Targeted Therapy for Cancer in the Genomic Era

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Abstract: The advent of cancer genomics has led to the development of many highly successful targeted therapies, primarily inhibitors of growth factor receptors and related kinases, including imatinib for chronic myeloid leukemia and trastuzumab for HER2-positive breast cancer. This approach has become highly successful for certain cancers. However, as the list of targeted therapies expands, their efficacy becomes more limited, and toxicity accumulates. What we have learned in the past decades is that while the targeted therapies approach may be highly successful in less complex tumors, cancers defined by carcinogen-induced genomic chaos, such as UV-induced melanoma or tobacco-induced lung cancer, are driven by a multitude of competing molecular pathways and, as such, are not as successfully managed by a similar approach. Luckily, in the past years, the field of cancer immunotherapy has become more fully developed with the emergence of checkpoint blockade inhibitor therapy. These promising new agents are particularly well suited for tumors with a high mutational burden due to underlying genomic disarray. While still in its infancy, we predict that cancer immunotherapy will offer a better alternative to our current targeted approach and eagerly await the results of several ongoing clinical trials that will elucidate this new direction in cancer therapy.

Key Words: Cancer genomics, CTLA-4, Erb2, immunotherapy, PD-1, PDL-1

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In the course of 2 decades, we have gone from an era where most new agents were relatively nonspecific chemotherapeutics to an era defined by therapies targeted against specific molecular targets, primarily growth factor receptors or related kinases. These agents altered our ability to treat patients across a broad range of diseases and thoroughly transformed the treatment of some specific cancers. As information regarding targeted molecular therapeutics grew, and the number of new agents increased, it became obvious that biology imposed real limits on the success of targeted therapeutics, a realization that led to another shift in cancer therapeutics. While targeted therapeutics continue to be developed, and signal successes still occur, they no longer represent the sole or even preeminent approach to new cancer treatment. This article provides a broad overview of these twin transformations.

The Basis of Targeted Therapies

Beginning late in the 1990s, the emerging biologic understanding of human cancer led to the development of numerous targeted therapies. Early successes included agents such as imatinib for chronic myeloid leukemia (CML) and trastuzumab for HER2-positive breast cancer and followed a novel and powerful approach to the cancer problem. The basic premise of this approach involved identifying a molecular lesion or pathway, measuring its activity in the clinic, and then developing an appropriate drug to inhibit its function. Many targeted agents took advantage of the oncogenic addiction pathways of cancer cells. One additional assumption underlying this approach was that molecularly targeted therapies would not only prove to be more effective than classic chemotherapeutics, but would also be associated with reduced toxicity, a function of their exquisite molecular sensitivity.

Perhaps the best known agent to use this approach was the tyrosine kinase inhibitor (TKI) imatinib for CML. Chronic myeloid leukemia, a myeloproliferative neoplasms characterized by the dysregulated production and proliferation of mature granulocytes, is associated with the fusion of the BCR gene (on chromosome 22) with the ABL1 gene (on chromosome 9), resulting in the BCR-ABL1 fusion gene. This abnormal fusion gene has a unique product, the BCR-ABL1 fusion protein, which is constitutively active and implicated in the pathogenesis of CML. The discovery of imatinib, an oral TKI that competitively binds to the BCR-ABL fusion protein and inhibits its activity, revolutionized the way in which we understood and treated CML. Even prior to a highly successful phase III trial,1 the first CML-focused trial clearly established imatinib as a world beater and game changer, replacing a much more toxic and less accessible transplant-based approach.2

Roughly simultaneously to the discovery of imatinib in the treatment of CML, a similar targeted therapy revolution was brewing in HER2-positive breast cancer. The ErbB protein family of receptors was among the first receptor tyrosine kinases for which ligand binding was studied and for which the importance of ligand-induced dimerization was established. This family of transmembrane receptor tyrosine kinases consists of the epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Binding of ligands to the extracellular domain of EGFR, HER3, and HER4 induces the formation of kinase active hetero-oligomers leading to the subsequent activation of a series of downstream signaling pathways.3 Heterodimerization of HER2 with EGFR or HER3 induces transphosphorylation and stimulates important intracellular pathways, including RAS/RAF/MEK/ERK and PI3K/AKT/TOR.4,5 Over the past decade, it has become increasingly evident that the ErbB family plays an important role in the development and maintenance of several cancers, and this has led to the development and implementation of specific ErbB inhibitors as important therapeutic agents (Table 1).

Trastuzumab was the first humanized monoclonal antibody targeting the HER2 transmembrane receptor and disrupting ligand-independent intracellular signaling.6 In a seminal study, Slamon et al7 first reported that about 20% of breast cancer patients had an amplification of the HER2 oncogene and that this was associated with poor outcome, suggesting a causal relationship to cancer virulence. After an initial phase III trial demonstrated a survival benefit in metastatic HER2-positive disease, clinical investigators rapidly launched and subsequently completed multiple phase III adjuvant trials for high-risk early-stage disease. These trials were crowned with success. The combined analysis of NCCTG (North Central Cancer Treatment Group) N9831 and NSABP (National Adjuvant Breast and Bowel Project) B-31 clinical trials has since shown that the addition of trastuzumab for adjuvant treatment of HER2-positive breast cancer...
results in a 37% improvement in overall survival (hazard ratio [HR], 0.63; 95% confidence interval [CI], 0.54–0.73) and a 40% improvement in disease-free survival (HR, 0.60; 95% CI, 0.53–0.68) with a median on-study time of 8.4 years.8

Subsequent successes followed in the HER2 domain. The addition of pertuzumab, a humanized monoclonal antibody that blocks ligand-dependent HER2 dimerization, to trastuzumab and docetaxel leads to a significant improvement in overall survival as first-line therapy in metastatic HER2-positive breast cancer with a median survival of 56.5 months, versus 40.8 months without pertuzumab (HR, 0.68; 95% CI, 0.56–0.84).9 More recently, the discovery of HER2 somatic mutations in non–HER2-amplified breast cancers has sparked an interest in its role in tumorigenesis with small molecule TKIs currently being explored in early-phase clinical trials (Table 1).10

Once again, the approach taken followed a pathway similar to that seen in CML: demonstrate that a cancer is highly dependent on a particular molecular alteration for growth, identify the lesion in a defined clinical population, develop an agent that interferes with the pathway, and perform well-designed clinical trials that test the importance of the pathway. This basic paradigm came to define drug development for the decade following the introduction of imatinib and trastuzumab.

Targeting other members of the ErbB family of transmembrane receptors has proven successful across tumor types. The role of EGFR in tumorigenesis was established more than a decade ago when somatic activating EGFR mutations were discovered in a subset of non–small cell lung cancers (NSCLCs).11–13 Non–small cell lung cancers harboring EGFR mutations, primarily localized within mutational “hot spots” in the kinase domain (exons 18–21), are highly sensitive to small molecule EGFR TKIs with RECIST (Response Evaluation Criteria In Solid Tumors) response rates of 55% to 75%.14–16 Interestingly, the prevalence of these activating somatic mutations vary across different patient populations, encompassing approximately 15% of NSCLC adenocarcinomas in the United States and occurring more frequently in women and nonsmokers, but with a substantially higher incidence in Asia, ranging from 22% to 62% of patients.17

The list of targeted therapies continues to expand, with new successes being introduced at annual meetings of the American Society of Clinical Oncology and the American Association for Cancer Research and their sister societies. These agents are widely used by practicing oncologists, are increasingly expensive (to the detriment of the American health care system as well as individual patients), and, with a few exceptions, are both less effective and (surprisingly) more toxic than targeted therapy archetypes such as imatinib and trastuzumab. Median improvements in survival for most targeted therapies are measurable in months rather than years, and the febrile neutropenia of the chemotherapy era has been replaced (or, rather, added to) by fatigue, diarrhea, skin disorders, pneumonitis, and a host of other toxicities. What went wrong?

The Problem With the Targeted Therapeutics Approach

On the efficacy side of the equation, the relative inefficacy of many targeted therapies is understandable in light of what we have learned from another technologic advance, genomics.

It is now relatively trivial to sequence whole-cancer genomes, thus allowing scientists to evaluate the relative burden and distribution of mutations across tumor types.18 The aim of such research is to explore genomic alterations, akin to HER2 or EGFR, which may lead to similarly successful targeted therapeutics.

What we have learned is daunting for targeted therapy. Few common cancers are driven by a single mutation, and indeed many cancers caused by a lifetime of genomic abuse (e.g., smoking-related NSCLC, BRCA-mutated triple-negative breast cancer, or UV radiation–induced melanoma) are best characterized in terms of genomic chaos: multiple driver mutations, rapid compensatory mechanisms of resistance, and inherent resistance to solitary targeted approaches. In retrospect, the failure of targeted agents to prolong life by more than a few months was predesigned.

<table>
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<th>TABLE 1. ErbB Classes of Drugs</th>
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<td><strong>Drug</strong></td>
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<td>Lapatinib</td>
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<td>Trastuzumab emtansine (T-DM1)</td>
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CRC indicates colorectal cancer; CT, chemotherapy.
Furthermore, while genome-wide sequencing is now readily accessible, identification of genes that drive tumor formation versus so-called “passenger genes” has become a major challenge. Miller\textsuperscript{19} recently reported on the use of commercial next-generation sequencing (NGS) to identify actionable mutations across solid tumor types by extracting DNA from more than 2200 routine formalin-fixed paraffin-embedded tissues. Two thousand one hundred twelve (95%) of 2221 tumors were successfully profiled, with alterations reported in 85% of the genes. The study found that 76% cases harbored more than 1 “actionable” mutation, that is, a potentially “druggable” target.\textsuperscript{19}

But what do we mean by “actionable”? Consensus guidelines defining what is “actionable” are currently lacking. “Actionable” usually refers to genetic variants with prognostic or diagnostic associations, whereas “targetable,” or “druggable,” implies that the specific genetic alteration is associated with a drug that is either approved for this indication or may be used as part of a clinical trial or as off-label use.\textsuperscript{20} Several so-called “basket” trials are now underway to study this commercialized NGS approach with the aim of personalizing care across cancer types based on the specific genetic alterations in the tumor DNA. The National Cancer Institute’s (NCI’s) Molecular Analysis for Therapy Choice (MATCH) initiative follows such an approach and will be using a protocol for multiple single-arm phase II trials with the aim of assigning more than 1000 patients that progress after 1 line of standard therapy to matched agents based on NGS of tumor DNA (Fig. 1).\textsuperscript{20,21}

It is not clear as of yet whether an approach such as the NCI MATCH will lead to sustained objective responses in patients. In fact, besides anecdotal experience, there is insufficient evidence to know whether targeting many of the so-called “actionable” genes will lead to antitumor activity in patients with advanced cancer. As evidenced by the specific BCR-ABL translocation driving the development of CML, for a target to be easily “druggable,” it seems there has to be a single dominant mutation with an overall small mutational load. However, as cancer evolution often depends on a massive number of alterations in the tumor genome, relative genomic complexity still guides how “druggable” a tumor is. Chronic myeloid leukemia is a relatively “stupid” cancer in that a single alteration at the genomic level drives tumorigenesis, and as such, monotherapy with imatinib leads to durable responses. In fact, if imatinib proves to be ineffective therapy, a switch to a second-generation TKI, such as dasatinib or nilotinib, both of which target the same kinase domain, is the current treatment recommendation, with the majority of patients responding to these newer agents.\textsuperscript{22} We are less fortunate in other human cancers.

The problem is that cancers vary in genomic complexity, and most evolve in response to a series of mutational events in different cell signaling pathways. While a smaller proportion of cancers are mutated at a high frequency, the majority of cancer genes that are mutated in most patients occur at an intermediate frequency of between 2% and 20%. The majority of these genes fall within known hallmarks of cancer, such as cell proliferation, apoptosis, genome stability, chromatin regulation, immune evasion, RNA processing, and protein homeostasis.\textsuperscript{23} Cancers defined by genomic chaos include those driven by exogenous carcinogens, such as a large proportion of malignant melanoma, NSCLC, and head and neck squamous cell cancers (HNSCCs). For example, in analyzing whole-exome sequencing of a series of HNSCCs, the tumors exhibiting a mutational profile consistent with a history of tobacco exposure display a doubling in the mutational burden as compared with tumors driven by infection by the human papillomavirus (mean of 4.83 mutations/Mb compared with 2.28 mutations/Mb, $P = 0.004$).\textsuperscript{24} The higher mutational burden of tobacco-induced HNSCC is comparable to that of other smoking-related malignancies, such as small cell lung cancer and lung adenocarcinoma.\textsuperscript{25,26}

Importantly, higher mutational tumor burden may be a predictor of reduced efficacy of single-agent, small molecule TKIs. In an elegant in vivo example, Wagle et al\textsuperscript{27} demonstrated acquired resistance to BRAF inhibition in a patient with metastatic melanoma, secondary to an activating somatic mutation in MEK1 downstream of the target kinase. Similar de novo secondary mechanisms of resistance have been demonstrated in preclinical and clinical studies of trastuzumab and lapatinib in HER2-positive breast cancer via activation of the PI3K pathway and EGFR-activated NSCLC via downstream activation.\textsuperscript{28,29}

Furthermore, genetic intratumoral heterogeneity can contribute to treatment failure and drug resistance. In a recent study, Gerlinger et al\textsuperscript{10} applied exome sequencing, chromosome aberration analysis, and DNA ploidy profiling to study multiple spatially

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**FIGURE 1.** The NCI MATCH study schema.
separated biopsy samples from primary renal cell carcinomas and associated metastatic sites. Interestingly, the authors found that genomics analyses from single-tumor biopsy specimens may underestimate the mutational burden of renal cell carcinomas and likely similar genomically complex tumors and, as such, may be unable to predict the degree of therapeutic resistance secondary to preexisting drug-resistant clones. In this study, a single biopsy revealed an average of 70 somatic mutations, which comprised only about 55% of all mutations that were detected in the tumor. Furthermore, only 34% of all mutations that were detected by multiregion sequencing in the nephrectomy specimen were present in all regions, again suggesting that a single biopsy was not representative of the mutational landscape of the entire tumor.

Similarly, by utilizing cell-free tumor DNA, a form of “liquid biopsy,” Diaz et al. demonstrated that preexisting KRAS mutant clones in metastatic KRAS wild-type colorectal cancer patients were “selected” in the setting of treatment with panitumumab, an anti-EGFR antibody, thus leading to drug resistance. These examples suggest that resistance to targeted drug therapy develops in the setting of selective Darwinian pressures in genomically complex tumors. The emergence of such compensatory mechanisms of resistance may defeat targeted therapies, especially as most tumors may contain multiple driver mutations.

Genomic Complexity and Cancer Immunotherapy

Given the genomic complexity of many cancers, compensatory mechanisms of resistance, including those delineated above, may defeat the targeted therapies approach. Most cancers are not as “simple” as CML, and as such, reliance on inhibition of a single pathway will not be sufficient for long-term disease control. Unfortunately, as we learn more about the multiple driver mutations, we realize that rare oncogenic pathways are actually rather frequent. As we explore the complex biology and potential “druggability” of cancer in this context, a collection of orphan diseases is formed, defined by newly discovered, rare mutations. Of course, to date, we do not know the efficacy of targeted therapies for a majority of these new cancer subtypes as large trials are difficult, if not impossible, to conduct given the small patient population affected by each unique mutation.

An example of such an “orphan disease” is breast cancer lacking HER2 amplification but with a somatic HER2 mutation driving cancer growth and metastasis. Through breast cancer genome sequencing studies using databases such as the TCGA, such activating mutations, which are thought to occur in less than 2% of all breast cancer patients, have now been characterized within distinct regions of the HER2 receptor. A phase II clinical trial investigating the use of neratinib, an irreversible TKI targeting HER2/EGFR, is now in progress (Clinicaltrials.gov NCT01670877).

The challenge for trials such as this is finding a proper balance between utilization of clinical trial resources and patient accrual given the very small prevalence of such somatic mutations in the general breast cancer patient population. In order to address some of these issues, study schemas, such as “basket” and “umbrella” trials, have emerged. Unlike conventional clinical trials in oncology, the so-called basket trial assesses the effect of a single targeted agent, on a single mutation or pathway, in a series of different cancers. The NCI MATCH trial, which consists of multiple phase II clinical trials based on molecularly defined eligibility criteria, falls within this category (Fig. 1). Umbrella trials, on the other hand, are designed to test the impact of different drugs on different mutations in a single type of cancer (Fig. 2).

Given the difficulties inherent in genomics-driven targeted therapeutics, especially for cancers with high genomic complexity, it seems that other approaches that move beyond the single-drug single-mutation model are needed. One exciting new direction involves manipulating the immune system to target human cancer. In particular, a new therapeutic paradigm has emerged with the introduction of immunologic checkpoint blockade with antibodies that target cytotoxic T lymphocyte–associated antigen 4 and the programmed cell death 1 pathway (PD-1 and PD-L1). Ipilimumab (cytotoxic T lymphocyte–associated antigen 4) and pembrolizumab (PD-1) are now approved by the US Food and Drug Administration for the treatment of advanced melanoma, and nivolumab (PD-1) was recently approved for the treatment of advanced squamous NSCLC. In addition to melanoma and NSCLC, both pembrolizumab and nivolumab have demonstrated durable response rates with minimal toxicity in large phase I studies with patients with other solid tumors including renal cell and urothelial carcinomas.

Recent work has focused on biomarkers of response to immune checkpoint inhibitors. While no single immunologic or tumor characteristic in a patient solely determines response to immunotherapies, exciting work has suggested a relationship between the somatic mutational burden of a tumor and its impact on treatment benefit from these novel therapies. Snyder et al. recently reported on the association of ipilimumab response in patients with advanced melanoma and degree of mutational burden and showed that there was a significant difference in mutational load between patients with a long-term clinical benefit and those with a minimal benefit. Interestingly, not all tumors with a high mutation burden responded to ipilimumab, which suggests that select neoepitopes derived from tumor somatic mutations may serve as neoantigens. Rizvi et al. similarly investigated the mutational burden of NSCLC with the hypothesis that tobacco-induced lung cancers have a higher rate of somatic mutations and, as such, will have an improved response to anti–PD-1 therapy. To examine this, they sequenced the exomes of NSCLC from 2 independent cohorts of patients treated with pembrolizumab, a humanized antibody to PD-1. As predicted, patients harboring deleterious mutations in a number of genes important in DNA repair and replication as well as those patients displaying a
previously validated molecular signature of smoking had the highest clinical efficacy to pembrolizumab. How does increased mutational burden affect tumor immunogenicity? In both studies, tumors from patients with clinical response had higher candidate neoantigen burden compared with nonresponders, suggesting a differential antitumor response to specific neoantigens that was augmented by immune checkpoint blockade therapy.

**Future Directions**

One may hypothesize that tumors with increased mutational burden secondary to a variety of mechanisms will have a robust response to immunotherapies similar to those seen in melanoma, given similar genomic chaos leading to the development of antigens foreign to the host immune system. Indeed, early-phase trials are now in development to investigate the role of such agents in these cancer subtypes. These recent discoveries in cancer immunology carry the potential to have a great impact on patients’ lives across multiple cancer subtypes, perhaps more so in cancers that are most difficult to treat because of the inherent genomic disarray within these tumors. Targeted therapies in the era of cancer genomics have offered exciting discoveries with agents such as imatinib, trastuzumab, and erlotinib leading to substantial improvements in the survival rates and the quality of life of cancer patients. However, given the genomic complexity of many cancers, the next chapter in oncology likely lies within the exciting discoveries and early-phase clinical trials in cancer immunotherapy.

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