in conjunction with an aromatase inhibitor or with fulvestrant in women who have received prior endocrine therapy. Studies of palbociclib's effect on breast cancer cell lines has shown that palbociclib works by inhibiting the phosphorylation of Rb protein which causes the cell to arrest in G1 phase thus prohibiting cell cycle progression and further proliferation [117]. In a phase 2, double blind, randomized trial called PALOMA-2, the data showed that the median PFS was significantly better for letrozole + palbociclib at 24.5 months, compared to letrozole + placebo, with 14.5 months [118]. Similarly, PALOMA-3 was a phase III, double-blind and randomizing trial which compared the efficacy of fulvestrant + palbociclib vs. fulvestrant + placebo. Results showed that median PFS was 9.5 months in the palbociclib + fulvestrant arm and 4.6 months in the fulvestrantmonotherapy arm. The most common adverse effect was neutropenia which occurred in 65% of participants in the treatment arm and 1% in fulvestrantmonotherapy arm. Thus, the results showed that palbociclib + fulvestrant was associated with significant improvement in PFS when compared to fulvestrant plus placebo [119]. Clinical trials involving palbociclib for other types of cancer are currently underway, specifically liposarcoma and glioblastomamultiformewhich will be discussed [120].

CDK4/6 mechanism

As mentioned above, abemaciclib, palbociclib, and ribociclib work by selectively binding to CDK4 and CDK6 and preventing them from forming a complex with cyclin D1. Overall, there are two regulatory mechanisms guarding the G1/S phase transition which include the retinoblastoma (Rb) pathway and the p53 pathway. The transcription factor p53, is often hailed as the "guardian of the genome" because it comes into play when DNA damage occurs and will halt the progression from G1 to S when it senses DNA damage. CDK4/6 and Cyclin D1 play a role in the Rb pathway. Normally, when cyclin D1-CDK4/6 complex forms, it works to phosphorylate Rb protein which then releases the transcription factor E2F. When pRb is bound to E2F, it inactivates E2F. Upon phosphorylation of Rb protein by cdk4/6-Cyclin D1 it causes the Rb protein to change conformation such that E2F is released. E2F transcription factor drives transition from G1 to S phase [121]. Cyclin D1 is a main target of the estrogen receptor, and thus these therapies have found a way to overcome resistance in breast cancer that has been previously treated with endocrine therapies [119].

Relevant cancer effect beyond breast cancer

Currently, abemaciclib, palbociclib, and ribociclib are in clinical trials to evaluate their efficacy in cancers other than HR-positive, HER2-negative breast cancer. There are also various other CDK4/6 inhibitors under clinical trial development for solid tumor therapy and blood cancers with expansive research in cancers that have been shown to have dysregulated CDK4/6 function, including multiple myeloma, glioblastomamultiforme, bladder cancer, high-grade gliomas that are RB+ tumors, and diffuse intrinsic pontineglioma. In addition, some clinical trials and studies evaluating CDK4/6 inhibitors expanded their inclusion criteria to include any type of solid tumors with CDK4/6 mutations or overexpression, making all solid tumors with specific markers a possible target of CDK4/6 inhibitors.

CDK4/6 inhibitors have seen the most success in cancer types that are known to commonly express CDK4/6-Rb-E2F pathway alterations. Furthermore, patients of any cancer that have unique molecular pathways related to abnormal CDK4/6-Rb-E2F are potential candidates for CDK4/6 inhibitor therapy. Squamous cell lung cancer is a classic example because a landmark study examining 178 patients squamous cell lung cancer showed that a vast majority had CDK4/6-Rb-E2F pathway alterations (Hammerman 2012). A 2019 study showed that CDK4/6 inhibitors in addition to taxanes, a standard of care treatment, were able to enhance overall anti-tumor efficacy by impairing pRB-E2F pathways more effectively than taxanes alone in squamous cell lung cancer cell lines [122]. Using gene expression analysis, the combination of taxanes and CDK4/6 inhibitors had novel mechanisms of action including blocking mitotic spindle assembly checkpoints, and impairing hypoxia-inducible factor 1 alpha (HIF-1alpha), which is tied to angiogenesis. These findings suggest that CDK4/6 inhibitors have potential in lung as well as other cancers that are treated with taxanes and show abnormal CDK4/6-Rb-E2F pathways.

Similarly, to squamous cell lung cancer, multiple myeloma is a cancer type known to often have dysregulation of CyclinD-CDk4/6 or dysregulation in CDK4/6-Rb-E2F pathway contribute to its pathogenesis, therefore making it an adequate candidate for CDK4/6 inhibitor therapy. Therefore, CDK4/6 inhibitors have There was a successful multicenter, open-label, Phase 1/Phase 2 study which first evaluated palbociclib in sequential combination therapy with bortezomib and dexamethasone in relapsed or treatment-refractory multiple myeloma. Bortezomib, a proteasome inhibitor, plus dexamethasone, a steroid treatment, is one of the standard therapies for multiple myeloma. Phase I was a dose escalation trial in twenty-one patients who were Rb positive and had relapsed or refractory MM. maximum tolerated dose and recommended dose were determined. Phase II enrolled thirty-two patients and had two arms based on different treatment schedules. The primary endpoint was ORR, and secondary endpoints included safety, PFS, and OS among others. During phase II the most common treatment related adverse events were thrombocytopenia, anemia, and fatigue; and thrombocytopenia, anemia, and neutropenia were the most commonly reported grade 3 or higher adverse events, with nearly all other treatment-related AEs being grade 1 or grade 2. In addition, the bone marrow suppression was shown to be reversible after palbociclib withdrawal. Results showed that 20% of 25 evaluable participants achieved an objective response and 44% had stable disease lasting a median of 3.9 months. Median Kaplan-Meier estimated time to progression was 3.9 months [123]. These results showed promise for CDK4/6 inhibitors as a future therapy for MM.

In addition to multiple myeloma, and squamous cell lung

cancer, another area of research for CDK4/6 inhibitors is in glioblastomamultiforme. Various studies have explored palbociclib and ribociclib as treatment options. For example, NCT01227434 was a phase II study which evaluated the efficacy and tissue pharmacokinetics of palbociclib in recurrent glioblastoma, it was planned to compare palbociclib followed by resection to palbociclib without resection. The study found adequate tissue PK in five out of twenty-two total patients, but had to be terminated early due to lack of efficacy with 95% of patients progressing within six months of trial start date. One issue this study faced was attaining adequate drug levels in the CNS, and the other was that it was evaluating a heavily pretreated patient population. Palbociclib still showed some promise for targeting the CDK4/6 pathway in glioblastomamultiforme treatment with further research needed [124]. Cincinnati Children's Hospital has an actively recruiting study investigating the effects of using ribociclib after radiation therapy in patients with high-grade gliomas. The study accepts RB+ tumors and non-biopsied Diffuse Intrinsic Pontine Glioma (DIPG), which are historically know to likely be RB+, thus making CDK4/6 inhibition a fitting target (NCT02607124).

A recent study looked at the role of CDK4/6 inhibitors as a therapeutic tool for patients with advanced bladder cancer who are not candidates for cisplatin therapy. The researchers used bladder cancer cell lines to test *in vitro* CDK4/6 inhibitor sensitivity and then used *in vivo* studies in a metastatic bladder cancer mouse model. The authors concluded that the bladder cancer cells lines were sensitive to CDK4/6 inhibition regardless of RB status, suggesting a novel pathway. In the mouse model, CDk4/6 inhibition correlated with FOXM1 knockdown, and FOXM1 is an oncogenic driver in bladder cancer [125]. This study warrants further exploration of CDK4/6 inhibitors in bladder cancer in human studies, and also research into the link between CDK4/6 inhibitors and FOXM1 knockdown, which shows promise as a novel therapeutic tool.

Squamous cell lung cancer, multiple myeloma, glioblastomamultiforme, and bladder cancer are clear examples of cancer types known to have a high predominance of abnormal expression of CDK4/6 or CDK4/6-Rb-E2F. Taking this idea one step further would be to examine patients of any cancer type who have abnormal CDK4/6 expression and study the effect of CDK4/6 inhibitor therapy.

For example, cell lines with loss of function mutations or abnormal mutations of CDKN2A, a gene that codes for p16, have been shown to be especially sensitive to CDK4/6 inhibitors. In three different studies of three different cancers; ovarian cancer, glioblastomamultiforme, and melanoma, the abnormal expression of p16 was linked to CDK4/6 inhibitor efficacy [126]. Specifically, this implies that loss of function of p16 is a potential target for CDK4/6 inhibitors, regardless of canner type.

In addition to p16, FOXM1is a protein that regulates the cell cycle and plays a role in CDK4/6 action. CDK4/6 has a role in phosphorylation of FOXM1 which activates its function as a cell cycle regulatory during G1, S, G2 and M. CDK4/6 inhibitor has been hypothesized to downregulated FOXM1 and cause cell senescence. This idea was discussed above in reference to a bladder cancer cell line. In a neuroblastoma cell line, CDK4/6 inhibitor was shown to decrease FOXM1 mRNA and this was associated with increased senescence, measured using senescence-associated beta-galactosidase [127]. Thus, FOXM1 shows promise as a novel target of CDK4/6 inhibition in various cancers.

One of the most promising studies actively recruiting is a Phase II trial titled, "Study of the CDK4/6 Inhibitor Abemaciclib in Solid Tumors Harboring Genetic Mutations and Amplifications of CDK4/6" (NCT03310879). Any solid tumor that that has mutations or overexpression of CDK4/6 or D-type cyclins can be included in the trial. This shift from studies with inclusion criteria based on type of cancer to inclusion criteria based on genetic makeup of the tumor is a step forward to explore CDK4/6 inhibitors' efficacy in a wide range of cancers.

Summary of CDK4/6

CDK4/6 inhibitors show great promise for improving cancer treatment outcomes among many different types of cancer. Abemaciclib, palbociclib, and ribociclib are three CDK4/6 inhibitors that are already FDA-approved for the treatment of HR-positive, HER2-negative breast cancer, often in combination with endocrine therapies, through the help of landmark clinical trials. There are currently various clinical trials underway to further evaluate CDK4/6 inhibitors outside of breast cancer, including in squamous cell lung cancer, MM, GBM, bladder, cancer, DIPG, and many others. There is also a shift from evaluating the role of CDK4/6 inhibitors in specific cancer types, and instead exploring its efficacy based upon genetic markers. Two proteins that shown potential include p16 and FOXM1, both regulators of the cell cycle. Loss of function mutations of p16 have been associated with CDK4/6 efficacy. Downregulation of FOXM1 with CDK4/6 inhibitors has shown an increase in cell senescence in cancer cell lines. The efficacy shown in the trials and the promise of the research studies reviewed shows promise for implementation of CDK4/6 inhibitors in Rb+, p16 mutated, and FOXM1 expressed cancers of all types. This will allow further evaluation of CDK4/6 inhibitors' role in treatment protocols for various cancers.

EGFR signaling

Epidermal growth factor receptor (EGFR), is a receptor tyrosine kinase that belongs to the same family as HER2-4. EGFR is activated to form homo or heterodimers through ligand dependent or independent mechanisms. Once phosphorylated, EGFR acts to activate multiple signaling pathways involved in survival, proliferation, apoptosis and migration. Importantly, EGFR activates the RAS/RAF/MAPK pathway, PI3K/AKT, STAT and Src family kinases. Over expression or mutation of EGFR results in over activation of these pathways which are critical for driving cancer development and progression. There are currently two categories of drugs that inhibit EGFR, monoclonal antibodies (mAb) which bind to the extracellular domain of EGFR and tyrosine kinase inhibitors (TKIs) which work intracellularly to block autophosphorylation.

Cancer relationship

EGFR is over expressed in approximately 75% of all colorectal cases and correlated with decreased survival and response to chemotherapy [128]. In patients with demonstrated over expression of EGFR there are two mAb antibodies, cetuximab and panitumumab that are FDA approved. Both antibodies bind the extracellular domain of EGFR and prevent dimerization and activation of the receptor. Cetuximab is approved for use in KRAS wildtype, EGFR expressing, metastatic colon cancer, in first line treatment in combination with FOLFIRI, or with irinotecan patients with refractory disease, or as single agent in patients who have failed oxaliplatin and irinotecan. It is also approved in head and neck cancer (SCCHN) in combination with radiation therapy (RT) or in combination with platinum-based therapy and fluorouracil. It is also approved as single agent for patients with recurrent or metastatic SCCHN who have failed platinum based therapy. Panitumumab is approved for RAS wildtype metastatic colon cancer in first line treatment with FOLFOX and as monotherapy following progression with fluoropyrimidine, oxaliplatin and irinotecan based chemotherapy. The ASPECCT study of over 1000 patients, investigated the response rate of cetuximab and panitumumab in KRAS WT, chemotherapy refractory patients and found that both drugs confer a similar survival benefit, 10.4 versus 10.0 months, respectively [129]. Results from another trial showed that cetuximab treatment increased OS and PFS benefit compared to best supportive care (9.5 vs. 4.8 months and 3.7 versus 1.9 months) respectively [130]. KRAS mutation status is important in this population as gain of function mutations in KRAS would activate the Ras/MAPK pathway below the level of EGFR inhibition. In addition, KRAS mutation, specifically KRASQ61 is often associated with acquired resistance to EGFR blockade [131].

In non-small cell lung cancer (NSCLC) mutations in EGFR are associated with roughly 30% of all cases [132]. Specifically, EGFR-positive NSCLC patients often harbor point mutations in exons 18 (G719A/C) and 21 (L858R, L861Q), as well as in-frame deletions in exon 19 [6,132-134]. These mutations lead to constitutively active tyrosine kinase activity resulting in increased cell proliferation, cell survival, metastasis, and angiogenesis and have proven effective molecular targets using tyrosine kinase inhibitors (TKIs) [135]. First-generation TKIs, such as erlotinib and gefitinib, and second-generation TKIs, such as dacomitinib, target the ATP-binding site and catalytic site, respectively, within the tyrosine kinase domain and have proven successful in patients with these mutations [136]. These drugs are EGFR specific inhibitors, while lapatinib, afatinib and neratinib are broad TKIs that also target other members of the ErbB family.

Multiple studies have shown the clinical significance of targeting EGFR mutation in NSCLC. A summary of randomized clinical trials by Lindeman et al. highlights the benefit of targeting relevant EGFR mutation with EGFR inhibition compared to standard of care in first line NSCLC [11]. Response rates of targeting EGFR with EGFR inhibitors varied from a high of 83% in the OPTIMAL trial involving 154 patients to a low of 56% in the LUX trial [137,138]. Similar significant benefit was shown in in multiple large randomized trials with progression free survival. Meta-analysis of 54 publications regarding FDA approved EGFR involvement in advanced NSCLC with and without the relevant EGFR mutation target further verified benefit by relationship of EGFR target match to EGFR targeted therapy [11]. Median survival of 23.3 ± 18.4 months was observed with target match of EGFR compared to no target match survival of 12.1±13.9 months [139]. The most common molecular change which involves approximately 50% of patients who develop resistance to EGFR TKI therapy is a T790M point mutation in EGFR. T790M confers resistance to gefitinib and erlotinib and are often present in a small sub-population of initially sensitive EGFR-positive cells [140,141]. This mutation increases the affinity of EGFR for ATP and attenuates the binding affinity of both first- and second-generation anti-EGFR tyrosine kinase inhibitors [142,143]. To address these patients, osimertinib, a third-generation TKI, was developed that effectively eliminates T790M-positive cells. Since osimertinib is also active against both T790M and common exon 18 and 21 mutations it has since become standard-of-care in EGFR-positive NSCLC patients with or without the T790M mutation [141,144-146]. In a phase I trial of 253 NSCLC patients with known EGFR mutations or who had prior benefit from EGFR TKI, the overall tumor response rate was 51%, and in tumors with the EGFR T790M mutation the response rate rose to 61% with osimertinib treatment. PFS was also significantly higher in patients with EGFR T790M mutations, 9.6 months versus 2.8 in patients without the mutation. In the AURA phase III clinical trial of patients with EGFR T790M mutations who had progressed on first-line EGFR TKI therapy, osimertinib was evaluated versus standard of care carboplatin or cisplatin with pemetrexed. In this study PFS and ORR was significantly longer in patients receiving osimertinib 10.1 months versus 4.4 months HR 0.30 and 71% versus 31% respectively. Osimertinib also increased PFS and OS from 10.2 to 18.9 months and 31.8 to 38.6 months respectively compared to either gefitinib or erlotinib in the phase III Flaura trial which enrolled stage IV NSCLC, EGFR mutant patients.

While there are conflicting reports of the frequency of EGFR mutations outside of NSCLC, one study found 6% of breast cancer, 5% of gastroesophageal adenocarcinoma, 1% of urothelial and 20% of other solid tumors sampled had EGFR mutations which indicates a wider population that may be considered to benefit from EGFR TKI inhibition [147]. In esophageal cancer (EC) EGFR mutations have been identified *in vitro*, Kyse450 a esophageal squamous cell carcinoma cell line that harbors a EGFR S768I mutation was demonstrated to be sensitive to gefitinib compared to WT controls [148]. Preclinical models have also demonstrated significant tumor response to osimertinib in both colorectal cancer (CRC) and glioblastomamultiforme (GBM) EGFR mutation positive cell lines [149,150].

A case report of a patient with HER2+ inflammatory breast cancer which was initially treated with standard trastuzumab with docetaxel and carboplatin. Following progression the patient was switched to several lines of systemic therapy before tissue from a repeat biopsy was sent for comprehensive genomic profiling (Foundation Medicine, Cambridge, MA). This revealed

several mutations including EGFR L858R and the patient was started on targeted molecular therapy with erlotinib. The patient responded well for 8 months with a decrease in FDG avidity. Upon progression, clinicians sent biopsy samples for repeat genomic testing which revealed no EGFR mutations, but did identify ERBB2, and Raptor amplification. This suggests that the clonal EGFR L858R mutation was lost due to negative selective pressure of EGFR inhibitor erlotinib [131]. There is also a case report of a patient with pancreatic ductal adenocarcinoma which harbored an EGFR L747_P753>S activating mutation. The patient was initially treated with neoadjuvent FOLFIRINOX and subsequent pancreaticduodenectomy followed by an additional 6 cycles of FOLFIRINOX. At the time of recurrent disease 8 months later, the patient was treated with gemcitabine and nab-paclitaxel followed by FOLFIRINOX. Upon progression a tumor specimen from the initial surgery was sent for genomic profiling at Foundation Medicine (Cambridge, MA) which revealed the EGFR L747_P753>S activating mutation. At that time the patient was started on erlotinib and maintained a PR for 32 weeks. Repeat biopsy revealed the common EGFR TKI resistance mutation EGFR T790M and the patient was started on osimertinib however did not achieve a response [151]. While rare, these case reports highlight the potential efficacy of targeting EGFR using TKIs outside of NSCLC in patients with relevant EGFR mutations.

As mentioned previously, the first documented resistance mechanism to EGFR TKI inhibition was the detection of the gatekeeper T790M mutation which led to the discovery of osimiertinib. Secondary mutations within EGFR are not the only resistance mechanism however. MET overexpression has also been identified as a mechanism for EGFR resistance through phosphorylation of ERBB3 [152]. In these patients, combination MET and EGFR TKI has shown to be effective. In the TATTON trial, NSCLC patients were enrolled if they had progressed on EGFR TKI and had MET amplification. Patients previously treated with first or second generation EGFR TKIs had an ORR of 52% (all PR) on osimertinib plus savolitinib (MET inhibitor). Patients previously treated with third generation TKIs had an ORR of 25% (all PR) on osimertinib plus savolitinib. This data indicates that combining targeted molecular therapy can overcome MET driven TKI resistance.

Another mechanism for EGFR TKI resistance is phenotypic transformation of cells which includes the epithelial tomesenchymal transition (EMT). EMT is involved in cancer progression and metastasis and results in the upregulation of mesenchymal genes and remodeling of the actin cytoskeleton [153]. AXL expression has been correlated with EMT and resistance to erlotinib. While the exact mechanism is unknown, AXL has been shown to strongly stimulate cell proliferation and migration. In preclinical work, AXL activates ERK, NF-kappaB, and Brg-1 which in turn activate MMP-9 a protein essential for invasion. Currently, there are several clinical trials evaluating the safety and efficacy of small molecule inhibitors of AXL.

Summary of EGFR

EGFR inhibition has shown clinical efficacy in NSCLC and colorectal cancer, and there are several case reports showing benefit across other tumor histologic types. While resistance to EGFR TKI has been seen, the molecular mechanism has been well characterized. Further studies to determine the efficacy in a tissue agnostic setting is required.

BRAF V600E mutation signaling

v-Raf murine sarcoma viral oncogene homolog B (BRAF) is a serine-threonine kinase that activates the mitogen-activated protein kinase (MAPK) signaling cascade [154]. This signaling pathway works to regulate cellular proliferation, differentiation, and survival, a mutation in this pathway results in uncontrolled cell proliferation and survival [155]. The MAPK signaling cascade can be characterized as the RAS-RAF-MEK-ERK pathway, which acts in response to growth factors [156]. RAS recruits RAF to the cell membrane and conformational changes result in phosphorylation and activation of MEK and subsequently ERK [157]. There are three RAF proteins (ARAF, BRAF, and CRAF or Raf-1) and out of these three BRAF has been shown to be the primary activator of MEK [158]. One difference in the distinct RAF isoforms is the regulation of their phosphorylation leading to activation. While CRAF and ARAF must be phosphorylated in the negative-charge regulatory region (N region) for maximal activation, BRAF's N region carries constitutive negative charge and phosphorylation is not required. This results in increased basal activity of BRAF when compared to CRAF and ARAF [159]. In colorectal cancers it was proven that BRAF mutations occur in the absence of KRAS, and that BRAF mutation is associated with the limited ability of the cancer to repair mismatched bases in DNA [160]. This suggests BRAF has an independent role in tumorigenesis [161].

Cancer relationship

Mutations of BRAF have been found in many malignancies and the mutation leading to a substitution of valine to glutamic acid at codon 600, referred to as the BRAF V600E (originally V599E), was first indicated in human cancer in 2002 [162]. BRAF V600E mutations are present in 40-60% of malignant melanomas and about half of metastatic melanomas [154]. This mutation is thought to increase its kinase activity, increase activation of the MAPK pathway, and lead to increased cell growth. Increased BRAF V600E has been seen in melanoma, colon adenocarcinoma, thyroid carcinoma, low-grade ovarian serous carcinoma, low-grade glioma, hairy cell leukemia, rare non-small cell lung carcinomas, gastrointestinal stromal tumors, and plasma cell myelomas [154].

The drugs targeting BRAF V600E mutations in cancer that are currently approved for use are small molecule inhibitors dabrafenib (Tafinlar) and vemurafenib (Zelboraf). Vemurafenib is currently approved for advanced stage skin cancer and Erdheim-Chester Disease while dabrafenib (often used in combination with trametinib, a small molecule inhibitor that targets MEK1 and MEK2) is approved for advanced stage skin cancer, anaplastic thyroid cancer, and metastatic non-small cell lung cancer. It is possible that these drugs may also have efficacy in the many other cancers that also harbor BRAF V600E mutations.

BRAF V600E inhibitors

Vemurafenib was the first drug targeting BRAF V600E approved by FDA. Vemurafenib functions to inhibit BRAF-V600 monomers and thus suppresses ERK signaling. This drug is not recommended to treat cancers with wild type BRAF (wtBRAF) or other BRAF mutations as some data has even shown that vemurafenib can enhance growth in tumors with wtBRAF by activating the ERK pathway [163]. The emergence of resistance mechanisms has made treatment with vemurafenibchallenging. Some of the resistance mechanisms that have been documented include dimerization of RAF through the increased wtRAF expression or RAS activity, a modified form of BRAF V600E (p61BRAF V600E) with greater dimerization in cells with low levels of RAF activation, BRAF V600E splicing variants lacking the RAS-binding domain, receptor tyrosine kinase-mediated activation of alternative or parallel survival pathways (like the PI3K/AKT pathway), activated RAS-mediated reactivation of the MAPK pathway, PDGFR beta upregulation, NRAS mutations, and COT activation of ERK [164-167]. Some other documented cases of primary resistance to vemurafenib include RAS/RAF/MEK complex formation and autocrine secretion of IL-6 via induction of JAK/STAT3 and MAPK signaling, oncogene mimicry via signaling plasticity, loss of PTEN, dysregulation of cyclin-dependent kinase 4, secretion of hepatocyte grow factor, loss of NF1, and RAC1 mutations [168,169]. Methods to overcome this resistance include using vemurafenib in combination with other targeted therapies including MEK inhibitors, sequential/intermittent treatment schedules, and using vemurafenib in combination with immunotherapy [169].

In the indicated population of advanced stage or metastatic melanoma patients receiving vemurafenib resulted in a significant increase in overall survival (HR, 0.44; 95% confidence interval (CI), 0.33-0.59; $P< 0.0001$). Progression-free survival was also significantly increased (HR, 0.26; 95% CI, 0.20-0.33; $P< 0.0001$). Overall response rates were 48.4% for vemurafenib versus 5.5% in dacarbazine (a previously approved drug for this indicated population) [170]. Additionally, when metastatic melanoma patients were treated with vemurafenib risk of death decreased by 63% and the risk of death or disease progression was decreased by 74%, as compared with dacarbazine ($P< 0.001$ for both comparisons) [171]. Another approved use for vemurafenib is in Erdheim-Chester disease (also known as Langerhans'-cell histiocytosis). With vemurafenibErdheim-Chester disease patients had a response rate of 43% (95% CI, 18 to 71), the median treatment duration was 5.9 months (range, 0.6 to 18.6), disease regression was seen in 12 out of 14 patients, no patients had disease progression during therapy, the preliminary 12-month progression-free survival rate was 91% (95% CI, 51 to 99) and the preliminary 12-month overall survival rate was 100% [172].

Many cancers outside of the indicated population have BRAF V600E mutations, and vemurafenib has been used for other types of malignancies. In an extensive study done by Hyman et al. nonmelanoma cancers (including non-small cell lung cancer, pleomorphic xanthoastrocytoma, anaplastic thyroid cancer, cholangiocarcinoma, salivary-duct cancer, ovarian cancer, colorectal cancer, breast cancer, multiple myeloma, and clear-cell sarcoma) were treated with vemurafenib and analyzed. In non-small cell lung cancer, the response rate was 42% (95% confidence interval [CI], 20 to 67), the median progression-free survival was 7.3 months (95% CI, 3.5 to 10.8), the 12-month rate of progression-free survival was 23% (95% CI, 6 to 46), the preliminary 12-month overall survival rate was 66% (95% CI, 36 to 85). In colorectal cancer, monotherapy with vemurafenib did not induce any responses, but in combination with cetuximab (an anti-EGFR antibody) one response was observed, but half the patients had tumor regression that did not meet standard criteria for a partial response. Median progression-free survival and overall survival for colorectal cancer patients receiving combination therapy was 3.7 months (95% CI, 1.8 to 5.1) and 7.1 months (95% CI, 4.4 to not reached), respectively. Three of four patients with anaplastic pleomorphic xanthoastrocytoma had partial responses. Responses were also observed in patients with the following tumor types: anaplastic thyroid cancer (two patients), cholangiocarcinoma (one patient), salivary-duct cancer (one patient), soft-tissue sarcoma (one patient), and ovarian cancer (one patient). In three of these patients (one each with anaplastic thyroid cancer, cholangiocarcinoma, and ovarian cancer), the responses have persisted for more than 12 months. Additional tumor regression that did not meet criteria for a response was observed in three patients with glioblastoma and one patient each with anaplastic ependymoma, pancreatic cancer, and carcinoma of unknown primary type [173]. In a case study, a 51-year-old man with BRAF-mutated anaplastic thyroid cancer showed nearly complete clearing of metastatic disease with adjuvant use of radiation [173]. In a case of low-grade serous ovarian adenocarcinoma, a cancer that is known to respond poorly to chemotherapy, vemurafenib allowed for more than 21 months of partial response and disease control with decrease in CA125 and relief of cancer-related symptoms [174]. In a case of a 34-year-old woman with glioblastomamultiforme (GBM) with spinal metastases vemurafenib was used. She was progression free for 11 months, there was significant improvement in intracranial leptomeningeal disease and in the nodular leptomeningeal enhancement along the conusmedullaris and multiple nerve roots of the caudaequina during that time, and quality of life was significantly improved [175].

Dabrafenib was the second BRAF V600E inhibitor medication approved by the FDA. Dabrafenib also targets and inhibits the upregulated BRAF kinase activity that results from the BRAF V600E mutation. Dabrafenib unlike vemurafenib is approved to inhibit BRAF V600K mutations as well [154]. The resistance mechanisms that have been discovered for dabrafenib are mostly the same to the resistance mechanisms for vemurafenib mentioned previously. Most notably the reactivation of MAPK pathway, upregulation of COT, NRAS or MEK mutations, dimerization or variant splicing of mutant BRAF V600, and MAPK-independent signaling through receptor tyrosine kinases, such as PDGFR beta, IGF-1 receptor, and hepatocyte growth factor receptor [176]. Dabrafenib is often given in combination with trametinib, this could help combat these resistance pathways.

In the indicated population of advanced stage or metastatic melanoma patients had a median progression-free survival of 9.3 months in the dabrafenib–trametinib group and 8.8 months in the dabrafenib-only group (HR for progression or death in the dabrafenib–trametinib group, 0.75; 95% confidence interval [CI], 0.57 to 0.99; *P*=0.03), the overall response rate was 67% in the dabrafenib–trametinib group and 51% in the dabrafenib-only group (*P*=0.002), and at 6 months, the interim overall survival rate was 93% with dabrafenib–trametinib and 85% with dabrafenib alone (HR for death, 0.63; 95% CI, 0.42 to 0.94; *P*=0.02) [177]. In the indicated population of non-small cell lung cancer in a multicenter, single arm, nonrandomized phase II study (BRF113928; ClinicalTrials.gov identifier: NCT01336634) cohort A (dabrafenib used as monotherapy) and cohort B (dabrafenib used in combination with trametinib) were studied. In cohort A objective response rate was 33%, disease control rate was 58%, median progression-free survival and overall survival were 5.5 and 12.7 months respectively [178]. In cohort B objective response rate was 63.2%, disease control rate was 79%, median progression-free survival was 9.7 months, and 65% of the patients achieved greater than 6-month progression-free survival [180]. In cohort C dabrafenib and trametinib was used in 36 treatment-naïve patients with BRAF V600E-mutant NSCLC. Overall response rate was 64% and disease control rate was 75%, median progression-free survival was 10.9 months, and the overall survival was 24.6 months [180]. In the indicated population of anaplastic thyroid cancer treatment with dabrafenib and trametinib was given. Patients had a confirmed overall response rate was 69% (11 of 16; 95% CI, 41% to 89%), median duration of response, progression-free survival, and overall survival were not reached as a result of a lack of events, with 12-month estimates of 90%, 79%, and 80%, respectively [181].

Dabrafenib has also shown success outside of indicated populations. In a phase I study of dabrafenib 28 non-melanoma BRAF mutant malignancies were treated. There were 14 patients with papillary thyroid cancer, 11 patients with colorectal cancer, and one patient each with non-small-cell lung cancer, gastrointestinal stromal tumor (GIST) and ovarian cancer included in the study. There were partial responses seen in three thyroid cancer patients and the non-small-cell lung cancer patient. Tumor regression that did not meet RECIST criteria for a partial response was observed in both the GIST and ovarian cancer patients. Of the BRAF V600E mutant colorectal patients, one patient had a confirmed response with dabrafenib. Additionally, when treating 10 patients with active, asymptomatic melanoma brain metastases, 90% of the patients showed a decrease in brain lesions with four of them achieving complete resolution. The median progression-free survival was 4.2 months and one patient had progression-free survival for 15 months [182]. In 43 patients with BRAF V600 mutant metastatic colorectal cancer combination dabrafenib and trametinib were studied. Of the 43, five (12%) achieved a partial response or better, including one (2%) complete response, with duration of response greater than 36 months; 24 patients (56%) achieved stable disease as best confirmed response. Ten patients (23%) remained in the study greater than 6 months. All nine evaluable during-treatment biopsies had reduced levels of phosphorylated ERK relative to pretreatment biopsies (average decrease ± standard deviation, 47% ± 24%) [183]. In a study that used dabrafenib in 32 children with relapsed or refractory low-grade gliomas the objective response rate was 38%, including one complete response and 11 partial responses, and an additional 14 patients had stable disease [184]. In a report of two clinical cases, two adults with BRAF V600E-positive high-grade gliomas were successfully treated with combination dabrafenib and trametinib. Both patients had significant clinical and radiographic responses, consistent with prior results using single-agent BRAF inhibitors in LGGs, one patient had disease control for 11 months before developing progressive disease. The report also lists 15 different cases of low-grade gliomas treated with monotherapy BRAF inhibitor (vemurafenib or dabrafenib) where 10 had partial response, 3 had complete response, and 2 had stable disease. Additionally, the duration of response ranged from 12 weeks (at time of publication) to greater than 24 months [185]. In another patient with chemotherapy and radiation-refractory BRAF V600E mutant intrahepatic cholangiocarcinoma with multiple metastatic lesions in the liver, lungs, pleura, and bone off-label combination dabrafenib and trametinib was used with proton-based radiation therapy to the right femur. CT scans 4 weeks after starting treatment showed spontaneous resolution of the left pleural effusion, almost complete resolution of the mass-like nodules and hilaradenopathy in the right lung, improvement in liver lesions, and the bone lesions appeared sclerotic due to treatment. CT and PET scans 10 weeks after treatment showed continuous response with residual reactive lesion in the left lobe and resolution of activity of other previous liver metastases, lung lesions and skeletal metastases. After 34 weeks of therapy, she remained almost completely asymptomatic [186].

Summary of BRAF V600E

BRAF is a kinase that plays an integral role in the MAPK signaling cascade that controls cell proliferation and survival. Mutations in BRAF are common in many cancers with the most common mutation identified as BRAF V600E. This mutation is predominantly linked to advanced stage or metastatic melanoma, but it is not limited to skin cancer. BRAF inhibitors targeting the V600 mutation (vemurafenib and dabrafenib) are proven to have clinical utility in targeting cancer identified to have the targetable BRAF mutation. Not only are there numerous studies detailing the drugs' effectiveness in approved uses, there are also several studies and cases showing utility in other cancer populations outside of FDA defined indication. These studies and case reports suggest therapy efficacy when the target is present.

Discussion

Results of CGP testing provide clinical guidance to national guidelines (NCCN) for cancer treatment (NCCN) indicated precision therapeutic approaches. As was discussed, "targeting the target" describes an approach of identifying actionable molecular aberrations within signaling pathways associated with cancer maintenance and progression. There are a limited number of hallmark biological capabilities necessary for

cancer development and persistence (sustained proliferation, evasion of growth suppressors, resistance to cell death, replicative immortality, angiogenesis, invasion ± metastability, reprogrammed energy metabolism and immune evasion) [187]. These capabilities support one or more of three core processes, i.e., cell survival, cell fate and genome maintenance, which are subserved by one or more of 12 signaling pathways [188]. Being able to identify and block one more of these cancer specific relevant pathway [s] underlying the core cancer survival processes will cripple cancer persistence and progression. The multigenomic/proteomic components of each of these relevant pathways are dependent on a limited number of aberrant and rewired "hub" elements comprising "driver" genes (oncogenes and suppressor genes) [4,188-190]. These are rate-limiting genes/proteins and high-information transfer genes/proteins, the targeting of which is feasible and has been demonstrated many times in the clinical arena as we highlighted in this review to effectively block cancer specific relevant pathways resulting in clinical cost effective survival and response benefit which appears to be independent of cancer histology [6,191].

As described, the introduction of genotyping studies (CGP) revealed genetic and molecular abnormalities in the various subtypes of cancer. These results are shifting the current and future paradigm of cancer management from histologically-driven therapeutic strategies, to molecular-driven precision therapies. Several driver mutations, far more than presented here, along relevant cellular signal pathways have been identified in cancer, including alterations and mutations involving EFGR, ALK, KRAS, PIK3CA, BRAF, RET, MET, AKT1, CDK4/6, CDKN2A/B, BRACA1/2, PDGFRα, mTOR and ROS1 [32]. These have been shown to be disabled in function by precision therapy. Furthermore, certain mutations have been identified as indicators of drug sensitivity, primary drug resistance and acquired drug resistance thereby necessitating increased need for medical bioinformatic involvement. Characterization of these signals allows proper tailoring of therapies to specific genomic alterations and facilitates re-direction of the course of therapy if and when resistance emerges.

Overall early initial cancer response of matched precision therapy vs. standard chemotherapy is ≥ 6 fold higher and survival of ≥ 1 year has been demonstrated as significant advantage in meta-analysis of EGFR inhibitors involving advanced NSCLC [191]. Since that time, as described, comprehensive genomic profiling with identification of relevant molecular target to matched molecular therapy has revealed survival advantage of between 8 months and 2 years in late stage solid cancer populations, response of ≥ 30% vs. ≤ 10% [192-200]and statistically significant progression free survival advantage over standard chemotherapy [193-200]. Moreover, correlation has been shown with less hospitalization, emergency room visits and fewer toxic deaths [201]. Both molecular signal and molecular targeted immune therapy are further supported by FDA recent recommendation for matched target to target therapy basket trial design to biotechnology industry as a new standard for drug development of the future [12,202-206].

Conclusion

Combination matching of multiple targets with multiple precision therapies is showing further enhancement in patient benefit. Highlight of PIK3CA, *BRCA 1/2*, EGFR, CDK4/6 and BRAF V600E mutations as indicators of broader use of precision therapy possibly expanding to advanced patients who have failed standard NCCN guideline options should be considered.

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