Precision medicine and cancer immunology in China

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One might argue that the concept of personalized medicine—in which a treatment is tailored for a specific individual based on their unique physiology as well as their specific disease and drug tolerance—is a foundational aspect of the ancient art of traditional Chinese medicine, practiced for centuries. Today, China’s medical practitioners depend less on ancient remedies and more on evidence-based practice. They bring with them an appreciation for the benefits and rationale of personalizing treatments to each patient. Therefore, to them, the shift from generalized therapies to precision medicine is perhaps an easier and more logical one to make than for those trained in westernized settings.

In order for precision medicine—the term that now appears to have dislodged “personalized medicine”—to be successful, accurate characterization of the patient is necessary. Various biomarkers provide the necessary data, collected through a variety of ‘omics techniques including next-generation DNA sequencing (genomics), analysis of protein levels in blood or tissues (proteomics), or determination of RNA levels (transcriptomics). However, identifying and characterizing biomarkers that accurately reflect a physiological state (normal or diseased), or response to a particular drug or therapy, has turned out to be challenging. Add to this the complication that biomarkers may differ between population groups, or indeed between individuals, and that tracking these biomarkers as the patient’s status changes can be onerous, and the future of precision medicine could be described as bleak.

This pessimistic outlook has not stopped researchers from pushing forward in their search for accurate and robust biomarkers. Big data analysis is helping, by providing a means to crunch millions of datapoints to yield associations that are not at first obvious. It is hoped that these associations will point to the presence of predictive biomarkers or potential targets for therapy, and also help to predict the risk of disease, ascertain the probability of positive clinical outcomes, and evaluate therapeutic efficacy. Such biomarkers are also the ultimate goal of many next-generation sequencing studies being performed on a range of samples, including tumor tissue and circulating cell-free DNA. Applications of this technology in the clinic are bringing researchers closer to real-time biomarker tracking, with implications for cancer detection and the development of safe, effective treatments.

New immunotherapy treatment modalities, such as the use of checkpoint inhibitors, cytokines, and chimeric antigen receptors, are being developed at an increasingly rapid pace, and the success of such therapies depends heavily on extensive knowledge of individual patients, for which high-quality biomarkers are especially important.

The articles presented in this booklet cover many of the topics above, with a focus on precision medicine research currently being performed in China. Researchers there are determined to overcome every obstacle to detecting and exploiting genomic and proteomic biomarkers in a clinical setting for the benefit of their patients. They also hope their insights will advance the practice of precision medicine both domestically and worldwide.

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BeiGene, Ltd. (NASDAQ: BGNE) is a global, commercial-stage biotechnology company focused on molecularly targeted and immuno-oncology cancer therapies. With a team of over 800 employees in China, the United States, and Australia, BeiGene is advancing a pipeline of novel small molecules, monoclonal antibodies, and combination therapies for cancer treatment. BeiGene also markets ABRAXANE (nanoparticle albumin-bound paclitaxel), REVLIMID (lenalidomide), and VIDAZA (azacitidine) in China under a license from Celgene Corporation.

BeiGene was founded in 2010 based on the premise that the confluence of two major developments—the revolutionary scientific breakthroughs in cancer medicine, and the emergence of the pharmaceutical market in China, where nearly a quarter of the world's cancer population has limited access to innovative therapies—may allow new biotech leaders to emerge. With Beijing-based R&D, BeiGene recruits from China's strong scientific talent pool and has developed a drug discovery platform incorporating tumor samples through local hospital collaborations. Its scientific advisory board consists of world-renowned scientists and clinicians and is chaired by Dr. Xiaodong Wang, cofounder of BeiGene, founding director and architect of China's National Institute of Biological Sciences, and a member of the Chinese Academy of Sciences and the U.S. National Academy of Sciences.

Over the past seven years, BeiGene has discovered and advanced into clinical development four investigational drug compounds: Bruton's tyrosine kinase (BTK) inhibitor zanubrutinib (BGB-3111), PD-1 antibody tislelizumab (BGB-A317), PARP inhibitor pamiparib (BGB-290), and RAF dimer inhibitor lifirafenib (BGB-283). Zanubrutinib is in registrational trials both globally and in China, and its global registration program includes a phase 3 head-to-head trial comparing BGB-3111 to ibrutinib, a currently approved BTK inhibitor, with the aim of demonstrating superior depth of response. Tislelizumab is the subject of a strategic collaboration with Celgene and is in registrational trials in China. BeiGene is also testing tislelizumab in combination with pamiparib and zanubrutinib, respectively. The company plans to initiate additional registrational trials of its assets, both in China and globally, and to advance additional preclinical assets into the clinic.

Building on its scientific roots and research foundation in China, BeiGene has established global clinical development capabilities with a significant presence in the United States, China, and Australia. Building on its scientific roots and research foundation in China, BeiGene has established global clinical development capabilities with a significant presence in the United States, China, and Australia. In addition, the company has domestic manufacturing capabilities, including a multipurpose manufacturing facility in Suzhou and a commercial-scale biologics manufacturing facility under construction in Guangzhou, established through a joint venture with the Guangzhou Development District. Through its strategic collaboration with Celgene, BeiGene also recently acquired Celgene’s commercial operations in China and gained exclusive rights to commercialize Celgene’s three approved therapies there, which is expected to help BeiGene prepare for the potential future commercialization of its internally developed compounds and any additional in-licensed compounds in China. BeiGene aspires to be a global biotech leader and is committed to bringing new, potentially life-altering treatments to patients worldwide.

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Targeted therapy for liver cancer: Challenges and opportunities

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Liver cancer is the sixth most prevalent cancer and the second leading cause of cancer-related death worldwide (1). China alone accounts for over half of the new cases and deaths. It is estimated that in 2015 alone, 466,100 new cases of liver cancer were diagnosed in China and 422,100 deaths occurred there (2). Of all the cancers, the survival rate of liver cancer is the poorest—the age-standardized five-year relative survival rate is only 10.1% (3). Due to difficulties in early diagnosis, most liver cancer patients are diagnosed at an advanced stage, losing the opportunity for curative treatments such as liver resection or ablative procedures.

Fortunately, the development of innovative technology such as next-generation DNA sequencing has enabled a rapid and dramatic increase in our understanding of the genetic, molecular, and morphological changes occurring in individual cancer patients, laying the foundation for the emergence of targeted therapy. Although targeted therapies such as sorafenib treatment have raised hope for advanced liver cancer patients, their clinical benefits remain modest at best (4, 5). It is hoped that targeted therapy will provide functional and even structural corrections at the molecular level, or at least offer a valid alternative to conventional treatment. However, liver cancer is an extraordinarily heterogeneous disease, which makes it difficult to properly stratify patients for optimal targeted treatment and increases the risk of side effects, leading to the persistent failure of targeted therapy (6). In this review, we summarize the progress made in targeted therapy for liver cancer treatment in China and focus on the challenges and opportunities thereof.

Sorafenib

Sorafenib is the first small-molecule targeted drug that has demonstrated a survival benefit in advanced hepatocellular carcinoma (HCC) patients (5, 7). It is a multikinase inhibitor of several tyrosine protein kinases, including vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR). Sorafenib can also target intracellular serine and threonine kinase signaling such as RAF proto-oncogene kinase, including the C-Raf and B-Raf pathways (8–10). Sorafenib was approved as the only standard systemic treatment for HCC mainly on the basis of two studies: the Sorafenib HCC Assessment Randomized Protocol (SHARP) phase 3 trial (conducted in Europe, North America, South America, and Australasia), and a phase 3 randomized trial conducted in the Asia-Pacific region. According to these two studies, the sorafenib treatment group showed prolonged median survival: 10.7 months in the SHARP study compared to 7.9 months for the placebo group (5), and 6.5 months in the Asia-Pacific study compared to 4.2 months for the placebo (7). However, further analysis of these two trials and results from other studies have shown undesirable tolerability of sorafenib caused by its severe adverse events, including gastrointestinal, dermatologic, hematologic, cardiovascular, and nervous system side effects (11–16), making patients reluctant to continue the treatment.

Drug resistance is another bottleneck issue for sorafenib treatment. Numerous studies have revealed significantly differing responses to sorafenib due to tremendous variability in the way HCC progresses (17, 18). Chinese researchers have made serious efforts to decipher the mechanism underlying resistance to sorafenib and to identify potential biomarkers predictive of sorafenib response. A study by our team demonstrated that HCC patients with high 26S proteasome non-ATPase regulatory subunit 10 (PSMD10) expression had worse prognosis and a poor response to sorafenib therapy. The overall survival time of sorafenib-treated HCC patients with high levels of PSMD10 was much shorter than those with low PSMD10 (p=0.0099), with the median survival time reduced by more than 40 months (p=0.0099). These results suggest that PSMD10 may be a potential molecular marker in the classification of HCC patients who may not respond effectively to sorafenib (17). In another study, our clinical investigation revealed that HCC patients with low Src-homology 2 domain-containing phosphatase 2 (Shp2) expression benefited from sorafenib administration after surgery. This study showed that Shp2 could promote liver cancer stem cell expansion by augmenting β-catenin signaling and might be a useful indicator when determining chemotherapeutic strategies (18).
The varied cellular metabolic phenotypes of tumor cells may also affect the efficacy of sorafenib. Investigation of the metabolic characteristics of tumor samples from 63 HCC cases showed huge variation in lipid content and glucose uptake. This study found that the rate-limiting enzyme acetyl-coenzyme A carboxylase alpha (ACCoA) enhanced glucose-derived de novo fatty acid synthesis (FAS) and promoted tumor cell survival under energy stress, which contributed to the heterogeneity of metabolic patterns in HCC. Inhibition of ACCα-driven FAS using a specific inhibitor, orlistat, improved the efficacy of sorafenib in xenograft-bearing mice, suggesting that interfering with ACCα-driven FAS could sensitize HCC cells to sorafenib (19). Another study has reported that blocking interleukin-6/signal transducer and activator of transcription 3 (STAT3)-mediated preferential glucose uptake could sensitize liver tumor-initiating cells to sorafenib treatment and enhance its therapeutic efficacy in vivo (20). These findings suggest that a combination of sorafenib and inhibitors of certain metabolic pathways could be a promising approach for some HCC patients.

EGFR inhibitors

Epidermal growth factor receptor (EGFR) is overexpressed in 40%-70% of human HCCs, a factor that has been proven to be closely linked to the formation and growth of tumors. But EGFR inhibitors have shown disappointing results in clinical trials with unselected patients (21). A study was conducted in Taipei to evaluate the efficacy and safety of vandetanib, an oral inhibitor of both VEGFR and EGFR, in patients with inoperable advanced HCC. The study observed no significant difference in the rate of tumor stabilization or vascular change between the vandetanib group and the placebo group, suggesting that vandetanib had limited clinical activity in HCC (22). Other clinical trials with erlotinib, gefitinib, or cetuximab showed only limited effects in advanced stage HCC or modest effects at most in phase 2 trials (21). A better understanding of the mechanisms underlying how EGFR signaling influences HCC progression is therefore needed.

A study of the role of EGFR in HCC formation showed that the absence of EGFR in macrophages impaired the development of HCC in mice, whereas mice lacking EGFR in hepatocytes unexpectedly developed more HCCs due to increased compensatory proliferation after cell damage. Following inflammatory stimulation, EGFR induces interleukin-6 expression in liver macrophages, triggering hepatocyte proliferation and the development of HCC (23). This study demonstrated that EGFR has different roles in tumor cells than in nontumor cells, providing some explanation of the disappointing results of anti-EGFR agents in HCC treatment. Other recent research by our team indicates that levels of choline kinase alpha (CHKA) are higher in human HCCs than in nontumor tissues, and that CHKA is associated with tumor aggressiveness and reduced overall survival. Further study has revealed that CHKA could facilitate a functional interaction between EGFR and mammalian target of rapamycin complex 2 (mTORC2), which could contribute to HCC metastasis by promoting AKT (Ser473) activation. In this way, overexpression of CHKA promotes resistance to EGFR-targeted drugs (gefitinib and erlotinib) in HCC, suggesting that dual inhibition of CHKA and mTORC2 could be a way to overcome the resistance of HCC cells to EGFR-targeted therapies (24).

Immunotherapy

GPC3-based immunotherapy

Glypican-3 (GPC3) can be detected in 72% of HCCs, but could not be detected in normal hepatocytes, cirrhotic liver, or benign liver lesions (25). In addition to being a marker for HCC, GPC3 plays a role in the progression of the disease. It activates Wnt signaling and stimulates cell cycle progression and cell survival (26), indicating that anti-GPC3 therapy could be a therapeutic strategy for HCC treatment. The potential usage of GPC3-derived antibody or peptide vaccines has been explored in HCC immunotherapy (27–29). Disappointingly, in clinical trials these agents showed only limited curative effect (30).

Chimeric antigen receptor T (CAR-T) cells have been heralded as a breakthrough technology due to the substantial benefits observed in patients with relapsed or refractory B-cell malignancies. More than 200 CART-cell clinical trials have been initiated so far, most of which are CD-19 specific CARs aimed at treating lymphoma or leukemia (31). Researchers interested in HCC have mainly explored the possibility of redirecting T cells to recognize GPC3 for the treatment of HCC. T cells with CARs or high-affinity T-cell receptors (TCRs) targeting GPC3 were therefore engineered. Such targeted cells can efficiently recognize and destroy GPC3-positive human HCC cells in vitro and in vivo (32, 33). In a recent study, Li and colleagues developed T cells carrying two complementary CARs—against both GPC3 and asialoglycoprotein receptor 1 (ASGR1)—to reduce the risk of on-target, off-tumor toxicity, while maintaining relatively potent antitumor activity (34). These preclinical studies suggested that
Adoptive transfer of GPC3-specific T cells presents a promising therapeutic strategy for treating HCC. Anti-GPC3 CAR-T therapy is now undergoing clinical trials in China.

Anti-PD-1/L1
Programmed cell death 1 (PD-1) is an immune coinhibitory receptor expressed on immune cells such as T cells, B cells, and natural killer (NK) cells. PD-1 suppresses antigen-specific T-cell activation through interaction with its ligand, PD-L1, which has been observed to be upregulated in tumor cells (35). Clinical trials of antibodies targeting PD-1 or PD-L1 for the treatment of HCC have shown some promising results (36). A recent report in The Lancet evaluated the safety and efficacy of PD-1 inhibitor nivolumab in patients with advanced HCC in an open-label, noncomparative, phase 1/2 dose escalation and expansion trial. The study showed that nivolumab produced durable objective responses in long-term survival rates in patients with advanced HCC (37). However, it is important to recognize that in previous studies the response rate to anti-PD-1 as a stand-alone therapy was 10%–30% overall, which included immunogenic tumors such as malignant melanoma that have a much higher response rate (36). One possible explanation for the low response rate might be the influence of molecules involved in immune escape other than PD-1 and PD-L1/2.

Potential targeted therapies
Recent findings have also shed light on other potential targets for HCC treatment. The development of HCC is a multistep process with high intratumoral heterogeneity, including alterations in tumor microenvironment, signaling pathways, and energy metabolism patterns. In
previous reports, it has been noted that adenosine monophosphate (AMPK) serves as an energy sensor in eukaryotic cells and plays a role in linking metabolism and cancer development (39–41). However, our recent research has demonstrated that activation of AMPK by the first-line medication metformin for the treatment of type 2 diabetes, not only inhibited HCC cell growth in vivo, but also augmented the growth inhibition induced by the chemotherapy drug cisplatin in these cells (42). Another study observed an imbalance of gut microflora as well as intestinal inflammation in chronic treatment of rats with the carcinogen diethylnitrosamine. Modulation of gut microbiota by probiotics dramatically mitigated liver tumor growth and spread in vivo (43). These studies indicate that an intervention strategy based on studies of HCC heterogeneity may present a new avenue for therapeutic intervention to treat the disease.

Perspectives
An improved understanding of the molecular pathways that drive development of HCC has led to the identification of various biomarkers and the evaluation of several agents specifically targeted to tumor cells with particular molecular features. However, clinical trials undertaken worldwide have documented only occasional positive responses to such treatments. To date, no single agent or single targeted therapy has been formally found to be a cure for HCC in clinical trials. An increasing number of studies have demonstrated that intratumoral heterogeneity in individual patients is a roadblock for HCC targeted therapy. Therefore, the efficacy of targeted therapy requires a thorough understanding of the tumor microenvironment, metabolism, and gut microbiota of an individual. Meanwhile, combined therapies may be more effective than the administration of a single agent. IL-6 and PD-L1 blockade, or sorafenib combined with anti-PD-L1 monoclonal antibody, have demonstrated better efficacy than a single inhibitor in mouse models (44, 45). It is hoped that the establishment of combined therapies can offer a way to successfully manage HCC patients in the future.

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The challenges of radiation oncology in the era of precision medicine

Ligang Xing and Jinming Yu*

Radiotherapy is an essential treatment in the management of cancer. Seventy percent of all cancer patients will get radiotherapy as at least part of their treatment (1). Advances in physics, mathematics, computer science, electrical engineering, and radiobiology have significantly improved the safety, precision, and efficacy of radiotherapy, the concomitant control of tumor growth, and the probability of a cure for many cancer sufferers. In recent years, the sequencing of the human genome has paved the way for precision medicine, which aims to deliver “the right treatment to the right patient at the right time.” Although discoveries arising from studying the genome have affected the delivery of chemotherapy and targeted biological agents (2), they have yet to impact the clinical use of radiotherapy. In this new era of precision medicine, radiotherapy poses both great challenges and great opportunities for physicians and researchers.

Progress in modern radiotherapy techniques

New technologies have been the main driving force behind innovations in radiation oncology over the last two decades. Technological advances have been put to clinical use, leading to better localization of the radiation dose and less damage to healthy tissue. These methods include technologies such as three-dimensional conformal radiotherapy (3D-CRT), intensity-modulated radiotherapy (IMRT), 3D brachytherapy, stereotactic radiotherapy, image-guided and adaptive radiotherapy (IGRT and ART), and charged particle radiotherapy using protons and carbon ions. Using 3D-CRT and IMRT, we can make the radiation conform to the shape of the target volume, solving the problem of irradiating complex targets that lie close to critical healthy structures. 3D-CRT and IMRT are routinely and successfully applied for head and neck cancers, prostate cancer, and many other common cancers including those of the lung, liver, esophagus, and breast (3, 4). In the past 10 years, there has been rapid growth in clinical research on and application of stereotactic ablative body radiotherapy (SABR), also known as stereotactic body radiation therapy (SBRT), for cancers such as lung, liver, spine, pancreas, and prostate. For example, by accurately delivering high-dose radiation to the tumor, SBRT has emerged as the standard of care for medically inoperable stage I non–small cell lung cancer (NSCLC) and may even outperform surgery in operable patients (5, 6).

Genomics for personalized radiotherapy

From 3D-CRT and IMRT to SBRT, two factors limit the efficacy of radiotherapy: (1) defining the target, or differentiating between tumor and normal tissue; and (2) determining the appropriate total dose of radiation and its “fractions”—the number of separate treatments into which the total dose is divided. In clinical practice, radiotherapy dosages and fractions have been determined empirically, which has resulted in reasonable disease control and acceptable levels of toxicity. However, results suggest that current radiotherapy dosing protocols can be further optimized with a modern precision oncology approach, such as gene profiling to detect relevant biomarkers or biomarker signatures that would inform clinicians of a particular patient’s sensitivity or resistance to radiotherapy. Existing tests that predict a patient’s sensitivity to radiation can be grouped into three categories: those that determine intrinsic radiosensitivity, those that determine tumor oxygen levels, and those that determine a tumor’s chance of growing (7). Unfortunately, none of these approaches is practical for clinical application. Radiotherapy is used in different settings depending on the site of the disease, so the clinical utility of a molecular biomarker signature indicating sensitivity to radiation would vary depending on the clinical application. The development of clinically relevant radiosensitivity molecular signatures is therefore challenging (8).

Recently, Scott and colleagues identified 10 genes that could index radiosensitivity (9). This could allow the radiation dose to be individually tuned to a tumor’s radiosensitivity and provide a framework for designing genomically guided clinical trials in radiation oncology. Being able to increase radiation dosage for more resistant tumors and lower it for more sensitive tumors would also
lower the risk of complications from the therapy. It should be emphasized that tumor genomic data gives information only about a tumor’s intrinsic radiosensitivity. Additional biological insights about the tumor and its microenvironment, as well as information about the patient, are also important for optimizing radiotherapy dosing.

**Combining radiotherapy with targeted therapy**

Biomarkers that can predict tumor sensitivity to therapy are considered the gatekeepers necessary to develop precise and personalized medicine (10). These biomarkers are therapy-specific, and can therefore aid in therapeutic decision-making. For example, mutations in the epidermal growth factor receptor (EGFR) gene have been shown to predict the benefit derived from using tyrosine kinase inhibitors (TKIs), while anaplastic lymphoma kinase (ALK) gene rearrangements have been shown to predict the efficacy of ALK inhibitors in treating NSCLC (11, 12). Building on the preclinical rationale that inhibitors of EGFR function create a strong sensitivity to radiation (13), serial clinical trials have been conducted to test combinatorial treatments using EGFR inhibitors plus radiotherapy. In one pivotal phase 3 trial, adding the chemotherapy drug cetuximab to radiation improved localization of therapy in locally advanced head and neck squamous cell cancer and improved overall survival (14). However, phase 3 trials that evaluated cetuximab in combination with chemoradiotherapy for NSCLC and esophageal cancer all failed to improve overall survival in an unselected patient population (15, 16).

Phase 1 and 2 trials of EGFR TKIs in combination with radiotherapy for locally advanced NSCLC or metastatic NSCLC have shown a favorable safety profile and some encouraging outcomes (17, 18). However, these trials were all performed in patients without information on whether their tumors carry the EGFR mutation, making the results less informative. These studies highlight the need for predictive biomarkers in cases where targeted therapy is combined with radiotherapy.

**Radiotherapy combined with modern immune-targeted therapy**

Understanding of the interaction between the immune system and tumor growth has led to the development of modern cancer immunotherapies. These include cancer vaccines, chimeric antigen receptor T-cell (CAR-T) therapy (in which immune system T cells are reengineered to act against a cancer), and immune checkpoint inhibitors, which interfere with proteins that prevent T cells from responding to cancer. Immune checkpoint inhibitors targeting several types of proteins, including cytotoxic T-lymphocyte antigen-4, programmed death-1 (PD-1), or programmed death ligand-1 (PD-L1), have demonstrated clinical efficacy against a broad spectrum of tumor types—a significant step for both science and medicine (19). Early studies revealed that radiotherapy could provoke an immune response not only at the irradiated site, but also at remote, nonirradiated tumor locations—the so-called “abscopal effect.” Cell death in the irradiated tumor can enhance antitumor immunity by inducing the expression of certain antigens on tumor cells and by activating lymphocytes to attack the tumor. Preclinical and clinical studies have demonstrated the efficacy and safety of radiotherapy combined with immunotherapy (20, 21). Currently, clinical trials of such combined treatments for a variety of tumor types are underway.

Most modern immunotherapies are not yet cost-effective, especially for patients in China, and immune checkpoint inhibitor therapy is not sufficiently precise yet. A crucial step in refining these therapies is the identification of biomarkers that can predict a tumor’s response to checkpoint blockades (22). The overexpression of the PD-L1 antigen, the presence of tumor-infiltrating immune cells, or a variety of molecules in the tumor’s microenvironment may be important predictive biomarkers and are being extensively explored. However, they are not yet sufficiently predictive to allow them to be used to routinely stratify patients. Gene analysis is a new approach for judging the potential clinical benefit of checkpoint inhibitors (23), but further preclinical and clinical studies are necessary before it can be applied in clinical practice. In order to move the strategy successfully into the clinic, it is also critical to clarify the appropriate fractions and doses of radiotherapy and the suitable combinations of radiotherapy and immunotherapy (24).

**Molecular image-guided precision radiotherapy**

Imaging plays a critical role in precision medicine, from screening and early diagnosis to guiding treatment, evaluating responses to therapy, and assessing the likelihood of disease recurrence (25). Rapid advances in imaging technologies permit better anatomical resolution and provide noninvasive measurements of functional and physiological properties of tissues and lesions at the molecular
level. The development and application of molecular imaging techniques brings new opportunities for creating more precise treatment.

Novel molecular imaging approaches are being developed and validated in many critical molecular pathways, such as glucose and amino acid metabolism, cell proliferation, hypoxia, angiogenesis, and receptor expression. The concept of “biological target volume” has been introduced as a factor when determining the intensity of radiotherapy needed for treatment (26). We are looking for the best way to use molecular imaging to guide radiotherapy for certain cancers, either to help define the target volume to be irradiated, or to aid in patient stratification. It was recently reported that an escalated radiation dose to treat a particular type of lung tumor detected by a mid-treatment positron emission tomography (PET) scan allowed clinicians to deliver higher-dose radiation to the more aggressive areas of the tumor and improve local control of tumor growth without increasing radiotherapy-induced lung toxicity (27). PET and computed tomography scans could also identify and delineate hypoxic areas that could be targeted for elevated dosing in lung cancer patients (28).

The role of cancer imaging in precision medicine is being explored from another angle as “radiomics,” which assesses a large number of imaging features that characterize the observable properties of a tumor, using descriptors beyond simply its size to predict clinical outcomes with increased prognostic power, or even correlate with gene expression profiles (29). This approach could be important in helping to stratify patients who are at risk of disease recurrence (30, 31).

In summary, precise radiation therapy is being explored at four different levels: (1) clinical features such as the molecular structure of the tissue, cancer stage, and tumor volume and location(s); (2) adaptive radiotherapy based on images collected during treatment; (3) biomarker-guided therapy; and (4) personalized radiotherapy delivery schedule (32). We believe that with multidisciplinary guidance, the strong support of science and technology, and an eye to cost-effectiveness, precision radiotherapy that incorporates radiobiology, bioinformatics, and molecular imaging will eventually be realized.

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The role of multidisciplinary efforts in precision medicine and immunology for clinical oncology

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Cancer is a group of highly heterogeneous diseases in which dysregulation of key cellular processes leads to neoplastic transformation. Tumor immunity and suppression of the immune response within the tumor microenvironment support its progression. In the past few years, dramatic advances in DNA sequencing technologies have facilitated key insights into the genomic alterations and somatic mutations that enable cancer formation and progression, allowing for significant advances in precision medicine. The broad lexicon of precision medicine describes the narrowing of medical care to the characteristics of an individual patient. It improves upon the current approach of stratifying patients into treatment groups based on only on phenotypic biomarkers, and instead makes use of a patient’s molecular information (including genomics and proteomics) to inform diagnosis, prognosis, treatment, and disease prevention for that individual.

Oncology is at the forefront of precision medicine. It is evolving from the previous model of administering cancer therapeutics through a unified treatment regimen based on cursory tumor classification, to one that applies the precise molecular profile of an individual's cancer genome to optimize personalized treatment. Another new and highly successful therapeutic approach to cancer, immunotherapy (Science’s 2013 Breakthrough of the Year), has created great excitement for clinicians, patients, researchers, and scientific journals. Current strategies in cancer immunotherapy include chimeric antigen receptor (CAR) T-cell therapy, immune checkpoint inhibitors, and cancer vaccines. These treatments have demonstrable clinical benefits and show great promise. However, there are still some challenges that precision medicine and immunology must confront.

For precision medicine the most pressing are:

1. Tumor heterogeneity and molecular evolution

Tumors exhibit tremendous genetic heterogeneity, both among different kinds of tumors and within a single tumor. This creates phenotypic variation, posing a significant challenge to personalized cancer medicine. Intratumor heterogeneity increases the complexity of cancer prognosis and likely contributes to tumor metastasis under therapeutic pressure. Exploring genomic alterations by time series analysis can identify the factors that drive a tumor’s evolution during treatment, which may identify the molecular targets of resistance or tumor progression. Rapid advances in technology for studying tumor heterogeneity would help us understand the tumor genomic landscape. Among such technologies are liquid biopsy assays of circulating tumor DNA, detection and analysis of circulating cancer stem cells, and multiregion next-generation sequencing.

2. Drug resistance

Understanding the clonal make-up of tumors, their molecular evolution, and their response and resistance to drugs poses perhaps the greatest challenge, not only to the application of traditional therapies, but also to personalized cancer medicine. To improve an individual’s treatment response and clinical outcomes, clinicians are obliged to inspect the evolving nature of the cancer genome. Moreover, combinational cancer treatments that target complementary signaling pathways, or harness the immune system through immunotherapy, have promise to overcome resistance while improving efficacy.

3. Shortage of agents that target specific genomic aberrations

Precise targeted therapy, which is based on the genomic characteristics of an individual patient and the mutations identified in their particular cancer, is limited by a shortage of biochemical agents that target specific genomic aberrations. In clinical practice, it is common to detect significant gene mutations in a cancer patient but to have no existing drug that can target those mutations. Or, as also happens, the targeted agent used has little antitumor efficacy. This may be a result of tumor heterogeneity (in which clones of resistant tumor cells survive and proliferate).

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and the variability in the drug’s effect on the targeted genes, or the presence of nonidentical molecular pathways in different patients due to inherent physiological and biochemical differences.

For cancer immunotherapy treatments, the most pressing challenges are:

1. **Lack of a definitive response to treatment**
   In many immunotherapy trials, durable responses and extended, long-term survival are seen only in a subset of patients, and it has been reported in some clinical cases that immunotherapy treatment could even promote cancer progression. These challenges highlight the importance of identifying distinguishing clinical and molecular characteristics that can explain the differential response. Clues to what factors might predict a patient’s response to therapy must be sought, and clinicians must be cognizant of overprescription in refractory patients.

2. **Identification of suitable patients**
   The limitations of immunotherapy approaches have aroused researchers’ attention. CAR-T therapy, for example, has had clinical success against lymphocytic malignancies, but not against more common solid tumors. Even though antibodies that modify immune function can help patients defend against tumors, overall response rates are still relatively low, ranging from 10% to 30%. Thus, precisely identifying which patients are likely to benefit from immunotherapy is an important issue to address. Identifying unique clinical, genomic, and molecular patient characteristics will help researchers identify those factors that might predict response, and presents an opportunity to heighten response in refractory patients. Biomarkers are crucial to identifying suitable patients, but the complexity of the immune system makes its development a challenge.

3. **Improving efficacy using combinational therapies**
   There is considerable current interest in combining treatment modalities to improve the effectiveness of immunotherapies in a broad array of patients. This includes immunotherapies used in conjunction with chemotherapy, radiotherapy, targeted therapy, and even other immunotherapies. But caution is recommended. Our understanding of biological mechanisms leads us to expect additive or synergistic responses from such combined treatments. However, the complexity of the immune response could lead to unexpected, even serious, adverse events. Crucially, immunomodulatory therapies carry distinct risks, including autoimmune reactions that can be fatal if doctors are not alert and ready with proper treatment.

To overcome these tough challenges, precision medicine and immunology for clinical oncology require multidisciplinary efforts. Large-scale genomic data needs to be integrated with clinical data, analyzed, and translated into information that can guide clinical decisions. Multidisciplinary efforts can also ensure the rational application of cancer immunotherapy and combinational therapies by using multi-omics data to improve the diagnosis, prognosis, and treatment of cancer.

The Department of Liver Surgery and the Center for Translational Medicine at Peking Union Medical College Hospital have been focused for several years on researching precision tumor medicine and immunotherapy and their translation to clinical use. As personalized cancer medicine moves to the clinic, conceiving of cancer as a systemic, highly heterogeneous and complex disease becomes even more apt.

Quality cancer care demands that we form a multidisciplinary team (MDT) of highly qualified health care professionals, with medical oncologists at its core.

We see the characteristics of our MDT as including:

1. **A multidisciplinary approach**
   Our MDT members come from specialties including hepatobiliary surgery, radiology, radiotherapy, medical oncology, and pathology. Also on the team are experts in cancer biology and bioinformatics who can help interpret information on genomic aberrations. We strive to translate dispersed knowledge into an integrated, coherent, and personalized treatment regimen.

2. **A patient-centric model**
   Patients’ perceptions of their care are created by the quality of the care, the outcome of the treatment, the empathy displayed by the physician and health care team during their interactions, and each patient’s individual world view. Therefore, the consulting model of our MDT practice is patient-centric. This means that all decisions consider the relationship between the patient, his or her family, and our health care team. We provide face-to-face counseling for every patient, and guarantee no less than a half-hour consultation for deciding on a personalized therapeutic regimen and answering patient questions.

3. **Guideline-first decision making**
   A core principle of our MDT decision-making is “guideline-first,” which means that every decision is evidence-based, and that each patient is treated according to clinically accepted and approved guidelines. The goal is to provide appropriate therapy for cancer patients to prolong their overall survival.
4. **A personalized therapeutic regimen**

Personalizing cancer care implies using MDTs for clinical decision-making, since personalized care requires input from a range of different scientific and care domains. In our teams, clinical information and genomic profiling results are reviewed by professionals to identify clinically significant results. Patients for whom traditional therapies such as surgery, chemotherapy, or radiotherapy have failed, or who have elected to give up such treatments to try targeted therapy, are matched with a targeted therapy or immunotherapy regimen if one is available. These treatments might be administered through a clinical trial or using a drug approved by the China Food and Drug Administration or the U.S. Food and Drug Administration. The team also creates personalized therapeutic regimens using cancer immunotherapy or combinational therapy based on individual clinical characteristics, genomic aberrations, and the specific microenvironment of each tumor.

5. **Public welfare**

The operation of our MDTs is supported by the Chinese Precision Medicine and Immunotherapy for Clinical Oncology Fund, which is a public welfare organization affiliated with the China Social Welfare Foundation. We primarily provide free services to hepatobiliary cancer patients, including multidisciplinary consultation, tumor genomic sequencing, and protein expression analysis to identify immune system biomarkers. Our mission is firstly to raise awareness of both current achievements using targeted therapies and immunotherapy, and of the potential and limitations of these therapies; and secondly to guide patients in seeking out clinical trials where their tumors can be better profiled and they can gain access to novel treatments. We also plan to develop more clinical trials to implement personalized therapeutic regimens.

Despite some obvious challenges, the largely encouraging clinical results from targeted therapeutics and biomarker-guided clinical trials are fueling further technological advancements in next-generation sequencing technology, data interpretation, and the associated preclinical and clinical cycles of drug development. All of this translates into a major shift in clinical practice. Moreover, cancer immunotherapy, especially using single-antibody immune checkpoint inhibitors, has shown promising efficacy against many solid cancers, and significantly improves the response rate against several solid cancers when used in combination with targeted drugs. These advances will improve the survival and quality of life of many cancer patients in the near future.

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**Current status of immunotherapy in advanced HCC**

Shukui Qin*

Primary liver cancer, consisting mainly of hepatocellular carcinoma (HCC), is one of the most common cancers worldwide, with especially high prevalence in China and a growing incidence: 782,000 new cases were reported worldwide in 2012 (1, 2). As one of the leading causes of cancer-related deaths, HCC was responsible for an estimated 746,000 deaths worldwide in 2012 (2). China alone accounted for more than 50% of both new HCC cases and HCC-related deaths globally (3). Chronic infection due to hepatitis B virus (HBV) or hepatitis C virus (HCV) contributes to an estimated 75% of all HCC cases (4). In the Asia-Pacific region, more than 75% of cases are associated with HBV infection (5).

Surgical resection or orthotopic liver transplantation (OLT) offer the best chance for successful treatment of HCC (6). However, surgical resection, liver transplantation, and radiofrequency ablation (RFA) are only applicable to a small portion of patients with well-preserved liver function who have early-stage or localized HCC (7). Due to difficulties in early diagnosis, most HCC cases are locally advanced or show distant metastases at the time of diagnosis. These patients generally have a poor prognosis, with median survival of 6 to 20 months (8), and a five-year survival rate of less than 16% (9).

For local advanced or metastatic HCC, systemic therapy is often used as an important palliative treatment. Various conventional systemic chemotherapy regimens such as doxorubicin have been used clinically, although there are few well-controlled studies demonstrating the efficacy of systemic chemotherapy in the management of HCC (10). In 2012, our group was the first to demonstrate that a regimen of “FOLFOS” (oxaliplatin, 5-fluorouracil, and leucovorin) significantly improved the objective response rate (ORR) and prolonged overall survival (OS) compared with doxorubicin alone in a randomized phase 3 clinical trial in Chinese HCC patients (EACH

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study) (11). Based on this trial, the FOLFOX4 regimen was approved by the Chinese Food and Drug Administration (CFDA) for use in first-line treatment of advanced or metastatic HCC patients in China.

Besides chemotherapy, molecular target therapies have also been evaluated in clinical practice globally. In 2007, sorafenib, a tyrosine kinase inhibitor against multiple targets including RAF, vascular endothelial growth factor receptor, and platelet-derived growth factor receptor, was shown to extend median overall survival in patients with advanced HCC, and was approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as a first-line treatment for advanced HCC. However, its use in the clinic has been limited due to low ORR, limited survival benefit, toxicity, and cost. After sorafenib, a series of molecular-targeted drugs including sunitinib, brivanib, lapatinib, linifanib, everolimus, axitinib, pazopanib, tivantinib, and ramucirumab were studied. Unfortunately, all failed in phase 3 trials. Additionally, trials combining either sorafenib with erlotinib or sorafenib with transarterial chemoembolization were also unsuccessful. In the decade since the approval of sorafenib, only two phase 3 studies, both involving multikinase inhibitors, generated positive results: regorafenib versus placebo as a second-line treatment (RESORCE study, ClinicalTrials.gov: NCT01774344) (12) and lenvatinib versus sorafenib as a first-line treatment (REFLECT study, ClinicalTrials.gov: NCT01761266) (13). Most recently, the U.S. FDA approved regorafenib for advanced HCC patients who have been previously treated with sorafenib. Lenvatinib is currently under regulatory review by FDA and EMA for first-line treatment of advanced HCC patients.

Although there are a growing number of molecular target therapies and a few cytotoxic drugs under rapid development, clinical outcomes have been unsatisfactory to date. There is a great unmet medical need for novel therapies with better response rates and survival.

**Immunotherapies for HCC**

Tumor immunotherapies, especially immune checkpoint inhibitors, have recently been established as effective treatments for several types of cancers. Immune checkpoint inhibitors release the “brakes” on the immune system and restore the ability of immune cells to eliminate cancer cells. Several immune checkpoint targets such as programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4) are being investigated extensively in various types of cancers. In the past few years, many clinical studies have shown immunotherapy to be a promising treatment option for many solid tumors and hematologic malignancies. Some of these agents are now approved for the treatment of melanoma, non–small cell lung cancer, renal cell carcinoma, head and neck cancer, bladder cancer, liver cancer, gastric cancer, mismatch repair (MMR)-deficient cancer, Merkel cell carcinoma, and Hodgkin’s lymphoma.

The human liver has a unique immunobiology whereby multiple regulatory mechanisms are in place to maintain an immunosuppressive environment. Normal liver tissue is inherently tolerogenic to environmental autoantigens and toxins in order to prevent aberrant immunity to pathogens encountered through arterial circulation and from the gut (14, 15). Clinical and nonclinical data showed increased numbers of immunosuppressive cells in HCC, including regulatory T cells and myeloid-derived suppressor cells, as well as increased expression of inhibitory signaling molecules such as CTLA-4 and PD-1 (16–18). HBV and HCV infections have also been associated with the increased proliferation of regulatory T cells and upregulation of PD-L1/PD-1 expression, thereby suggesting a role for this pathway in HBV or HCV-mediated hepatocarcinogenesis (15, 18–21). Overexpression of PD-L1 or PD-L2 has also been shown to be associated with tumor aggressiveness, disease progression, and high mortality in HCC patients (17, 22). Therefore, therapeutic agents that target immune checkpoints may potentially improve clinical outcomes by reversing the immunosuppressive nature of the HCC tumors and stimulating host immunity against the tumor cells. Interestingly, encouraging antitumor activity has now been reported in several clinical studies with anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies.

Tremelimumab is a fully humanized immunoglobulin G2 (IgG2) monoclonal antibody that blocks CTLA-4. In a phase 2 study reported in 2013, of 21 HCV-positive patients, 17 were assessable for tumor response (23). The tumors shrunk or disappeared in three patients (17.6%), and the disease control rate was 76.4%, with a median time to progression of 6.48 months. This study provided the first evidence of the antitumor activities of immune checkpoint inhibitors in HCC, and greatly encouraged further investigations and clinical trials. Several anti-PD-1 antibodies are currently being evaluated in HCC patients globally. In September
2017, the U.S. FDA approved nivolumab for the treatment of patients with HCC who have been previously treated with sorafenib. Accelerated approval for this indication was granted based on the tumor response rate and durability of response observed in the CheckMate-040 trial (24). In this trial, 14.3% [95% confidence interval (CI): 9.2–20.8; 22/154) of patients responded to treatment with nivolumab. The percentage of patients with a complete response was 1.9% (3/154), and the percentage with a partial response was 12.3% (19/154). Among responders (n=22), duration of response ranged from 3.2 to 38.2+ months; 91% of those patients had responses lasting six months or longer and 55% had responses lasting 12 months or longer. The median time to response was 2.8 months (range: 1.2–7.0). The overall response rate (based on modified RECIST criteria) was 18.2% (95% CI: 12.4–25.2; 28/154). Complete response rate was 3.2% (5/154) and partial response rate was 14.9% (23/154), also based on modified RECIST criteria. A phase 3 randomized trial (CheckMate-459) is now ongoing to assess the clinical activity of nivolumab versus sorafenib in first-line HCC treatment (ClinicalTrials.gov: NCT02576509) (25). Another anti-PD-1 antibody, pembrolizumab, is also under investigation for treatment of HCC patients. The KEYNOTE-240 study is a phase 3 study to assess pembrolizumab versus placebo plus best support care as a potential second-line therapy in patients with previously systemically treated advanced HCC (ClinicalTrials.gov: NCT02702401) (26). This study is currently ongoing.

In addition to anti-PD-1 antibodies, anti-PD-L1 antibodies are also being evaluated in HCC patients. A phase 1/2 clinical trial of durvalumab in predominantly sorafenib-pretreated HCC patients showed an ORR of 10%, with a median OS of 13.2 months and a well-tolerated safety profile (ClinicalTrials.gov: NCT01693562) (27). A separate phase 1/2 study of durvalumab in combination with tremelimumab in unresectable HCC showed an ORR of 25%, with no unexpected safety signals (ClinicalTrials.gov: NCT02519348) (28). Overall, the trial demonstrated that a regimen of durvalumab plus tremelimumab was well-tolerated in this unresectable HCC patient population.

Many combinations of immunotherapies with molecularly targeted therapies are being evaluated in advanced HCC patients. Examples include pembrolizumab/lenvatinib (ClinicalTrials.gov: NCT03006926) (29), nivolumab/galunisertib (ClinicalTrials.gov: NCT02423343) (30), nivolumab/yttrium Y 90 glass microspheres (ClinicalTrials.gov: NCT02837029) (31), and nivolumab/cabozantinib (ClinicalTrials.gov: NCT01658878) (32).

In addition to checkpoint inhibitors, chimeric antigen receptor T cell (CAR-T) therapy has also made significant progress in the field of cancer immunotherapy. Remarkable clinical outcomes have been shown with CAR-T treatment, especially CD-19–directed CAR-T in various hematologic malignancies (33). However, to date, the clinical activity of adoptive CAR-T treatment in solid tumors has been less impressive. In HCC, CAR-T therapies targeting various antigens, including CEA, MUC1, GPC3, EGFR, EpCAM, and CD133, are under investigation in early stage clinical trials (34).

**Immunotherapies for HCC in China**

HCC remains a cancer with a high mortality rate and a clear unmet medical need in China, despite the fact that multiple HCC prevention programs supported by the Chinese government have been in place for many years (35, 36). Based on data retrieved from the China National Central Cancer Registry, estimated new cases of liver cancer numbered about 356,000 in China in 2011, and the incidence rate was 26.39 per 100,000. There was also an increasing trend in the incidence rate of liver cancer in China from 2000 to 2011. As a result, the burden of liver cancer is still very high in China (37). Development of effective therapies for HCC patients remains a big challenge and an important unmet medical need.

With the boom in the Chinese biotech industry over the past five years, several China-based biopharmaceutical companies have been actively developing innovative immuno-oncology drugs for the treatment of cancers, including HCC. BGB-A317, developed by BeiGene, is an anti-PD-1 antibody engineered to minimize Fc-gamma receptor binding on macrophages, with the aim of abrogating antibody-dependent phagocytosis, a potential mechanism of T-cell clearance. Results from its ongoing phase 1 study were recently reported (38). At the time of data cutoff, 40 patients with unresectable HCC had been enrolled. A majority (85%) of these patients were infected with HBV. The median treatment duration was 64 days, with a range of 1 to 471 days. BGB-A317 was well tolerated: Twenty evaluable patients remained on the treatment at the data cutoff date; partial responses were observed in three patients (all HBV-positive) and nine patients achieved stable diseases, some of whom also had significant reductions in α-fetoprotein (AFP) levels, which indicates a positive
treatment response. Based on these initial results, a randomized, open-label, multicenter phase 3 study has been initiated to assess the efficacy and safety of BGB-A317 versus sorafenib in first-line patients with unresectable HCC.

In addition, SHR-1210, a humanized anti-PD-1 IgG4 antibody developed by Jiangsu Hengrui Pharmaceutical Co., is under investigation either as a single agent or in combination with apatinib or FOLFOX4 in HCC. There are also at least 15 other anti-PD-1 and anti-PD-L1 antibodies from different pharmaceutical companies that have either received authorization from CFDA or are currently under review to initiate clinical studies in China (39).

Glypican 3 (GPC3), a member of the glypican family of heparan sulfate proteoglycans, is highly expressed on the surface of liver cancer cells with minimal expression on normal tissues (40). A clinical trial was initiated by Chinese investigators from Renji Hospital in collaboration with CARsgen Therapeutics to investigate GPC3-targeted CAR-T in Chinese patients with GPC3-positive refractory or relapsed HCC. Its preliminary phase 1 results showed that GPC3-targeted CAR-T was well tolerated in 13 Chinese patients with GPC3-positive HCC (41). Of these 13 patients, eight were treated with lymphodepleting conditioning (LDC) at baseline. Among these eight patients, one partial response, three showed stable diseases, two had progressive diseases, and two were unevaluable. Taken together, GPC3-positive CAR-T therapy showed encouraging preliminary clinical activity in Chinese patients with advanced HCC.

Conclusions

HCC remains one of the most devastating malignancies in the world, and a disease with significant unmet medical need, particularly in China. Immunotherapies, especially immune checkpoint inhibitors, have demonstrated preliminary but promising clinical activity in patients with HCC, and several large randomized phase 3 clinical trials of these treatments are currently underway. Despite the limited progress and challenges in the development of HCC treatment in the past decades, there is hope on the horizon. With the joint efforts of pharmaceutical companies and academic institutions, we look forward to the continuous development of safer, more effective treatments for patients with advanced or metastatic HCC.

References

Challenges and prospects for precision cancer immunotherapy in China

Zhihao Lu, Jianling Zou, Shuang Li, and Lin Shen*

With increasing incidence and mortality, cancer has become a major public health problem in China (1). Surgery, radiotherapy, chemotherapy, and targeted therapy remain standards of care for cancer patients. However, the effectiveness of these treatments is not completely satisfactory. The success of immunotherapy, especially anti-PD-1/PD-L1 treatment, which blocks the ability of tumor cells to shield themselves from attack by the immune system, has led to some important strides in cancer care in recent years. At the same time, immunotherapy has become a hot area in cancer research and treatment in China. Here, we discuss the challenges and prospects for precision immunotherapy in the treatment of cancer, based on the unique characteristics of the Chinese population.

Current status of cancer immunotherapy

Chinese pharmaceutical companies and researchers are highly motivated to explore new immunotherapy agents and therapies. Even though no PD-1 or PD-L1 inhibitor has yet been approved by the Chinese Food and Drug Administration (CFDA), the Chinese government is making earnest efforts to improve the overall clinical research environment, and the CFDA is actively reforming the regulatory framework to accelerate approval of novel agents (2). By July 2017, Chinese pharmaceutical companies had developed 10 anti-PD-1/PD-L1 inhibitors, eight of which have been approved by CFDA for phase 1 to phase 3 clinical trials in patients with advanced solid tumors (Table 1). In November 2016, clinical trials of PD-1 inhibitor KN035 were approved by the U.S. Food and Drug Administration (FDA) (3). In the past five years, around 1,000 international clinical trials of PD-1/PD-L1 therapies for solid tumors have been registered in the ClinicalTrials.gov database, of which 100 involved Chinese sites (2). Thus, Chinese...
Clinical trials are developing rapidly and catching up with those in other parts of the world. However, there are some problems with the current situation. The promise of these drugs is leading to a crowded market. Numerous companies are jockeying for position, while many others are hoping for profitable out-licensing deals. All of this makes quality control a serious challenge. Furthermore, whether in pharmaceutical development or regimen design, China has been producing too many “me-too” drugs—innovation has been lacking. Finally, because of overreaching pragmatism and the current system for evaluating research (which places great importance on publication numbers), it is possible that applied research could impede the progress of pure science.

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**TABLE 1.** PD-1/PD-L1 blockades in clinical trials in China. NA, none available; IND, investigational new drug.
Challenges facing cancer immunotherapy

Anti-PD-1/PD-L1 treatment is one of the most successful immunotherapies against solid tumors, but it faces many difficulties in China. Several of these relate to biomarkers:

Lack of predictive biomarkers for treatment response

Multiple tumor types have been shown in early phase trials to have a long-lasting response to anti-PD-1/PD-L1 treatment (4, 5). However, the overall response rate to this treatment is only 10%-44% (6, 7). Therefore, better biomarkers are needed to guide patient selection and provide early indicators of treatment response.

Lack of predictive biomarkers for adverse events

Adverse effects, including lethal complications, have been reported during anti-PD-1/PD-L1 treatment (8). Most clinical trials focus on potential predictive biomarkers of treatment response, but no reliable biomarkers for adverse events have been identified. However, they are equally necessary in the clinic.

Lack of predictive biomarkers for hyperprogressive disease

Champiat and colleagues reported for the first time instances in which a cancer progressed unusually rapidly in patients receiving immune checkpoint inhibitors like anti-PD-1/PD-L1, described as “hyperprogressive disease.” The incidence varied from 9% to 29% (9, 10) of patients receiving the immune checkpoint therapy. As this study indicates, it is necessary to identify biomarkers for hyperprogression in order to selectively avoid those patients who might be harmed by immune checkpoint inhibitors. As of now, unfortunately no such explicit biomarkers have been found.

In addition to these difficulties, Chinese patients have some special characteristics that need to be considered in designing immune therapies for them. They have relatively high rates of hepatitis B virus infection, for example, and more frequently use Chinese traditional medicine. Chinese patients also have more “driver gene” mutations that are linked to cancer development, better clinical responses to chemotherapy, and different toxicity profiles (11-14), which may have an effect on anti-PD-1/PD-L1 therapy. These factors have rarely been considered in trial designs. Furthermore, Chinese patients have limited access to immune checkpoint inhibitors such as antibodies against PD-1 and PD-L1, so there has been only a small patient pool in which to explore biomarkers in the Chinese population.

Toward precision cancer immunotherapy in China

Because of the dynamic nature of the immune system and the multiple elements involved in the complex immune response to cancer, it is much more challenging to develop biomarkers for immunotherapy than for other treatments. Thus, it is imperative to establish a paradigm for precision immunology to steer patients through immunotherapy. Some strategies for precision cancer immunotherapy include the following:

1. Mechanism-based predictive biomarkers for patient selection

Mismatch repair deficiency

DNA mismatch repair (MMR) describes a specific genetic aberration that results in a many-fold increase in tumor mutational burden in various tumor types and has been explored for responsiveness to anti-PD-1 (15). The high level of microsatellite instability (MSI-H)—a predisposition to mutation that is characteristic of MMR-deficient (dMMR) cancers—or the absence of one of the MMR proteins, is used to identify this genetic subset of patients.

In 2017, the U.S. FDA granted accelerated approval to pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors that have been identified as MSI-H or dMMR. The approval covers patients whose disease has progressed after prior treatment and who have no satisfactory alternative treatment options. However, only about 4%-5% of patients with advanced solid tumors have been identified as MSI-H or dMMR, while their therapeutic response is approximately 40% (16). Thus, further exploration of novel biomarkers is needed to refine anti-PD-1 treatment strategies.

PD-L1 expression

The protein PD-L1, detected on patient tumor cells using immunohistochemistry (IHC), is currently the most common clinically detected biomarker for predicting patient response to anti-PD-1/PD-L1 therapy (17).

A recently published randomized phase 3 trial in advanced non–small cell lung cancer (NSCLC) revealed a correlation between high PD-L1
expression and longer progression-free survival and overall survival in immunotherapy treatment regimens (18). Nevertheless, a recent review of immune biomarker utility in NSCLC therapy indicated a lack of consensus on how to use PD-L1 expression as a biomarker to help in selecting treatment regimens. In fact, patients with little or no detectable expression of this biomarker can also benefit from anti-PD-L1 therapy (19). A similar scenario has been observed in other tumors such as gastric cancer (20), renal cell carcinoma (21), and urothelial cancer (22). However, due to the complexities of PD-L1 IHC, further studies are needed to carefully validate these observations and other characteristics of the tumor microenvironment.

**Mutational landscape and mutational burden**

Various studies have shown how the number of mutations in a tumor’s genome—the mutational burden—correlates with greater efficacy of diverse anti-PD-1/PD-L1 drugs (23, 24). Analyzing the genetic signatures of tumors might therefore identify patients who have a better chance of responding to checkpoint inhibition (25). NSCLC patients with a high nonsynonymous mutational burden showed long-lasting clinical benefits from treatment with pembrolizumab. Furthermore, another study showed that almost all patients with a low nonsynonymous mutational burden did not benefit from the same treatment (24). These results make the mutational landscape of the tumor one of the most promising predictive factors both at the beginning of treatment and after an initial favorable response.

**Other predictive biomarkers**

It is well known that there is a relationship between inflammatory cells, cancer, and proinflammatory proteins such as chemokines. Some findings demonstrate that the genetic signatures of genes related to the cytokine interferon γ appear to be reasonable tumor biomarkers that could soon be incorporated into predictive models of how patients will respond to checkpoint inhibitors (26, 27).

Recent studies have also shed light on the previously unknown effect of the gut microbiota on immunotherapy for patients being treated with checkpoint blockade inhibitors (28, 29). Future studies of the gut microbiota of cancer patients who respond to checkpoint inhibitors could provide invaluable information to aid in patient selection.

Findings from 2017 show that CKLF-like MARVEL transmembrane (CMTM) domain-containing proteins CMTM6 and CMTM4 are associated with the PD-L1 protein and enhance the ability of tumor cells that produce PD-L1 to inhibit T cells, suggesting a potential therapeutic target in the effort to overcome immune evasion by tumor cells (30, 31).

Results of completed trials indicate that numerous biomarkers that predict toxicity to antibodies against immune checkpoint inhibitor cytotoxic T-lymphocyte antigen 4 (CTLA-4) should be considered for further evaluation as an anticancer treatment in larger prospective trials. Of these promising biomarkers, neutrophil activation markers and several immunoglobulin genes warrant further investigation as biomarkers predictive of toxicity to anti-CTLA-4 treatment. There is currently no predictive biomarker for toxicity to anti-PD-1 treatment. Evaluating the gene expression profile of patients who develop toxicities to anti-PD-1/PD-L1 treatment may also be an interesting landscape to explore in the future.

2. Predictive biomarkers related to hyperprogression

Champiat and colleagues at Université Paris Saclay found a greater incidence of hyperprogression in patients 65 years old or older than in those younger than 65 (9). That may be because the functioning of immune cells, chemotaxis, phagocytosis, and intracellular killing of pathogens is decreased in older patients (32), but the mechanism by which this happens is not clear. Saada-Bouzid and colleagues (10) found that hyperprogression correlated more significantly with the presence of a recurrence near the original tumor than with a recurrence farther away (90% versus 37%) in patients with recurrent or metastatic head and neck squamous cell cancer. Kato and colleagues (33) investigated potential genomic biomarkers associated with hyperprogression after immunotherapy. Their results showed that murine double minute 2 (MDM2) family amplification or epidermal growth factor receptor (EGFR) alterations were correlated with hyperprogression in patients previously treated with PD-1/PD-L1 inhibitor, but not CTLA-4. However, the exact mechanism linking MDM2 family amplification and hyperprogression is unclear. Pretreatment and early postimmunotherapy biopsies would be valuable in exploring the underlying mechanism in the future.
3. Combination Immunotherapy

In recent years, there has been an increase in the number of novel combination therapies used in cancer immunotherapy treatments. The goal of combination approaches is to expand the spectrum of patients who would benefit from immunotherapy, to enhance its curative effects, and to reduce the number of side effects compared with single therapy regimens (Figure 1).

Several hundred clinical trials are currently exploring the effects of anti-PD-1/PD-L1 used in combination with experimental immune modulators, such as other monoclonal antibody checkpoint inhibitors, cancer vaccines, epigenetic drugs, and drugs targeting metabolic pathways, and also with surgery, radiotherapy, and chemotherapy. Preliminary evidence [for example using a combination of PD-1 and CTLA-4 in treatment of melanoma (34) and NSCLC (35)], has highlighted the potential to enhance the clinical benefits of monotherapies by combining them with agents that have synergistic mechanisms of action. Initial analyses from the international, multicohort, phase 2 KEYNOTE-059 study showed that objective response rate (includes both partial and complete responses) was 60% across all patients (36).

Despite the promise of combination therapies, several issues need to be further investigated:
1. **Combination agents.** Although there are dozens of clinical trials of immune modulators used in combination with anti-PD-1/PD-L1 treatment, there is little research in China on the rationale, dosages, schedules of treatment, and configuration of the specific combinations used.

2. **Predictive biomarkers for combination immunotherapy.** Although studies have found the rate of response to combination therapies to be much higher than that of monotherapies, there are still a proportion of patients who do not survive. Finding predictive biomarkers for combination immunotherapy is therefore of prime importance, and will probably require dynamic monitoring of the immune response and a fuller understanding of the changes in tumor microenvironments before, during, and after treatment.

3. **Predictive biomarkers for adverse events.** Combination immunotherapy usually brings more instances of adverse events. In the KEYNOTE-059 study, grade 3–4 treatment-related adverse events (TRAEs) including neutropenia, stomatitis, anemia, thrombocytopenia, anorexia, and fatigue, occurred in 76% of patients—a much higher frequency than with chemotherapy or targeted therapies. We should therefore pay more attention to TRAEs associated with combination therapy, and attempt to identify novel predictive biomarkers for them.

**Conclusions**

The ultimate goal of precision immunology for cancer is to select a subset of patients with a common biological basis of cancer: the patients who are most likely to benefit from a particular immunotherapy. Hopefully, with the development of new technologies to dynamically monitor the cancer–immune system interaction and by considering the special features of different populations, precision cancer immunology will be applied more widely, improving diagnosis, stratification, and treatment of cancer patients in China.

**References**

The rise of engineered T-cell therapy in China

Jianshu Wei¹, Yi Zhang²³*, and Weidong Han⁷

There are many treatment methods for controlling or eliminating cancer cells, including tumor vaccines, chemotherapy, radiation therapy, and tyrosine kinase inhibitors. The advancement of basic research into the progression of malignancies has resulted in significant improvements in treatment. However, complete and durable remission for inoperable malignancies remains rare. There is a tremendous effort underway to find novel therapies that can offer better prospects for destroying tumors.

Recently, immunotherapy as a tumor treatment has come to the forefront because of the significant successes of therapies using immune checkpoint antibodies and chimeric antigen receptor modified T (CAR-T) cells. Simply speaking, immune checkpoint antibodies fight tumors by activating the naturally occurring lymphocytes in the body that can recognize and kill the tumor cells. CAR-T cells are the patient's own cells educated in vitro by genetic manipulation to kill tumor cells. The immune system is so sophisticated that the mechanisms underlying cancer immunology are far from fully explained, although there has been steady progress over the past 30 years. As clinical applications have developed, T cell–centered cancer immunotherapy has proven to be an effective antitumor treatment.

In 2017, the U.S. Food and Drug Administration (FDA) approved the drug CTL019 (tisagenlecleucel-T, Novartis), the first CAR-T treatment to get the regulatory nod. It was a milestone in tumor immunotherapy, marking the start of the CAR-T era. With strong support from the government, CAR-T therapies are developing very rapidly in China, where the number of registered clinical trials recently surpassed that in the United States.

Cancer immunotherapy has been in clinical use in China since before 1988, in the form of a method using lymphokine-activated killer (LAK) cells. LAK cells are white blood cells stimulated to grow in vitro and cultured in the presence of interleukin 2 (IL-2). In 1988, Rosenberg and colleagues first reported a method for preparing LAK cells (1). Since then, LAK cells have been used to treat multiple types of tumors (2).

In 1991, Schmidt-Wolf reported on cytokine-induced killer (CIK) cells (3). CIK cells were more cytotoxic than LAK cells and exhibited better proliferation efficiency. Several clinical trials on CIK cells have been conducted since then. In China, the first research article on the subject was published in 2002 (4). Subsequently, CIK therapy has been widely utilized for a variety of reasons, including its safety, relative technical simplicity, and the support it received from the government. During this period, other treatments using natural killer (NK) cells and dendritic cell–activated CIK cells (DC-CIKs) were also in clinical use, providing a solid foundation for the development of newer CAR-T treatments. In general, the tumor-killing cells utilized during that time were natural lymphocytes—small white blood cells—with no modifications, and the killing effect was weak and depended largely on nonspecific tumor cell recognition mechanisms. As a result, the overall clinical benefits were minimal.

To improve the clinical efficacy of these therapies, Chinese scientists have tried a variety of ways to increase the number of T lymphocytes that recognize a specific tumor, of which CAR-T technology is undoubtedly the most attractive. The generation of the first CAR molecule was reported in 1989 by Gideon Gross (5). This first-generation CAR-T showed poor activity in phase 1 clinical trials. The first domestic research using improved second-generation CAR-T was published in 2009, and involved a human epidermal growth factor receptor 2 (HER2)-specific CAR that was designed for the treatment of breast cancer (6). The first article on CAR-T clinical trials in China was published in 2014 by the Han laboratory of the People’s Liberation Army General Hospital (PLAGH) (7). With the government restricting the clinical use of CIK and similar minimally successful immune therapies, the use of natural lymphocytes gradually diminished, and China entered a new era of precision immunotherapies, exemplified by CAR-T.

By early 2015, 12 clinical trials of CAR-T in China were registered on the ClinicalTrials.gov website, of which eight were conducted by PLAGH, two by Beijing Cancer Hospital, and one each by Renji Hospital and the 307 Hospital of the People’s Liberation Army. By January 2016, the total number of trials had risen to 26. As of September 2017, 121 CAR-T trials have been registered by domestic

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hospitals, academic institutions, and private companies, and China has become the country with the most registered CAR-T trials worldwide.

Of the 121 registered trials, 53 are CAR-T therapies targeting CD19 for treatment of leukemia, and 44 of these are still ongoing. CD19 is a membrane protein found on all B cells and critical for B-cell activation and proliferation. Generally speaking, acute lymphoblastic leukemia (ALL) is most responsive to this therapy, known as CART-19. Of the nine patients enrolled in the first domestic CART-19 trial saw complete remission (CR) for two to nine months (8), and cytokine release syndrome (CRS) was observed in most cases. CRS occurs when large numbers of white blood cells release proinflammatory cytokines, which go on to activate more white blood cells, creating a potentially fatal cascade effect called a “cytokine storm.” Two patients who had previously received hematopoietic stem cell transplantation (HSCT) presented with grade 2–3 graft-versus-host diseases (GVHD) after infusion of donor-derived CART-T cells, suggesting that HSCT-treated patients without 100% chimerization (i.e., in which the hematopoietic system was not completely reconstructed from donor-derived bone marrow cells) had a higher risk of GVHD when receiving donor-derived CAR-T cells. Overall, the rate of complete remission is a bit lower than that seen in results obtained at the Memorial Sloan Kettering Cancer Center (MSKCC), the University of Pennsylvania, and Novartis. It is worth noting that the patients recruited in this trial were more aggressively treated and in later stages of disease than the participants in the United States. Results from several subsequent domestic CART-19 trials have shown that overall CR rates were comparable to those in the United States. For example, the First Affiliated Hospital of Zhejiang University enrolled 15 patients with relapsed or refractory (R/R) ALL (9), 12 of whom had CR responses one month after CART-19 infusion.

Relapse is the major obstacle to long-term survival of patients hoping for complete remission. Researchers in China have been exploring ways to avoid relapse. The first option is to use bispecific CAR-T cells that are active against two different antigens. A group from the Southwest Hospital in Chongqing has registered a trial in which CART-19 and CART-20 (targeting CD-20) will be infused sequentially into patients with diffuse large B-cell lymphomas (DLBCL). And the PLAGH group has carried out two trials in which tandem CART-19/20 and tandem CART-19/22 were used for patients with R/R B-cell malignancies. Another common approach is to initiate HSCT treatment after obtaining a CR response using CAR-T therapy. A group at Lu Daopei Hospital in Beijing has treated 27 patients with this regimen (10). Of the 27 CR patients treated with allogeneic HSCT following CART-19, 23 remained disease-free for at least 45 days (median follow-up time was 206 days; range was 45–427 days). This suggested that further use of allogeneic HSCT could effectively reduce the risk of relapse. The Southwest Hospital group has also registered a trial in which HSCT will be given after CART-19 treatment for patients with B-cell malignancies. In collaboration with PLAGH, a team led by Huisheng Ai launched the world’s first treatment to use coinfusion of haploidentical donor-derived CART-19 cells and mobilized peripheral blood stem cells (PBSCs)—stem cells stimulated to move from the bone marrow into the bloodstream—for a 71-year-old female with R/R ALL (11). Any residual disease was undetectable following treatment, and CRS and GVHD were controlled. This result suggested that coinfusion of allogeneic CAR-T cells and PBSCs may induce full donor engraftment in R/R ALL, offering a novel option for combining CAR-T and HSCT.

CD20 and CD22 have expression patterns similar to those of CD19, which are expressed in all B lymphocytes except B progenitor cells, making these proteins potential targets for managing B-cell malignancies. At present, there are five CD20 and six CD22 CAR-T trials registered in China. The initial article on a CD20 CAR-T trial was published in 2014 by researchers at PLAGH (7), being the first to report results of CART-20 therapy for DLBCL. Seven patients with DLBCL were treated with CART-20 cells, and five of the six evaluable patients showed an objective remission response (ORR).

ALL and B-cell lymphoma are the cancers most sensitive to CAR-T therapy, so they are prime candidates for further research, both in China and abroad. But China’s research groups have also done pioneering work on other types of blood-based malignancies.

Multiple myeloma (MM) is a fatal plasma cell cancer and the second most common hematological malignancy. Typically, CD19, CD20, and CD22 are not detectable in MM. Clinical results have suggested that CD138, a transmembrane proteoglycan found in certain hematopoietic cancers and carcinomas, may be an effective target for refractory and progressive MM. In 2015, researchers at PLAGH reported the first results of CART-138 therapy on MM. To date, five patients have been treated, and in four the disease was stabilized for more than three months, while one patient with progressive MM had a significant reduction in myeloma cells in peripheral blood—from 10.5% to less than 3% (12). This study
suggested that CART-138 could be a promising approach for treating MM. There are three CART-138 clinical trials presently registered in China.

B-cell maturation antigen (BCMA), a protein reported to be expressed in B cells, including MM cells, is another potential target for MM treatment. At the 2017 conference of the American Society of Clinical Oncology (ASCO), Nanjing Legend Biotech Co., Ltd. presented data from their trial of BCMA-targeted CAR-T therapy (CART-BCMA) in 35 participants with R/R MM. Thirty-three evaluable patients saw significant clinical responses (defined as a complete response or a very good partial response) within two months following CAR-T infusion. The overall ORR was as high as 100%. Four CART-BCMA clinical trials are currently registered in China.

Acute myeloid leukemia (AML) is the most common acute leukemia affecting adults (13), and CD33 antigen is expressed on leukemic cells in more than 90% of patients with the disease (14). In 2015, a clinical trial was conducted at PLAGH to assess the feasibility and efficacy of CART-33—CAR-T therapy targeting CD33—for the treatment of R/R AML. A marked decrease of myeloid blasts in the bone marrow two weeks after cell infusion was observed, but the disease progressed after nine weeks (15). This was the first demonstration of the potential of CART-33 for the treatment of AML. Five clinical trials for treating AML with CART-33 are currently registered in China.

In Hodgkin’s lymphoma (HL), the CD30 protein is a potentially potent target. This year, the first phase 1 clinical trial results on CART-30—CAR-T therapy directed at CD30—were reported by PLAGH researchers. Eighteen patients with progressive R/R HL were enrolled. The data showed that the treatment was well tolerated without severe toxicity. Seven patients achieved partial remission and the disease was stabilized in six patients (16). There are now four CART-30 clinical trials underway in China.

Compared with the performance of CAR-T seen with hematologic malignancies, there is general pessimism about the prospects of this therapy for treatment of solid tumors. Chinese researchers have nonetheless pursued this possibility with enthusiasm and have made many original contributions. Currently, 44 clinical trials of CAR-T cell treatments of solid tumors have been registered in China, covering 14 different targets.

Among them, the glypican-3 (GPC3) protein has attracted the most interest, with nine trials underway. GPC3 is generally overexpressed in multiple solid tumors, including lung cancer and hepatocellular carcinoma (HCC) (17, 18). A group led by Zonghai Li from Renji Hospital has done several preclinical studies to verify the feasibility of CART-GPC3 in the treatment of lung cancer and HCC (18, 19).

Epidermal growth factor receptor (EGFR) is a glycoprotein that is overexpressed in many malignancies that derive from the epithelium. These include non-small cell lung cancer (NSCLC) and multiple digestive tract cancers (20). A preclinical study of CART-EGFR was carried out in 2013 by Yuquan Wei’s laboratory at Sichuan University, which provided an experimental basis for further clinical study (20). The first CART-EGFR clinical results were published by a group at PLAGH in 2016. Patients with EGFR-positive (>50% expression) R/R NSCLC received escalating doses of CART-EGFR. Among 11 evaluable patients, two achieved partial remission and five had a stabilized disease response for two to eight months. Significantly, eradication of EGFR-positive tumor cells was observed in tumor biopsies after the CAR-T infusion, providing the most direct evidence supporting the effectiveness of this treatment (21). However, this result also emphasized that the heterogeneity of solid tumors may present a formidable obstacle to the CAR-T treatment. Six CART-EGFR trials have been registered in China as of now.

Several other domestic clinical trials of CAR-T treatment for solid tumors have been published. In 2016, a group headed by Lin Yang published a case report on CAR-T therapy targeting mucin 1 (MUC1) for a patient with MUC1-positive metastatic seminal vesicle cancer in which significant tumor necrosis was observed (22). In 2017, a group at PLAGH published results from a phase 1 clinical trial using CAR-T cells to target HER2 in patients with advanced biliary tract cancers and pancreatic cancers (23). Cheng Qian’s group published results of a phase 1 trial of CAR-T cells targeting carcinoembryonic antigen (CEA) therapy for patients with CEA-positive metastatic colorectal cancers (24). Data from all of these studies demonstrated the safety and feasibility of CAR-T therapy for a variety of cancers.

What can be done to provide patients with greater clinical benefits following CAR-T treatment? To answer this question, Chinese research groups have done some pioneering work. For example, cancer stem cells (CSCs) are believed to play an important role in tumor growth and metastasis. Theoretically, CAR-T therapy targeting CSCs might effectively restrain tumor metastasis. The antigen CD133 is thought to be a specific marker for CSCs, and is also expressed by various carcinomas including cholangiocarcinoma (CCA), a cancer of the bile ducts. A 2017 article reported on a 52-year-old}

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**PRECISION MEDICINE AND CANCER IMMUNOLOGY IN CHINA**

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patient with advanced CCA who was treated with CART-133 after seeing a partial remission following CART-EGFR treatment. A further 4.5-month partial remission was observed (25). Although the killing of CSCs by CART-133 was not conclusively shown, the novel sequential use of the CAR-T cocktail indicated intriguing possibilities. Epithelial cell adhesion molecule (EpCAM)—a transmembrane glycoprotein involved in epithelial cell adhesion—is another CSC marker, and several preclinical studies on CART-EpCAM cells have been published in China (26, 27). Currently, four Chinese CART-EpCAM trials are underway.

CAR-T research in China has been developing rapidly in recent years. However, we cannot overlook the gap in domestic research relative to that seen internationally.

For example, although CAR-T treatment for hematological malignancies is efficient, researchers in the United States continue to make improvements. A group at the Fred Hutchinson Cancer Research Center in Seattle, Washington has been working to optimize the composition of CAR-T treatments. Clinical data has indicated that optimizing the types of T cells used (e.g., including higher percentages of naive or central memory T cells) or utilizing a defined ratio of CD4-positive to CD8-positive T cells gives better clinical results (28, 29). Along these lines, Xinqiao Hospital in Chongqing has registered a CART-19 clinical trial that will use a CAR-T therapy in which a higher percentage of memory T cells will be used. A case report from Carl June’s group at the University of Pennsylvania demonstrated the possibility of treating MM with CART-19 cells, something previously considered to be impossible (30). Such innovative attempts to subvert traditional ideas rarely occur in China.

For CAR-T cell preparation, the vast majority of domestic clinical trials use viral transfection. In contrast, the University of Texas MD Anderson Cancer Center has been promoting the use of the so-called “sleeping beauty” system, a synthetic DNA transposon vector designed to introduce DNA sequences into chromosomes following electroporation (31). These scientists believe this system can better meet future demands for lower cost and greater gene-editing capabilities.

The challenges related to solid tumor treatment have motivated researchers in the United States to develop new strategies and clinical applications that have been rapidly translated into the clinic. One such strategy was developed by a group from City of Hope National Medical Center (CHNMC) in Duarte, California. In order to overcome the immunosuppressive microenvironment, they designed a PD-L1:CD28 chimeric receptor, converting an inhibitory receptor to a costimulatory receptor by exchanging the transmembrane and cytoplasmic protein domains of PD-L1 with that of CD28. This PD-L1:CD28 chimera exhibited enhanced functional attributes and demonstrated a novel strategy to overcome PD-L1-mediated immunosuppression (32). Another new strategy was developed by a group from MSKCC to avoid “on-target, off-tumor” toxicities; the group designed an antigen-specific inhibitory CAR that could constrain the activation of T cells when a specific antigen was encountered (33). To reduce the risk of off-target toxicity, affinity-tuned CARs that could more precisely distinguish tumor cells from normal cells were tested by Carl June’s group (34). Unlike in the United States, original research in this area is lacking in China. However, it is gratifying to note that some innovative clinical trials have also been initiated there. For example, six trials in which CAR-T cells will be modified to express soluble PD-1 or CTLA-4 antibodies to overcome the immunosuppressive microenvironment have been registered.

It is true that clinical trials in the United States are more varied than those in China; China’s clinical trials are relatively elementary and mostly similar to one another. Yet the diversity of U.S. trials has brought us valuable insights into how to design effective CAR-T treatments. For example, original U.S. studies have illuminated the benefits of using a preconditioning regimen with the drugs fludarabine and cyclophosphamide to improve the proliferation and persistence of CAR-T cells, and have shown how to optimize CAR-T preparation protocols and the framework of the CAR molecule.

Compared with traditional drugs, CAR-T therapy offers more opportunity for businesses—especially small companies—because of the great diversity of targets and the many possible ways to optimize CAR molecular structures and preparation methods. American businesses have also made outstanding contributions to the industrialization of CAR-T therapy. Of the seven ongoing phase 2 CAR-T trials, six are being conducted by three companies (Kite Pharma, Juno Therapeutics, and Novartis). However, in China, most CAR-T trials are led by institutions or hospitals. Business participation has been mainly limited to providing technical support or supplying viruses. The most effective way to encourage domestic enterprises to participate in the industrialization of CAR-T therapy is a topic deserving further discussion.
The U.S. FDA has issued clear guidelines and regulations for clinical application of CAR-T therapy after extensive input from experts in relevant fields. In China, no formal regulations on CAR-T therapy have yet been issued. This policy uncertainty is a cause for concern for businesses. Achieving consensus within the field and deepening communication with regulators has become a top priority for the industry. In recent years, several related associations and conferences have been established, including the Chinese Research Hospital Association (CRHA), the China Biotherapy Conference (CBC), and the China Disease Biotherapy Conference (CDBC). Meetings over the past few years have effectively promoted communication, cooperation, consensus building, and the standardization of clinical trials. It is gratifying that the regulatory authorities have issued a policy draft on CAR-T therapy, and that formal regulations are expected by the end of 2017. We believe that CAR-T therapy will be regulated as a novel drug type in China as it is in the United States. Furthermore, the government has been actively supporting CAR-T therapy; last year, it announced 14 key research projects, and CAR-T therapy was among them.

The future of CAR-T therapy
The side effects of a CAR-T cell infusion can include serious consequences, even death. Without proper control, serious clinical accidents could cause significant human and financial losses. For example, a phase 2 CART-19 trial sponsored by Juno Therapeutics was terminated this year because three patients died from cerebral edemas. It is therefore very important to establish more clinical teams with experience in immunotherapy. And it is strongly recommended that the businesses involved in producing the therapies establish close cooperation with experienced clinical teams who can help reduce the risk of clinical accidents.

The preparation of CAR-T cells is complicated and greatly affected by variation among patients. This places a heavy burden on the preparation technologies, which may be an obstacle for some companies wishing to carry out CAR-T clinical trials. To address this problem, some domestic enterprises have begun to develop automated and standardized CAR-T preparation platforms. For example, Sinobiocan (Beijing) Biology Technology is building a one-stop CAR-T preparation apparatus, including an automated cell-preparation platform, and is also providing a steady supply of reliable reagents. As an alternative, “off-the-shelf” universal CAR-T cells can be prepared using the CRISPR/Cas9 system and other gene-editing technologies. Currently, there are two clinical trials utilizing universal CAR-T technology ongoing in China.

The deficiencies mentioned above point to a clear path for improvements to the current status of CAR-T research in China. We believe that this powerful form of cancer treatment will undergo rapid development with the Chinese government’s active support and with our joint efforts.

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**Precision cancer medicine and immunology in China**

Xu-Chao Zhang¹² and Yi-Long Wu*¹

Precision medicine in the context of clinical oncology treats patients by devising therapies based on information about genetic and epigenetic changes to the genes in their tumors. Canonical examples of such mutated genes include epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) genes in lung cancer, human epidermal growth factor receptor 2 (HER2) in breast cancer, and the BCR-ABL gene fusion in chronic myeloid leukemia (CML), among many others. The term “precision medicine” incorporates personalized medicine, since in a clinical setting it implies choosing the right drug for the right molecular target in the right patient. Precision medicine has dramatically changed clinical practice and is further shaping the landscape of oncology treatment standards.

Chinese investigators have contributed significantly to the establishment of standard treatments for non–small-cell lung cancers (NSCLCs) in which the EGFR and ALK genes are mutated. In 2009, the world’s first phase 3 study was carried out showing the efficacy of first-generation EGFR tyrosine kinase inhibitor (TKI) drugs in patients whose tumors had a mutated EGFR gene—a landmark in the study and treatment of lung cancer (1). Since then, there have been further studies of second- and third-generation EGFR TKIs in China (2-5). In addition to EGFR, several trials of drugs targeting ALK, ROS1, and other so-called “driver genes” for lung cancer have generated good evidence for the establishment of new standards of care for patients with specific genomic alterations, which also promoted the approval from the China Food and Drug Administration (CFDA) for new drugs to be marketed in China (6, 7). Research into immune therapies has also been making rapid progress, and some have already been approved for use in the United States and other countries. Early clinical trials of these therapies in China are coming to fruition, and it is hoped that the resulting treatments will be approved by the CFDA in the near future. This area is very competitive in China because of the number of similar drugs available internationally; several local antibody-based drugs are entering the arena, although they are still only in early trials.

Progress in precision medicine has already changed the practice of oncology in China, which is seeing broader use of genotyping and TKI-based treatments. Nonetheless, the availability of targeted therapies is affected by several factors and could benefit from certain improvements. For example, the processes by which Chinese patients are selected to receive immunotherapy need both regulatory and clinical consensus.

In this article, we will talk about the current state of precision medicine in China, specifically in the treatment of lung cancer. We will also address future directions and strategies in this area.

**Precision medicine in lung cancer treatment**

Lung cancer is a highly heterogeneous group of diseases. In Chinese lung cancer patients, the major driver genes—including EGFR, ALK, RET, MET, HER2, BRAF, and KRAS—are very similar to those listed in the guidelines published by the National Comprehensive Cancer Network (NCCN), an alliance of American cancer centers (8, 9). However, there are notable differences. The EGFR mutation rate, for instance, is around 35% in Chinese patients, and only 15% in Caucasian patients, while the KRAS mutation is around 5% to 10% in Chinese lung cancer patients versus 30% in Caucasians. There is no obvious difference in prevalence between populations for other mutated genes that occur with lower frequency (Figure 1, next page).

*EGFR* mutations were identified as a focus for targeted therapy for lung cancer in 2004. With the first report of *EGFR* mutations in the Chinese population and preliminary data about the response rate to TKIs, Chinese investigators Tony Mok, Yi-Long Wu, and others led a milestone phase 3 randomized study, named the Iressa Pan-Asia Study (IPASS), published in the *New England Journal of Medicine* in 2009 (1). The study showed for the first time that an EGFR TKI was effective in treating *EGFR* mutant cancers. Based on IPASS and follow-on studies, EGFR TKI was established as a first-line standard treatment for patients with *EGFR* mutated tumors, and was incorporated into the treatment guidelines of numerous international cancer organizations including NCCN, the American Society of Clinical Oncology (ASCO),

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and the European Society for Medical Oncology (ESMO). In 2007, oncologist Yi-Long Wu, who is also on the board of directors of the International Association for the Study of Lung Cancer (IASLC) and president of the Chinese Society of Clinical Oncology (CSCO), founded the Chinese Thoracic Oncology Group (CTONG). CTONG now has 31 member hospitals across China and has become an important platform for organizing trials and translational research studies. It contributed significantly to the development of targeted therapies in China, not only participating in several international synchronized trials, but also taking the leading role in dozens of national studies.

All the above studies bolstered the registration and approval of targeted therapies in China as well as the capacity for designing and organizing trials. The CFDA has now approved three first-generation EGFR TKIs (gefitinib, erlotinib, and icotinib), one second-generation EGFR TKI (afatinib), and a third-generation EGFR TKI, osimertinib, for EGFR mutant tumors. Crizotinib, aimed at Chinese patients who have ALK or ROS1 mutations, was also approved by the CFDA. Other ALK TKIs are in clinical trials led by investigators from CTONG, while TKIs targeting numerous other mutated genes are in different stages of clinical trials or already in use in clinical settings.

**Novel trial design for oncology**

A pragmatic question faces developers of targeted therapies: How can they ensure that a sufficient number of patients with specific genetic alterations are enrolled in the clinical trials for each drug? The idea of simultaneously screening patients for a set of genetic biomarkers linked to the disease and matching the drugs under investigation to each specific genetic or pathway alteration has now been adopted worldwide.

Yi-Long Wu, as the president of CSCO, has advocated this kind of trial design for many years, attempting to push molecular genotyping and targeted therapies into clinical practice. In 2015, Wu launched the CLUSTER 1.0 trial, which is the first multiple-biomarker–driven clinical research study to be conducted in Asia (10). The enrolled patients were assigned to groups receiving different agents according to their genetic
profiles. Five agents targeting different molecular abnormalities are being studied based on the mutation(s) found, making up the five arms of the study. Each arm will be analyzed independently. If the response rate to an agent reaches 40%, a phase 2 trial will be conducted. This multiple-arm, parallel assignment is intended to reduce the overall drug screening failure rate. This study drew much attention from the Center for Drug Evaluation of the CFDA, and is a significant milestone for precision medicine in China. Design of the follow-up CLUSTER 2.0 lung cancer trial is complete, and will be launched in the fourth quarter of 2017. CLUSTER 2.0 is also biomarker-driven and will evaluate novel targeted agents in at least 10 study arms focused on specific genetic or pathway alterations.

During the process of developing new anticancer agents such as icotinib, avitinib, and apatinib, Chinese researchers have already had experience with innovative clinical trial design. One example is a phase 1/2 study that aims to determine the safety profile and recommended phase 2 dose (RP2D) of avitinib in patients carrying a particular EGFR mutation. Instead of using only the maximum tolerated dose, as is generally done, in this study RP2D was determined by the pharmacokinetics, preliminary efficacy, and safety profile of the agent. This innovative design established a new paradigm that allows the testing of a wider range of dosages showing indications of efficacy and low toxicity at an early stage. Based on the expedited determination of RP2D, in 2016 researchers applied for approval from the CFDA to conduct a phase 2/3 trial with avitinib—the fastest-ever application for a novel agent in China.

Currently, the CFDA has approved only five EGFR TKIs (gefitinib, erlotinib, icotinib, afatinib, and osimertinib) and one ALK/ROS1 TKI (crizotinib). No drugs are clinically available for lung cancer patients with other common mutations. Therefore, to be enrolled in a new trial offers such patients a way to gain access to precision medicine. Indeed, through these ongoing innovative clinical trials, patients with actionable genomic alterations can quickly access drugs under study internationally, as well as drugs approved in the United States, Japan, or Europe (but not in China).

**Guidelines for biomarker testing, diagnosis, and treatment**

In precision oncology, molecular diagnosis precedes precise treatment. EGFR and ALK testing, and their corresponding treatments, have been included in the guidelines of numerous cancer associations, including NCCN, ASCO, and ESMO. IASLC has released the EGFR and ALK testing atlas, a series of consensus documents on molecular testing (11).

In China, local guidelines for different tumor types have been formulated. Since 2016, CSCO has taken the lead in compiling the annual Guidelines for Diagnosis and Treatment for patients with lung and other cancers. The Ministry of Health and Family Planning Committee, formerly the Ministry of Health, also organized national experts to produce Clinical Path and Guidelines for Diagnosis and Treatment for Primary Lung Cancer Patients. Yi-Long Wu played a leading role in compiling both of these guidelines as they pertain to lung cancer. In the CSCO guidelines, due to the limited availability of drug and testing technologies, and disparities and imbalances in resources distribution, only EGFR mutations and ALK and ROS1 rearrangements and their targeted drugs are listed for routine clinical practice. Circulating tumor DNA from plasma is regarded in the report as an alternative biomaterial to direct tumor sampling for screening of EGFR mutations, but its potential for producing false negatives is emphasized. The national and local guidelines for testing, diagnosis, and treatment are modified annually to account for progress and new evidence from trials.

Several EGFR and ALK gene testing kits have been approved by CFDA and are also recommended by national or local guidelines. According to one as-yet unpublished report, the overall rate of testing for EGFR mutations in patients in China is around 50%, which although higher than the previous rate, is still not on par with the rate in developed Western countries, Japan, Korea, and Singapore.

Many leading hospitals carry out molecular testing in their own laboratories under license from the Ministry of Health and Family Planning Committee, using CDFA-approved kits or tests developed in-house. Some hospitals, especially those with fewer resources, outsource molecular testing to third-party laboratories. As sophisticated next-generation sequencing (NGS) technology comes into wide use, it is reported that many companies are emerging across the country to deliver that service. The CSCO biomarker committee, led by Yi-Long Wu and Xu-Chao Zhang, is paying attention and making efforts to standardize the clinical use of NGS technology and to ensure testing quality for clinical oncology practice. In a draft of Consensus of the Use of NGS in Clinical Oncology, to be released in the near
future, NGS technology is regarded as a complex genotyping tool for which quality control is critical. In addition, diagnostic kits based on NGS used to stratify patients with specific gene mutations are currently being developed by many companies. They will be validated as companion diagnostic tools in the CLUSTER 2.0 trial.

For patients with driver genes or genetic alterations other than EGFR, ALK, or ROS1, the CSCO guidelines recommend participation in trials in which investigational drugs are provided. Patients with disease relapse or those who are resistant to the available TKIs are encouraged to undergo additional molecular screening to clarify the resistance mechanism and to participate in clinical trials pertinent to their diagnosis.

Precision medicine in China has made substantial progress in the treatment of lung cancers harboring EGFR, ALK, and ROS1 alterations. Chinese investigators have contributed their efforts to the global and national establishment of standard targeted therapies. Yet if precision oncology is to improve, more effort is needed to improve the detection rate of key driver genes like EGFR, to develop new drugs, to launch novel trials, and to standardize molecular testing.

**Immunology of cancer**

Internationally, immune therapy has been recognized as a potentially powerful treatment since the establishment of the field of immuno-oncology more than 100 years ago. In the United States, Europe, and Japan, immune checkpoint inhibitors—such as drugs that target CTLA-4, PD-1, and PD-L1, proteins known to inhibit T cells from fighting cancer—have already been approved by regulatory agencies for a variety of cancer types including melanoma and lung cancer. By the end of December 2016, the U.S. Food and Drug Administration (FDA) had approved therapies that block the PD-1 pathway, including nivolumab, pembrolizumab, and atezolizumab. The working principle of this kind of inhibitor is to remove the brakes on immune activation or block the inhibition of immune attack in a tumor’s microenvironment. Antibodies against other immune checkpoints like TIM3 and IDO are in the preclinical or clinical stage of development.

**Immune therapy for lung cancer**

In China, no PD-1 or PD-L1 inhibitors have been approved by CFDA. The contrast with the United States indicates that Chinese R&D in immuno-oncology biologics (protein-based drugs) lags behind, as it does in the area of small-molecule drugs. However, various clinical trials are actively investigating both international and domestic drugs in Chinese patients with a range of cancer types including lung cancer.

Between January 1, 2013 and April 6, 2017, ClinicalTrials.gov registered 270 international clinical trials of PD-1/PD-L1 therapies for NSCLC, including trials for nivolumab, pembrolizumab, atezolizumab, and durvalumab. These 270 trials included 61 studies that involved East Asian sites and 14 that involved Chinese sites, 12 of which were multinational trials and 2 that evaluated only Chinese patients (12).

In the 14 international trials that enrolled Chinese patients, six involve first-line/primary therapies, four involve second-line therapies (used if primary treatments fail), two are studies of adjuvant therapies, and two are phase 1 studies using only Chinese patients. There are five studies for atezolizumab, four for durvalumab, three for pembrolizumab, and two for nivolumab. It is noteworthy that PD-L1 expression was set as a criterion for recruitment in some trials, but not in others, and that different diagnostic antibodies and screening platforms were used in this determination.

Nonuniform PD-L1 expression testing and cutoffs make it extremely difficult to compare results from different trials. Nonetheless, it is encouraging that Chinese pharmaceutical companies are working intensively to develop PD-1 and PD-L1 drugs. With the efforts of CFDA to reform the regulatory framework for clinical trial approval of novel agents and to adjust its policies to respond to advances from international trials, immunotherapy trials are now getting underway faster. Chinese pharmaceutical companies had developed eight PD-1 or PD-L1 inhibitors by January 7, 2017, and four drugs have been approved by CFDA for phase 1 trials among patients with advanced solid tumors including NSCLC. Another four drugs are now being considered by CFDA for clinical trial approval. In November 2016, the PD-1 inhibitor KN035, which is administered by subcutaneous injection, received approval from the U.S. FDA for clinical trials.

Notably, the immuno-oncology market is very competitive for domestic Chinese pharmaceutical companies. As of October 2017, there are 17 inhibitors targeting immune checkpoints PD-1,
PD-L1, or CTLA-4 from 14 domestic corporations treating a range of cancers (Table 1).

**Improving patient selection for immune checkpoint inhibitor therapy**

PD-L1 protein expression on tumor cells or immune cells in the tumor microenvironment has been widely used as a companion diagnostic, or evaluated retrospectively in clinical trials. The overall response rate to PD-L1 inhibitor therapy in patients selected for using PD-L1 expression levels increased from 20% to 40%–60%, a smaller increase than expected (13). However, testing for PD-L1 protein expression by immunohistochemistry (IHC) was performed using different platforms, antibodies, and scoring methods, making it difficult to compare results from different trials. Hirsch and colleagues reported that in one of the studies, the discrepancies caused by testing for PD-L1 on different platforms could result in misclassification of PD-L1 status (14).

In China, no PD-L1 protein expression tests or kits have been approved by CFDA. No PD-L1 test is recommended in the CSCO guidelines for any cancer type. In contrast, a PD-L1 IHC test has been approved by the U.S. FDA. In ongoing trials in China, a PD-L1 test is taken as one criterion for inclusion in certain trials but not in others. Whether PD-L1 should be used as a biomarker to select Chinese patients for PD-L1 inhibitor treatment requires further investigation.

Are there any potential biomarkers other than PD-L1 to be developed for the Chinese population? Theoretically, molecules in the microenvironment of a tumor and in the circulating blood could potentially be predictive markers. Such molecules could be involved in any one of the many steps between the appearance of an antigen and the launch of an immune attack on tumor cells. Biomarkers such as tumor mutational burden, DNA mismatch repair status, high microsatellite instability, and circulating T-cell characteristics are under extensive investigation in trials using several cancer types.

In ongoing clinical trials in China, investigators have a good opportunity to study these biomarkers by testing biomaterials collected from tumor and blood samples. In one study (with the acronym CHOICE) led by Yi-Long Wu, researchers looked at the immune scores of 250 heavy smokers with lung cancer by analyzing the RNA sequencing data of the immune cells isolated from the tumors. Preliminary data showed a correlation between

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immune score and disease prognosis (unpublished data). This RNA-sequencing-based method can be further evaluated in clinical trial patients to see if the immune scores can be predictive for positive response to immunotherapy. It is important to note that some biomarkers require sophisticated technologies like multicolor IHC, whole-exome deep sequencing, or the use of cancer gene panels. One other challenge is how to integrate several different kinds of immune-related biomarkers, for example, combining tumor mutational burden with PD-L1 expression.

Overall, the development of novel biomarkers to predict and guide immune therapy using PD-1/PD-L1 inhibitors needs further exploration, particularly in trial patients who can provide ongoing samples and whose treatment and follow-up data can be monitored.

Summary and future directions

Over the past 10 years, the practice of clinical oncology in China has gradually incorporated the attributes of precision medicine, as is happening in other countries. There has also been significant progress in clinical research and drug development. Chinese investigators have contributed in important ways to international studies, and have conducted trials based on the particular characteristics of Eastern populations. A series of novel targeted agents with good efficacy has been synthesized and introduced for clinical use. In the arena of immune therapy, dozens of national and international trials are underway in the hope of moving PD-1/PD-L1 inhibitors into clinical use. Eight such inhibitors from local Chinese companies are in the early stages of clinical development for use against a variety of cancer types. And a handful of other small-molecule TKIs and immune checkpoint inhibitors are undergoing validation in clinical trials. National and local guidelines for clinical practice have been written in recent years to standardize the use of these effective new treatments.

Nonetheless, China is lagging behind in some areas, including development of novel drugs, availability of TKIs and antibody drugs that have already been approved in other countries, development of companion diagnostics, and availability of clinical biomarker tests. Furthermore, many domestic novel anticancer agents are structural modifications of agents that are already on the market or are undergoing research; innovative design is rare.

However, there are many possible future directions. The pharmaceutical industry should shift its focus from generic agents to the development of pioneering agents. New technologies like NGS and liquid biopsies of ctDNA can be effective ways to push innovative biomarker-driven trials forward, especially for those patients with rare genetic alterations in cancer-related genes. Attention should also be paid to the mechanisms that cause resistance to EGFR and ALK TKIs, and to drugs that can overcome this resistance. More translational studies on immunotherapies including checkpoint inhibitors and adoptive cell therapy need extensive exploration. Finally, development of biomarker companion diagnostics and standardization of biomarker tests in a clinical setting should be addressed.

It is well known that much effort is needed to improve drug development by local companies, as well as the competitiveness of those companies. The Chinese government has policies to encourage innovative R&D on new drugs and to incubate pioneering startups in China. Influenced by the Precision Medicine Initiative declared by former American President Obama and the Cancer Moonshot Initiative led by former Vice President Biden, China initiated a set of national science and technology programs designed to stimulate R&D on precision medicine in oncology.

To further drive precision oncology forward, all stakeholders, including the government, academic investigators, pharmaceutical companies, innovative biotechnology companies, patients, and disease advocates should come together and bring their resources to bear. Only this way will we be able to bring precision medicine to the patients who need it most.

References
From big data to knowledge in precision medicine

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The explosive growth of medical and ‘omics big data

The biomedical field is experiencing an era of significant growth due to the large amount of clinical, biomolecular, and health data being collected. The increased adoption of electronic medical record systems worldwide has enabled large volumes of clinical data to be captured and stored. These data include screening, diagnostic, and treatment results as well as medical and family histories, and data from biochemical testing, imaging, and follow-ups. Furthermore, advanced technologies that collect biomolecular data from studies in genomics, transcriptomics, proteomics, epigenomics, metabolomics, metagenomics, and exposomics—collectively termed “omics”—can provide results within hours or days, dramatically speeding up clinical analysis. "Multi-omics" molecular data are widely used in the field of precision medicine and related clinical applications. These data constitute one of the fastest-growing data types in biomedicine. Moreover, a large amount of daily biological data related to personal health is being continuously collected by wearable devices and smartphones. These multidimensional data require efficient methods of storage and analysis if they are to be useful tools for exploring the mysteries of disease and health.

Large cohort studies in Chinese precision medicine

As a consequence of major advances in gene sequencing technology, programs promoting precision medicine have been launched worldwide, with the aim of improving accuracy in screening, diagnosis, intervention, and treatment. Scientists around the world, but particularly in the United States, the United Kingdom, and China, are collecting the genomic data of millions of people to establish a database of genetic variants. In 2016, the Chinese Ministry of Science and Technology founded the Precision Medicine Research Key Special Project (PMRKSP) and announced an investment of billions of Chinese yuan before 2030. At present, three types of large cohort studies have been launched as part of this effort. The first is the study of natural national populations, in which millions of participants from seven main regions covering four municipalities and 24 provinces of China will be analyzed. The second is a study of eight cohorts affected by critical diseases, including cardiovascular, cerebrovascular, respiratory, metabolic, neurological, psychosomatic, and immune system disorders as well as seven common malignant tumors. A primary goal of this study is to collect samples from a total of 700,000 people. The third investigation is a clinical cohort study of 50 rare diseases that aims to collect more than 50,000 patients in a single cohort. In addition, the Chinese Academy of Sciences (CAS) has announced a precision medicine research plan targeting the collection of genetic information from 4,000 Chinese volunteers within four years.

From big data to knowledge

The meticulous analysis of big data is essential to extract accurate information and ultimately convert them into knowledge (1) that can assist in clinical decision-making and health management (Figure 1, next page). In order to support this transformation, the Chinese government has developed a series of plans that focus on key technologies involved in processing large amounts of data. The 13th Five-Year National Science and Technology Innovation Plan, issued by the State Council, will develop technologies for precision medicine, integrate them into a multilevel knowledge database, and create a national platform to share biomedical big data. PMRKSP has also initiated a series of research projects that focus on the most important technologies for big data integration, storage, use, and sharing. The program has taken three steps toward transforming data into information, and information into knowledge. This program first started to build fundamental data infrastructure (data management, processing systems, and
disease databases) in 2016. At the beginning of 2017, scientists began processing the raw data generated from PMRKSP into multilayered biomolecular information repositories, which assist in the integration of detailed standardized information and metadata, including clinical patient information and its associated genomic, proteomic, metabolic, and other ‘omics data. Finally, by the start of 2018, knowledge mining will begin on the results of the first two stages of work, with the purpose of discovering important genetic diseases, key mutation sites, and epigenetic information. This project will facilitate the implementation of research in precision diagnosis and the treatment of disease, and will lead to important developments in precision medicine.

**Construction of big data platforms for precision medicine**

With the support of a national policy concerning big data platforms, China has been able to construct a comprehensive infrastructure to successfully implement precision medicine across both state-owned and private health care sectors. Under the guidance of China’s National Health and Family Planning Commission, a new company known as China Healthcare Big Data Development Co., Ltd. (CHBDDC) was established. CHBDDC designated two provinces and four cities to participate in a pilot program of national health care big data centers. The China National GeneBank (CNGB), whose establishment was approved by the National Development and Reform Commission, was launched in 2016 and will store and manage the country’s unique genetic resources. In addition to the national big data centers, domestic biological big data platforms such as BGI Online (www.bgionline.com) and JingYun (www.gene.ac) have been built to provide fast and accurate computing services in bioinformatics. Information technology cloud service providers, including Alibaba and Huawei, provide specialized cloud storage and computing services for biomedical
data, which allow information technology and biotechnology to be closely integrated.

**The potential of medical big data**

Medical and 'omics big data is improving health care efficiency and quality by transforming data into knowledge (2–4). There are three aspects of big data that best describe its potential value. Firstly, the data can describe the entire process of a disease by finding the hidden associations and patterns within complex data, uncovering molecular mechanisms, revealing biomarkers, identifying disease-associated genetic factors, and evaluating therapeutic effectiveness. Secondly, the genomic and clinical data can be used to predict the risk of genetic diseases, the occurrence of cancers, and the clinical outcome of treatments aimed at novel disease targets. Finally, big data can help doctors to prevent disease by prescribing personalized health care regimens using multidimensional data from individuals as well as real-time health and disease information available through the dynamic collection of data acquired daily through personal health monitoring devices.

**Challenges of big data use in precision medicine**

**Data sharing**

To maximize the contributions of big data, generate reproducible results, and improve research practice, it is essential to allow data to be open and shared among different institutions (3). PMRKSP requires that data from clinical studies must be unconditionally shared through a national big data center. However, a mutually beneficial alliance is necessary to encourage research institutions and businesses to share key data generated outside of PMRKSP.

**Security and privacy of data**

In the future, a large volume of multi-omics information on populations and electronic medical and health records will be stored in big data platforms. If personal privacy is not guaranteed, ethical issues are bound to arise. To avoid privacy disputes, the government should formulate strong data protection laws. Additionally, methodologies that do not reveal an individual’s information, such as multiparty computation, are indispensable and should be established (5).

**Standardization of data generation and analysis**

Currently, clinical research based on data from next-generation sequencing (NGS) lacks standards and specifications for sample collection, storage, handling, and analysis. These issues lead to poor data quality, errors in analysis, and misinterpretation of results. In 2016, the Chinese Society of Clinical Oncology released the first draft of “Consensus on Second Generation Sequencing Technology Applied in Precision Diagnosis and Treatment of Clinical Cancer” in order to promote the standardization of NGS in clinical testing. The following year, experts from West China Hospital of Sichuan University and Peking Union Medical College Hospital published the “Expert Consensus on Second Generation Gene Sequencing in Clinical Molecular Pathology Laboratories.” These documents will greatly accelerate standardization of the generation and interpretation of NGS data. However, more informative industry standards are needed to ensure the reliability and reproducibility of precision medical data.

**Integration of multidimensional 'omics data**

To overcome the deficiencies of the single 'omics approach, it is crucial to introduce redundancy by generating multi-omics data that allow information on different types of biomolecular interactions to be extracted. In addition, expanding the scope of the methods used to collect data, such as including data from noncoding DNA regions, will further enhance the power of the big data analyses being performed.

**Deep integration with information technology**

Medical big data raise significant challenges in storage and computation, which urgently require innovative information technology approaches to provide solutions. A key problem associated with big-data knowledge mining is the rapid rate at which the size and complexity of the information network expands, resulting in bottlenecks in computing speed. One resolution to this issue has recently been offered by the Chinese technology company Hanwuji Intelligence, which improved computing speed by using a dedicated processor that analyzes data through a type of machine learning known as “deep learning” (6). This solution suggests that resolving the unique problems associated with medical big data processing will require a range of cutting-edge information technology, potentially requiring dedicated hardware and software to be embedded in the big data platform.

**Future trends in big data for precision medicine**

Future big data technology will be closely inte-
grated with artificial intelligence (AI) technology to promote the development and application of precision medicine. The clinical application of IBM’s Watson supercomputer has demonstrated that AI can enable clinical decisions to be made quickly and accurately through the integration and analysis of big data. At present, China has insufficient medical resources and is facing problems with an irrationally structured medical system as well as unbalanced distribution of its medical resources. This situation makes it difficult for citizens to seek medical treatment, as is explained by the State Council in its position paper Key Tasks to Deepen the Reform of the Medical and Health System in 2016. To address these issues, the government is establishing diagnosis and treatment guidelines, strengthening basic services, and promoting better allocation of high-quality medical resources for Chinese citizens. The intelligent diagnosis and treatment system constructed with big data, in conjunction with AI, will incorporate the experience of experts, effectively enhancing the quality of grass-roots services available and addressing the deficit in the availability of medical resources in China.

**References**


**The role of circulating cell-free DNA in the management of cancer in China**

**Ying Hu**1, Yanhui Chen1‡, Lei Zhang2‡, Haitao Zhao3, Hui Zeng1*, and Henghui Zhang1,2*

**Clinical needs in the management of cancer in China**

Cancer is a major public health problem in China. Wanqing Chen and colleagues reported that an estimated 4.9 million new cancer cases and 2.8 million cancer deaths occurred in China in 2015, with lung cancer being the most common and the leading cause of cancer death (1). Antitumor treatments currently being developed in China aim to improve overall survival and quality of life for patients.

Some of the most important clinical needs for effectively managing cancer include:

1. Biomarkers that are sensitive and specific to particular kinds of cancer cells, enabling earlier diagnosis and identification of the tissue of origin for unknown metastases and helping scientists distinguish between benign and malignant tumors.
2. Real-time tracking of biomarkers that can assist in clinical decision making, predict the safety and efficacy of a therapy, monitor the patient’s response to a therapy, allow early detection of any resistance to treatment, and aid in prognosis.
3. Biomarkers that can define the molecular characteristics of a cancer and enable monitoring of these characteristics as the cancer progresses or shrinks following treatment.

Cell-free DNA (cfDNA)—nucleic acids not bound inside cells but circulating in the plasma—was detected in the bloodstream of patients with cancer as early as 1948 (2). Clinical studies indicated that plasma cfDNA could be used as a biomarker for diagnostic screening, predicting responses to therapy, monitoring the size of the tumor, and diagnosing a relapse at an early stage (3–5).

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**cfDNA in cancer diagnosis**

Circulating cfDNA as a noninvasive biomarker has been used for early diagnosis and monitoring of tumor burden (6, 7). Several studies have shown that levels of cfDNA are higher in cancer patients than in healthy people (8–10). However, the use of circulating cfDNA in early cancer diagnosis has some limitations, since it is thought to originate from both tumor cell DNA and normal cell DNA. In early stage tumors, the low abundance of tumor-derived cfDNA may make detection difficult. Somatic mutations—nonheritable genetic alterations—found in cfDNA may provide information to help distinguish benign from malignant tumors. In practice, some variants identified in gene tests are difficult to classify clinically as pathogenic or benign, making it challenging to clinically distinguish benign from malignant tumors.

**Using cfDNA to identify the tumor origin**

Recent studies have demonstrated that epigenetic information embedded in cfDNA can provide information useful for predicting a tumor’s tissue of origin (11, 12). Guo and colleagues performed a systematic search of tissue-specific methylation regions across the entire human genome. Using a reference database of known methylation signatures, the authors identified tissue-specific methylation markers and demonstrated accurate determination of tissue of origin from cfDNA from patients with lung or colorectal cancer (11).

**Using cfDNA in selecting patients for targeted therapy**

cfDNA has been used in clinical practice to stratify non–small cell lung cancer (NSCLC) patients for targeted therapy. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved the use of epidermal growth factor receptor (EGFR) mutations detected in circulating cfDNA to select NSCLC patients with EGFR-actionable mutations for EGFR tyrosine kinase inhibitor (EGFR-TKI) therapy. However, this presents challenges for clinical decision-making, including the detection of discordant mutations in the tumor tissue compared to circulating tumor DNA (ctDNA). A large retrospective study of Chinese NSCLC patients showed that among 2,463 NSCLC patients with matched tumor tissue (T) and ctDNA (C) specimens, 1,017 patients carried the EGFR mutation in tumor tissues and/or ctDNA. Of these patients, 472 received EGFR-TKI treatment and were divided into three groups based on whether they carried the mutation in the tumor, in circulating cfDNA, or both: T+/C+ (n=264), T+/C- (n=28), and T-/C+ (n=180). The median progression-free survival across the groups were similar. Further PCR and next-generation sequencing (NGS) validation from microdissected surgical specimens suggested that intratumor heterogeneity and relatively low sensitivity of the mutation detection assay contributed to discordant EGFR mutant status between tissues and ctDNA. In fact, neither tissue nor ctDNA analysis cover all EGFR mutations, suggesting that a combination of data from both tissue and ctDNA might be needed to accurately detect the presence of EGFR mutations. This study suggested that primary screening using ctDNA, followed by tumor tissue analysis in the case of a negative result, may be a preferable approach to determine whether EGFR-TKI therapy is warranted (13).

Previous studies have detected RAS mutations in ctDNA, using this information to stratify metastatic colorectal cancer (mCRC) patients for cetuximab or panitumumab treatment (4, 14). Recently, Xu and colleagues found a novel phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutation (p.K944N) in patients with resistance to cetuximab. These results indicated that patients harboring PIK3CA or RAS mutations in ctDNA showed shorter progression-free survival than those with wild-type sequences (15).

**Role of cfDNA in monitoring clonal evolution**

The early detection of relapse following primary treatment for cancer and characterization of emerging molecular subsets might offer new therapies to limit tumor recurrence. ctDNA analysis is a potentially powerful method to noninvasively track tumor clonal evolution. Results from the TRACERx [TRAKing Cancer Evolution through therapy (Rx)] lung cancer study showed that phylogenetic cfDNA profiling closely tracks the subclonal nature of lung cancer relapses and metastases. It also found that the tumor volume was correlated with the variant allele frequency determined from plasma cfDNA. cfDNA profiling can allow for tracking of tumor evolutionary dynamics and detection of adjuvant chemotherapy resistance in NSCLC (5). Recently, Jiang et al. reported that the mutational landscape of cfDNA is associated with therapeutic response to first-line, platinum-based doublet chemotherapy in patients with advanced NSCLC (16).

The CRC patients with wild-type RAS could be stratified based on the tumor tissue genotype (17). However, RAS mutations often occur after EGFR blockade treatment, and it is challenging to obtain repeated tumor biopsy tissue to track tumor
evolution. Siravegna et al. observed RAS clonal evolution in circulating cfDNA of mCRC patients. Interestingly, they found that the abundance of mutated KRAS clones in plasma increased during EGFR blockade treatment, but decreased following withdrawal of EGFR-specific antibodies (4).

**cfDNA in immune checkpoint therapies**

Recent clinical data support the use of new immune checkpoint inhibitors in several types of cancer. These drugs interfere with proteins that prevent the immune system from attacking cancer cells, and include such drugs as anti-PD-1 and anti-PD-L1 antibodies (18–22). A number of biomarkers have been developed to stratify patients likely to benefit from these immune checkpoint blockers. Their use has improved the survival of cancer patients and reduced treatment costs. Among these biomarkers, the PD-L1 protein is the most well known. Numerous clinical trials suggest that patients expressing PD-L1 are more likely to benefit from treatment with PD-1/PD-L1 inhibitors (23–25). However, these studies showed that approximately 10% of PD-L1-negative patients can also benefit from PD-1/PD-L1 inhibitors, suggesting that there might be other biomarkers that can better predict drug efficacy (23–25).

Several studies have reported other biomarkers that can also predict drug efficacy. A deficiency in DNA mismatch repair (dMMR) is a classical biomarker of colorectal and other cancers, and is associated with the efficacy of chemotherapy. High microsatellite instability (MSI-H) or dMMR leads to the accumulation of genetic mutations that may increase the risk of immune system identification and destruction of the tumor, suggesting that patients with dMMR progressive tumors can benefit from treatment with pembrolizumab or other PD-1 inhibitors (20, 26). Recent studies identified the mechanisms of acquired immune resistance after anti-PD-1 monoclonal antibody treatment and found two specific genetic variants in JAK1/JAK2 and beta-2-microglobulin to be involved (27, 28). A good response to immune checkpoint inhibitor treatment was positively associated with significantly increased tumor-infiltrating CD8 T cells or T-cell receptor (TCR) diversity (29, 30). In these studies, comparison of TCR clonality at baseline and postdosing biopsies showed that samples from responders had over 10 times as many clones expand after anti-PD-1 therapy; peripheral blood TCR diversity increased in responders after anti-CTLA-4 therapy. Peripheral blood T cells are relatively easy to obtain from cancer patients, so TCR diversity testing might be a good predictor of response to immune checkpoint inhibitors. In NSCLC, the amount of tumor variant load or tumor mutational burden (TMB) can impact the efficacy of immune checkpoint inhibitors (such as anti-PD-1 antibody) (31–36).

Biomarkers such as those mentioned above, which can predict the efficacy and prognosis of immune checkpoint inhibitor treatments, are found in many tumor tissues. It is well known that the predictive efficacy of biomarkers from tumor tissue is limited because of the difficulty of obtaining samples from advanced-stage cancer patients and because of tumor heterogeneity (37). Clinical studies have shown that cfDNA can effectively reflect the tumor’s size, malignant state, and ability to metastasize. It can also provide real-time information on causative mutations, providing more comprehensive gene mutation data than can be derived directly from tumor tissues (38). Researchers at the 2017 American Society for Clinical Oncology conference reported that TMB data obtained from sequencing of cfDNA could effectively predict those lung cancer patients who would benefit most from anti-PD-1 antibody treatment (39). In this study, ctDNA TMB was associated with a significantly higher number of DNA repair mutations. Additionally, smoking was associated with a higher TMB score. However, lower TMB detected in ctDNA predicted that a subset of patients would respond better to checkpoint inhibitors, in contrast to results from other studies (31–36). Potential reasons for this result include the small sample size, the possibility that ctDNA does not accurately reflect tumor burden, and the limited length of DNA sequenced (78,000 bp–138,000 bp). Larger prospective studies are necessary to validate these findings.

In the field of commercial gene testing, NGS-based MSI-H detection in cfDNA samples has been used to guide treatment choices, such as whether to use anti-PD-1/PD-L1 antibodies. In China, lung cancer, colorectal cancer, and cholangiocarcinoma are highly prevalent, providing a good opportunity to apply MSI-H and TMB detection assays. In addition, since the mechanism of tumor development in Chinese cancer patients has unique characteristics, research into tumor heterogeneity based on cfDNA analysis may identify biomarkers more suitable for these patients.

Recently, Ghoneim and colleagues reported that de novo DNA methylation plays a role in establishing the PD-1 blockade-nonresponsive
state of CD8 T-cell exhaustion, and that administration of DNA-demethylating agents (such as decitabine) prior to PD-1 blockade therapy may enhance the reinvigoration of antitumor CD8 T cells. This suggests that combining a PD-1 blockade drug with a DNA-demethylating agent may improve the therapeutic response (40).

In evaluating the efficacy of immunotherapy, it is worth noting that changes in cfDNA may occur earlier than changes in tumor size. Radiological evaluation of the efficacy of immune checkpoint inhibitors during therapy is therefore limited, and delayed clinical responses have been observed in some patients (18, 19). These results suggest that conventional radiological imaging may not reliably predict overall survival in response to immune checkpoint blockers. By contrast, ctDNA could be a promising means to monitor treatment efficacy. In one prospective proof-of-principle study, lack of detection of ctDNA at week 8 following treatment was a significant predictor of progression-free survival and overall survival in patients with lung cancer, uveal melanoma, and some forms of colorectal cancer following treatment with nivolumab or pembrolizumab (41).

Some Chinese companies, including Innovent Biologics (Suzhou) Co., Ltd., Jiangsu Hengrui Medicine Co., Ltd., and Shanghai Junshi Biosciences Co., Ltd., are developing new PD-1/PD-L1 inhibitors that are currently in clinical trials. The authors believe that the biomarkers described above would be very helpful in these trials.

**Role of cfDNA in T-cell transfer immunotherapy**

T-cell immunotherapy is a potentially life-saving treatment for patients with advanced stage tumors. As with immune checkpoint inhibitor treatments, there are still no good biomarkers that can identify responders or predict patient response. Since changes in ctDNA can be used to monitor the course of a disease, treatment responses, and recurrence (42–45), ctDNA levels might be useful

<table>
<thead>
<tr>
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<th>Cancer type (number of studies)</th>
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<tr>
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<td></td>
<td></td>
<td>Gastric cancer (2)</td>
</tr>
<tr>
<td></td>
<td>Not yet recruiting</td>
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<td>Hepatocellular carcinoma (2)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Lymphoma (2)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Breast cancer (1)</td>
</tr>
<tr>
<td></td>
<td>Active, not recruiting</td>
<td>4</td>
<td>Colon cancer (1)</td>
</tr>
<tr>
<td></td>
<td>Completed</td>
<td>4</td>
<td>Biliary tract cancer (1)</td>
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<td>Lung cancer (1)</td>
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<td>Enrolling by invitation</td>
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</tr>
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<td>Completed</td>
<td>2</td>
<td>Head and neck cancer (1)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Leukemia (1)</td>
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<tr>
<td></td>
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<td>Others (2)</td>
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</table>

for monitoring efficacy of T-cell immunotherapy. A recent study showed that malignant melanoma patients who showed an early peak in ctDNA, but then cleared their serum of BRAF V600E ctDNA, were highly likely to show a complete treatment response over the next one to two years. This result may be instructive for other kinds of T-cell transfer immunotherapy approaches (46).

In China, dendritic cell/cytokine-induced killer-cell therapy is still the most common T-cell immunotherapy, used mainly for the treatment of blood malignancies (47, 48), but its efficacy for treatment of solid tumors has yet to be demonstrated. There is an urgent need for appropriate biomarkers to predict efficacy and to screen patients to find those potentially responsive to T-cell immunotherapy. cfDNA- and ctDNA-related biomarkers might be good options to explore.

**Clinical trials of cfDNA in China**

Not surprisingly, clinical studies of ctDNA in precision cancer research are growing in number and importance both in China and worldwide. As shown in Table 1, there are currently 59 clinical trials involving ctDNA in China, including the Chinese mainland (41 studies), Hong Kong (1 study), and Taiwan (17 studies). Only 6 have been completed, while most are recruiting or preparing to recruit patients. Twenty-nine clinical trials involve lung cancer patients (particularly NSCLC), accounting for half of all studies underway, no doubt because

<table>
<thead>
<tr>
<th>Institution</th>
<th>Research description</th>
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<tbody>
<tr>
<td>Peking University People’s Hospital</td>
<td>Comparison of ctDNA and tumor tissue DNA by targeted sequencing in non-small cell lung cancer (NSCLC)</td>
</tr>
<tr>
<td></td>
<td>Urinary ctDNA detection in NSCLC: a prospective study</td>
</tr>
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<td></td>
<td>ctDNA detection in surveillance of surgical lung cancer patients</td>
</tr>
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<td></td>
<td>Dynamic changes of ctDNA in surgical lung cancer patients</td>
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<tr>
<td>Sun Yat-sen University</td>
<td>ctDNA dynamic monitoring and its prognostic role in stage 1 NSCLC by NGS</td>
</tr>
<tr>
<td></td>
<td>A prospective, observational trial of the diagnostic and prognostic uses of lung neoplasms and meningeal carcinomatosis</td>
</tr>
<tr>
<td></td>
<td>ctDNA for the prediction of relapse in gastric cancer</td>
</tr>
<tr>
<td></td>
<td>Detecting cell-free DNA in lung cancer patients</td>
</tr>
<tr>
<td>Peking University</td>
<td>Liquid biopsy in monitoring the therapeutic efficacy of targeted therapy in advanced/metastatic gastric cancer</td>
</tr>
<tr>
<td></td>
<td>PD-1 knockout engineered T cells for castration-resistant prostate cancer</td>
</tr>
<tr>
<td></td>
<td>PD-1 knockout engineered T cells for muscle-invasive bladder cancer</td>
</tr>
<tr>
<td>Peking Union Medical College Hospital</td>
<td>Clinical application of ctDNA in operable breast cancer patients</td>
</tr>
<tr>
<td></td>
<td>Effectiveness of circulating DNA for predicting the relapse and overall survival in NHL patients</td>
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<tr>
<td></td>
<td>Circulating cell-free DNA as a predictive biomarker for hepatocellular carcinoma</td>
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<tr>
<td>Beijing Cancer Hospital</td>
<td>Blood detection of EGFR mutation for Iressa (gefitinib) treatment</td>
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<tr>
<td></td>
<td>Study of small doses of etoposide as maintenance treatment in small cell lung cancer (SCLC)</td>
</tr>
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</table>

TABLE 2. Leading research institutions doing clinical studies of circulating tumor DNA (ctDNA) on the Chinese mainland.

Data from ClinicalTrials.gov (https://www.clinicaltrials.gov). NHL, non-Hodgkin’s lymphoma; EGFR, epidermal growth factor receptor; NGS, next-generation sequencing.
lung cancer causes more deaths worldwide than any other carcinoma. Certain cancers such as hepatocellular carcinoma and nasopharyngeal cancer get more attention in China than the United States or Europe, because of higher domestic incidence. Most sponsors of Chinese clinical trials involving ctDNA are domestic universities, hospitals, or companies, with the exception of the multinational pharmaceutical company AstraZeneca. Leading research institutions conducting clinical studies of ctDNA on the Chinese mainland are shown in Table 2.

**Future directions: cfDNA studies in the real world**

The application of cfDNA in the management of cancer diagnosis and treatment in China faces some challenges. First, it is important that cfDNA-related clinical data be gathered from Chinese patients, as data from other populations might not translate well to the local population. Studies should cover the entire process, from diagnosis through treatment, including early diagnosis; identification of tumor tissue sources; choice of therapy, whether chemotherapy, radiotherapy, targeted therapy, or immunotherapy following surgery; and decisions about optimization of treatment options. Complete clinical and follow-up data should be collected within a researcher-centered, normative multicenter clinical study, following international guidelines for NGS. A guide to the use of NGS with cfDNA samples must also be established so that results from these studies are comparable, accurate, reproducible, and can be easily applied in the clinic. Finally, government and industry should invest substantially to carry out this research and help to establish a model for collecting large amounts of data, including clinical data on cfDNA use, early cancer screening, treatment options, guidance on personalized treatments, prognosis, monitoring of drug resistance, real-time treatment adjustment, and monitoring of small lesions that may develop during treatment.

**References**

Next-generation sequencing–based testing for cancer precision medicine in China: A review of technologies and validation procedures

Weifeng Wang, Weiwei Shi, Ming Yao, and Kai Wang

With the advent of next-generation sequencing (NGS), which is much faster than traditional sequencing methods, our understanding of cancer genomics has grown rapidly and is revolutionizing our ability to treat and manage cancers with precision medicine. NGS has proven to be a reliable and precise diagnostic technology in developed countries. But in China it still faces challenges in the form of quality control and assurance problems, bioinformatics analysis pipelines, clinical annotation, and the broad selection of competing NGS platforms. Here, we review NGS-based testing in China, looking at the challenges it faces, and its clinical utility in precision medicine for Chinese cancer patients now and in the future.

Advantages of NGS-based testing technologies

NGS-based testing is an efficient and cost-effective technology that can inform cancer treatments by detecting genomic alterations in tumor tissues or liquid biopsies. The prevailing belief in cancer biology is that genetic alterations spur cancer initiation, evolution, and progression. However, early attempts at understanding these alterations were hindered by the cost and inefficiency of available technologies, such as Sanger sequencing. NGS-based testing, in contrast, can detect all classes of genomic alterations in a single test, and at a lower per-base cost. Those alternations detected include base substitutions, long or short insertions and deletions (indels), gene copy number variations, and gene fusions/rearrangements. Such comprehensive profiling provides a detailed genetic picture of a cancer patient. For instance, researchers have been unraveling the various mutation forms of the epidermal growth factor receptor (EGFR) gene, known to play a role in a number of different cancer types. In the past 15 years, researchers have developed therapies that target EGFR mutations, including base substitutions (1, 2), short insertions (3), duplications (4), and fusions (5) (Figure 1). Detection of such complex mutation profiles in individual patients would have been impossible without NGS-based testing. The seminal discovery about 10 years ago of the correlation between EGFR mutations and the clinical response to drugs called EGFR tyrosine kinase inhibitors (TKIs) in non-small cell lung cancers (NSCLC) ignited the pursuit

![FIGURE 1. Timeline for the past 15 years of detecting EGFR genomic alterations and corresponding targeted therapies.](image-url)
of precision medicine, which tailors individualized therapies according to the genetic and cellular characteristics of the patient. Thanks to its technical accuracy and clinical utility, NGS-based testing has become the primary method of informing precision cancer therapy.

In addition to its clinical utility, NGS-based testing advances the identification of those genomic changes that drive cancer, as well as the even larger number of passenger mutations that do not provide a growth advantage but simply “ride along,” significantly expanding our knowledge of the causes of cancer. With worldwide efforts from such projects as The Cancer Genome Atlas (TCGA) in 2005 and the International Cancer Genome Consortium (ICGC) in 2008, as well as vigorous bioinformatics data analyses, researchers are constructing a complete mutational atlas of all cancer types and their associated alterations. In the treatment of NSCLC, for example, analysis of the EGFR and anaplastic lymphoma kinase (ALK) genes has become part of routine testing to help doctors choose the appropriate targeted therapies in first-line treatment, substantially improving patient survival and quality of life. In addition to its proven value in targeted therapy, NGS-based diagnosis panels are finding a place in the rapidly developing field of immunotherapy by identifying those patients who are most likely to benefit from the PD-1/ PD-L1 inhibitor treatments. Tumor mutational burden (TMB)—the total number of mutations present in a tumor specimen—is becoming an important metric for predicting a patient’s response to immunotherapy (6). Tumors that harbor defects in the core DNA mismatch repair (MMR) protein complex are classified as being deficient in MMR and as microsatellite instability-high (dMMR/MSI-H) (7). In June 2017, the U.S. Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab, a drug targeting adult and pediatric patients identified as MSI-H or dMMR. This was the first time the agency had approved a cancer treatment based on a common biomarker rather than the tissue type in which the tumor originated. In light of these developments, comprehensive NGS-based gene profiling has the potential to act as a versatile tool for detecting not only gene alterations for targeted therapy, but also predictive markers associated with response rates to immunotherapy. This suggests that a well-designed combination of targeted therapy and immunotherapy based on genomic profiles could provide an impetus for new clinical trials to explore the relationship between the genomic data and the therapies.

With the anticipated approval of additional drugs, the U.S.-based National Comprehensive Cancer Network (NCCN) has been updating its list of recommendations for actionable gene testing in the treatment of NSCLC at an unprecedented pace (Figure 2). For example, a structural gene variant called “MET exon 14 skipping,” which is caused by a defective splicing of the gene’s RNA transcript, was added to the NCCN guideline on NSCLC shortly after the publication of papers that showed significant clinical benefits from MET inhibitors to patients with such mutations (8). In practice, NGS-based diagnosis can detect all of the NCCN-recommended targets, while having the flexibility to incorporate screening of
many more mutations as they become known. Laro-trectinib, the first small-molecule inhibitor specifical-ly targeting oncogenic chimeric proteins expressed from fusion genes containing neurotrophic tyrosine receptor kinase (NTRK) plus other gene partners, has demonstrated consistent and durable antitumor activity in clinical trials of both adults and children with 17 different cancers. It is thus expected that the detection of NTRK fusions, TMB, or MSI-H using NGS sequencing panels will probably be approved as companion diagnostic tests in the near future (Figure 2). All of this will allow clinicians to identify more cancer patients with unique deleterious gene variants and help them determine therapeutic op-

tions quickly and cost-effectively.

NGS offers a single universal test that identifies predictive biomarkers for both targeted and immu-notherapies. The high-throughput capacity of NGS-based testing is evident when it is compared with traditional molecular assays, such as fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR). FISH primarily detects amplifications and rearrangements of known genes, while PCR merely detects variations of a single nucleotide and known insertions or deletions (Table 1). So far, NGS is the best diagnostic technology for examining all types of genomic alterations in a single experiment, regardless of prior knowledge, and at much lower per-base cost than traditional techniques.

**Challenges facing NGS adoption**

Cancer is a major public health problem in China. According to a recent report, it is the leading cause of death in the country, with new patients diagnosed at a rate as high as 12,000 cases each day (9). As a developing country with the world’s largest population of patients, China has a pressing need to provide precise and efficient therapies. Targeted therapy has been increasingly used in recent years in addition to traditional chemotherapy. Unfortunately, the genetic characteristics of tumors can vary widely, which means that not all tumors have the same therapeutic targets. Therefore, precise matching of treatments to each patient’s clinically actionable targets will be crucial to the success of precision cancer medicine. This is of particular importance in developing countries like China where the cost of therapies is a big concern. Many of the top hospitals and cancer centers in the United States have established NGS screening platforms for cancer patients. In China, clinical molecular diagnostic laboratories are also adopting NGS-based diagnostics. The technology is at the epicenter of a paradigm shift, and has attracted a tremendous amount of attention from doctors, scientists, and entrepreneurs, which, in turn, is catalyzing its refine-

<table>
<thead>
<tr>
<th>ARMS PCR</th>
<th>Digital PCR</th>
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<th>IHC</th>
<th>Sanger sequencing</th>
<th>NGS (target sequencing)</th>
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<tbody>
<tr>
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<td>Inefficient</td>
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<td>1%-5%</td>
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<td>Known CNV and fusion</td>
<td>Protein expression</td>
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<td>No</td>
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</tr>
</tbody>
</table>

* TABLE 1. Comparison of detection techniques in clinical application.* ARMS, amplification-refractory mutation system; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; SNV, single-nucleotide variant; indel, insertion or deletion; CNV, copy number variant; MAF, mutant allele frequency. *Some special protocols not included. MAF sensitivity of FISH and IHC influenced by subjective interpretations.
of participating laboratories were able to detect all known variants in standard samples provided by the National Center for Clinical Laboratories of the Chinese Ministry of Health. This prompted the Chinese Society of Clinical Oncology and the China Actionable Genome Consortium to release a consensus statement on the application of NGS technology to precision cancer medicine. Similarly, in 2017, the U.S. Association for Molecular Pathology and the College of American Pathologists published a joint recommendation on guidelines for validating NGS-based cancer sequencing screening panels. As expected, both sets of recommendations emphasized the importance of standard NGS operating procedures and reliable NGS equipment to guarantee high-quality genetic information from cancer samples. Stringent standard operating procedures and quality control checkpoints should be imposed at each step of the NGS-based testing pipeline. This includes sample preparation, DNA library preparation, sequencing, data analysis, annotation of results, and reporting of results. Additional attention should be paid to the criteria by which NGS panel tests are judged to be valid. These include types and number of samples, reproducibility and repeatability of variant detection, reportable range (region of the genome with acceptable quality results), reference range (the spectrum of nonpathogenic base changes observed in a population), limits of detection, interfering substances, clinical sensitivity and specificity, and if appropriate, validation of bioinformatics pipelines and other parameters. As emphasized by OrigiMed, a Shanghai-based diagnostics service provider, NGS-based diagnosis in China should have meticulously designed, well-tested platforms run by trained personnel, and backed up by adequate infrastructure. Another recommendation was that annotations explaining genomic alterations and potentially actionable sites or signaling pathways should be clearly described for each gene.

The depth and breadth of sequencing coverage are two important technical factors affecting the quality and cost of NGS-based testing. The numbers of target genes analyzed can differ substantially depending on the type of screening used, with the breadth of sequencing ranging from just a few variants to a few hundred using sequencing panels, to the entire coding gene region [whole-exome sequencing (WES)] or even to the entire genome [whole-genome sequencing (WGS)]. The selection of which genes to screen for in clinical applications more or less obeys the “Goldilocks principle,” namely that a small number of genes may generate an insufficient number of clinical clues, while a large number of genes could produce too much information. Specifically, the excessive genomic information generated by large sequencing ventures provides limited additional clinical value while posing the huge challenge of having to interpret incidental findings of variants of unknown or uncertain significance. Furthermore,
sequencing too wide a DNA region drives up expense due to the need to sequence to sufficient depth for the data to be valid, and can lead to poor sensitivity if depth of coverage is insufficient. More narrow sequencing coverage costs less, but significantly decreases detection sensitivity and may ultimately result in data that is difficult to interpret. If sequencing coverage is less than 300x, only 84% of low-frequency single-nucleotide variants (<5% mutant allele frequency) are detected, and the rate of detection for short variants is even poorer (12). Previous publications have shown that druggable mutations with low mutant allele frequency cannot be detected by low-coverage WGS/WES, or by sequencing panels, and that this may affect treatment options. On the other hand, most gene fusions, such as those found in the ALK gene, cannot be detected by WES alone unless the breakpoints are within or in close proximity to exons, and may require WGS.

A small NGS panel offers an affordable way to sequence fewer than 10 genes containing mutations common in certain cancer types, but risks missing several critical treatment options due to incomplete gene profiling (Table 2). The incorporation of more genes into the panel improves the quality of genomic mutation profiling and increases the number of treatment options, but it still fails to reflect the overall TMB, which is an important predictor of how effective immunotherapy will be. In order to maximize a patients’ treatment options, an even larger panel consisting of several hundred relevant genes is recommended. However, this is not always available in China. The leading NGS panel-based product in the Chinese market, YuanSu from OrigMed, sequences 450 well-defined cancer genes known to be important for targeted therapy, and analyzes TMB, microsatellite instability, and potential resistance to immunotherapy treatment (also incorporating RNA sequencing to detect more gene fusions and splice variants).

The present and future of NGS-based testing in China

In 2015, the Chinese Ministry of Science and Technology held the first nationwide conference of experts to devise and promote a strategy for precision medicine. One of the primary accomplishments of the conference was a pledge from the government to invest 60 billion RMB (US$9.24 billion) in the field of precision medicine by the year 2030. NGS-based technology is one of the key components of this investment.

China is preparing for the expanded use of NGS-based technology in precision medicine, especially in the area of cancer. The American company Illumina retains the largest share of the market for sequencing hardware, with around three hundred NGS machines installed so far; other vendors provide the majority of reagents and consumables. But several domestic Chinese companies have entered the NGS market in recent years. More than 10 companies have developed their own NGS machines, some of which have been approved by the China Food and Drug Administration. These include the BGISEQ-500, developed by BGI, and the BioelectronSeq 4000, made by CapitalBio Corporation. However, domestic companies still have a long way to go to reach the high bar set by their foreign competitors. In the sequencing services and clinical testing segment of the market, the lack of good training and supervision means that most of the approximately 160 sequencing service companies in China’s eight major cities fail to meet reliability standards for their sequencing data analysis.

China is still behind the United States in the clinical use of NGS-based testing. Most hospitals in China remain heavily reliant on traditional molecular diagnostics like PCR, immunohistochemistry, and FISH as the major cancer diagnosis and prognosis tests. There are three main reasons why this situation exists. First, NGS-based testing involves a series of procedures—including sample preparation, DNA library construction, sequencing, bioinformatics analyses, and clinical annotation—that each require stringent standards of operations and quality control and assurance. As discussed above, China is not readily set up for these requirements in most domestic hospital laboratories. While the business of commercial assays has boomed in the past two years, only a few companies have the necessary analytical and clinical validation for their NGS-based assays. And the importance of validation is not yet appreciated by every doctor in China.

Second, numerous targeted cancer therapies have been approved by the U.S. FDA, and many more are being studied in clinical trials, but experience in the appropriate use of the new drugs is relatively limited in China. Doctors and patients tend to opt for traditional diagnostics like single gene-based tests, instead of comprehensive diagnostics like NGS-based assays. Finally, the cost of NGS-based testing is still too high for most Chinese patients. Generally, the price of a comprehensive NGS-based assay ranges from US$1,500 to US$3,000, depending on the size of the panel assay and the quality.

Although NGS-based cancer testing is still in its infancy, it is soon expected to become the major component of worldwide clinical NGS diagnostics,
including in China. It is expected that academia and industry in China will continue their efforts at building sequencing hardware and designing reagents. More importantly, China will be expanding the academic and clinical application of NGS-based assays with a focus on exploring cancer etiology and pathogenesis, and providing a genetic basis for personalized therapy with the help of cancer genome sequencing, as well as integrating various NGS-based technologies to aid ongoing research in conventional clinical medicine. The hope is that these steps will lead to significant improvements in precision medicine, including disease diagnosis, treatment, and clinical decision-making for Chinese cancer patients.

Looking to the future

NGS-based testing is one of the most exciting developments in oncology research and clinical application in recent years. It provides a more comprehensive and efficient approach for characterizing the genomes of individual cancer patients and matching them with treatments for optimal clinical management. Most hospitals in China are not fully ready to run NGS platforms in-house. But with the concerted efforts of the government, researchers, and companies, precision cancer medicine in China is developing rapidly. More detection technologies are emerging for clinical applications, including ultrasensitive circulating tumor DNA detection and exosome and epigenome profiling. We envision a future where each cancer patient will be tested by a comprehensive gene panel at several points in time and wherever they are receiving treatment, with a range of sequencing technologies that will detect multilevel molecular abnormalities and help clinicians design a personalized, precise therapy tailored to their individual needs.

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A wealth of information about a cancer can be obtained using liquid biopsy, a noninvasive blood test. The diagnostic potential of liquid biopsy is best illustrated by the development of the companion diagnostic for osimertinib (a third-generation targeted therapy for patients with non–small cell lung cancer). Osimertinib was approved by the Chinese Food and Drug Administration in just seven months, a landmark achievement in lung cancer therapeutics. A major contributor to its success was the codevelopment of a companion assay for detecting the EGFR T790M mutation (1–3), which commonly emerges in lung tumors after treatment with first-generation therapies. When tissue samples were unavailable, mutation detection could be done using liquid biopsy technology, which required only a small sample of patient blood. Liquid biopsy testing has been so effective in tracking EGFR T790M that the practice is now strongly recommended by the European Society for Medical Oncology (4) and in the National Comprehensive Cancer Network (5) guidelines. In addition, the Chinese Lung Cancer Summit Committee formally endorsed the use of liquid biopsy for the detection of EGFR T790M in 2016 (6). Although the emergence of a tissue-based molecular diagnostic market in Asia has been driven largely by the high occurrence of epidermal growth factor receptor (EGFR) mutations in the region, EGFR mutation detection is just the beginning. With a concerted effort, liquid biopsy technology has the potential to redefine the field of clinical molecular diagnostics not just in Asia, but worldwide.

Challenges for Chinese ctDNA-NGS clinical diagnostics

The tremendous potential of liquid biopsy is offset by the technological challenges it presents. Any practical liquid biopsy diagnostic assay based on circulating tumor DNA (ctDNA) must...
facilities authorized to perform ctDNA-NGS assays), 33 passed the evaluation and among these, only 17 received perfect marks (15). The lack of technical development and operational protocols at these pilot sites has resulted in the use of off-site, third-party testing services, reducing adoption. Thus, the rapid adoption of ctDNA testing in China is contingent upon the standardization of testing protocols.

One solution: hospital-owned ctDNA-NGS clinical labs

One way to promote the use of ctDNA-NGS is by providing widely accessible testing guidelines or kits that would enable hospitals to confidently perform in-house NGS testing. This “kit-able” solution would include such essential features as a “low-touch workflow” requiring little or no NGS expertise and a self-contained data analysis pipeline. Such a kit could then be easily translated into an automated technology for informing personalized therapeutic treatment at scale.

Although a few kit options are available for tumor tissue sequencing, kit options for NGS-based ctDNA sequencing are far scarcer. Historically, performance comparisons of NGS-based ctDNA kits have highlighted their relative trade-offs. Capture-based assays have demonstrated high specificity and multiplexing capacity but limited sensitivity and a complex workflow. A single AVENIO capture-based assay from Roche (11, 16), for example, can cover >100 kilobases and detect single-nucleotide variants, insertion-deletions, and fusion alterations at a rate of 96%-99%, at allele frequencies of 0.5%-1%. In contrast, amplicon-based assays (17) offer high-performance sensitivity and ease of use (the Thermo Fisher

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<td>Example</td>
<td>AVENIO ctDNA Expanded Kit</td>
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<td>Oncomine cfDNA Lung Cancer Panel</td>
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TABLE 1. Comparison of liquid-biopsy ctDNA sequencing technologies. SNV, single nucleotide variant; indel, insertion or deletion; kb, kilobases.\(^a\)30-ng input, 0.1% allele frequency (AF) (27).\(^b\)20-ng input, 0.1% AF (28).\(^c\)AccuraGen analytical data for Firefly technologies. No false positive in 50 noncancerous individual plasma samples and 24 wild-type cfDNA standards. Average of 96% detection rate for 20-ng input at AF of 0.1.

have an extremely high and readily reproducible performance sensitivity and specificity (7, 8). CtDNA—fragmented DNA from a tumor that is free-floating in the peripheral blood—is present in minute quantities (on the order of a few nanograms per milliliter of plasma). As a result, isolating ctDNA signals amid the noise generated by noncancer DNA fragments and sequencing errors is difficult. Droplet digital polymerase chain reaction (ddPCR) and next-generation DNA sequencing (NGS) are among the only quantitative technologies able to meet the sensitivity demands of low-frequency mutation detection in ctDNA (9, 10). Between the two, NGS offers several advantages. It has greater multiplexing capacity (where multiple genes can be analyzed in parallel from the same input) and is better at detecting de novo mutations. The performance sensitivity and specificity of NGS technologies, however, varies significantly (11–13).

CtDNA testing is also not without regulatory constraints. Unlike in the United States, medical facilities in China perform most clinical testing in-house. While this is an effective cost-saving practice for established testing protocols, the ctDNA-NGS diagnostic assay is difficult to deploy on-site without highly specialized technicians. As a result, the use of ctDNA-NGS testing is carefully monitored by the Chinese National Health and Family Planning Commission (NHFPC) (14). To date, 26 facilities have received government authorization to offer NGS testing for the purposes of oncological treatment. While the availability of these services is a promising first step, a formal assay performance evaluation conducted in 2015 by NHFPC revealed an alarming variability in results. Of the 64 sites (including the 26 facilities authorized to perform ctDNA-NGS assays), 33 passed the evaluation and among these, only 17 received perfect marks (15). The lack of technical development and operational protocols at these pilot sites has resulted in the use of off-site, third-party testing services, reducing adoption. Thus, the rapid adoption of ctDNA testing in China is contingent upon the standardization of testing protocols.
Oncomine kit has a turnaround time of just two days, but provide limited coverage and few opportunities for assay customization. Amplicon-based assays also tend to produce more false positives. We believe that AccuraGen's technology, Firefly, brings together the best of amplicon- and capture-based assays (18). Firefly can detect a breadth of genetic alterations with a sensitivity approaching the physical limit and a specificity 10-fold better than benchmark, all while maintaining the added benefits of flexible panel design and workflow integration. It has a consistent error rate of 1 in 1 million at the molecular level and can uncover 0.1% of variants in 20-ng samples of cfDNA at a detection rate greater than 90%.

The backbone of the Firefly technology is its use of rolling-circle amplification to empower consensus-based concatemer error correction. Single-stranded ligation without the usage of any barcode results in a high conversion rate, and rolling-circle template amplification minimizes the proliferation of enzymatic errors common in PCR. Finally, tandem repeats in concatemers make mutation detection both accurate and economical.

**Emerging challenges facing NGS-based ctDNA mutation assays**

Although the clinical utility of ctDNA sequencing is currently oriented toward targeted therapy selection, mounting evidence suggests it may also be used to track the progression of cancer (19). Two studies monitoring postsurgical residual disease in breast (20) and colorectal (21) cancer patients demonstrated the predictive value of ctDNA in determining the likelihood of cancer relapse was far greater than estimates made using traditional clinical imaging evidence. While these studies foreshadow exciting future applications of ctDNA liquid biopsy assays, their limited genetic scope and small patient sample sizes impede their clinical actionability. Fortunately, new tools with increased panel sizes and product accessibility have since emerged. Recently, UK-based TRACERx (TRAcking Cancer Evolution through therapy (Rx)) reported the superiority of a 30-plex panel over a single plex or lowplex panel (up to 4 amplicons) in detecting cancer relapses and tumor progression (22, 23). Empowered by Firefly technology, AccuraGen currently offers Accu-Act, a 61-gene capture-based panel for residual disease monitoring and strategic cancer management. AccuraGen's amplicon-based ctDNA kit, Accu-Kit, which detects only genes included in NCCN guidelines, is easy to use, customizable, and affordable.

Increasing panel size, however, creates new challenges for developing a viable assay. The underlying genetics of tumor cells vary tremendously both within a single tumor and from one tumor to another. How will intra- and intertumoral heterogeneity be addressed? How can assay results be both highly sensitive and easily reproduced so they can be clinically actionable? How will diversified subclone mutation profiles be integrated to generate a holistic overview of the disease? Although these problems are complex, they are not insurmountable.

A combination of ctDNA-NGS kits and laboratory-developed tests will likely address the majority of known clinical needs for ctDNA. Though China's NHFPC has yet to publish official guidelines, several clinical committee consortiums have released their own recommendations to guide independent clinical testing (24–26). AccuraGen is uniquely positioned to empower NHFPC-certified facilities to act on these recommendations by equipping them with clinical kits for on-site testing. By making liquid biopsy technology more accessible and lowering barriers to workflow integration, we believe that AccuraGen can help bring cutting-edge research to the bedside.

Although the most effective way to encourage widespread liquid biopsy adoption would be CFDA approval of a ctDNA-NGS in vitro diagnostic kit, the likelihood of this occurring in the near future is low. In the short-term, ctDNA-NGS assays will need to be performed in-house at NHFPC-approved clinics. Those facilities will need the expertise to generate high-quality mutation profiles; they already regularly perform a wide array of diagnostic tests. At AccuraGen, we believe that with superior technology, ctDNA-NGS assays can be as easy to use as any other tools in the clinic's toolbox.

Regulatory processes, while slow, are necessary safeguards for delivering quality services. But regulation should not stonewall innovation. New products envisioned by teams of scientific experts and seasoned physicians will still be developed. AccuraGen is committed to advancing the frontiers of molecular diagnostic testing using ctDNA-based NGS technology, and we believe that China is in the vanguard of a revolution in this area.

**References**

Adoptive cell transfer therapy: A strategic rethinking of combination cancer therapy

Minghui Zhang

In recent decades, significant progress has been made in immunotherapy treatment, which is regarded by some as the latest battlefield in a long war against cancer. In 1890, the father of immunotherapy, American bone surgeon William Coley, used a mixture of two dead infectious bacteria strains to treat patients with inoperable cancers and achieved a complete remission rate of 10%. American biologist Paul Ehrlich speculated about the possible existence of immune tumor surveillance—the process by which the body’s immune system is constantly seeking out and destroying tumor cells. As more tumor antigens are identified and characterized, we gain a deeper understanding of how the immune system fights tumors. Recently, several “immune checkpoint blockers,” which promote the ability of certain cells to attack cancer, have had clinical success. Adoptive chimeric antigen receptor (CAR) T-cell transfer, a therapy in which genetically modified T cells are transferred into a patient [and a type of adoptive cell transfer (ACT)], has been approved by the U.S. Food and Drug Administration. But the efficacy of these treatments needs improvement, and researchers are still trying to mitigate their adverse side effects.

Scientists are also looking for ways to develop effective therapies that combine immune treatment with conventional cancer therapies to increase therapeutic benefit, minimize harm, and provide a cure or long-term remission. Tumors use multiple means to evade detection by the immune system. A better understanding of the mechanisms of tumor development and the reasons for success or failure of current therapeutic strategies is needed for the development of more effective treatments. ACT, which in essence provides more effective immune cells to fight against tumor cells, is playing a key role in cancer treatment today. However, there are sever-
al different forms of ACT. Here, we will discuss ways that ACT might be exploited as a broad-spectrum therapy and be combined with traditional treatments to increase its efficacy.

**Tumor development**

It is well accepted that cancer is a disease that develops and progresses because of the accumulation of genetic and epigenetic changes that affect the way genes are expressed. Genetic drivers of tumor formation and growth are confirmed by gene sequencing profiles and the study of proteins expressed in and on the tumor cells. However, deeper understanding of the effects of the immune response on tumors is leading to the realization that the immune system is the most important extrinsic influence on the fate of mutant cells: If immunosurveillance works correctly, mutant cells will be recognized and eradicated (Figure 1), explaining why certain people don’t get cancer. If the immunosurveillance system cannot recognize or eradicate the mutant cells, these cells will have the opportunity to develop into a tumor. This principle is also expressed as the “three Es” of cancer immunoediting: elimination (the immune system finds and destroys cancer cells), equilibrium (cancer cells that are not destroyed right away may exist in a delicate balance between growth and control by the immune system), and escape (the tumor cells escape immune system surveillance and begin growing in an environment in which the immune response is suppressed).

Substantial genetic heterogeneity has been detected both in different samples from the same kind of primary tumors and metastases, and in individual cancers from the same patient. Precision cancer medicine in its current form is heavily dependent on genetic testing, and promotes the molecular characterization of tumors in order to develop more effective and beneficial drugs for patients carrying certain mutations. However, the benefits often don’t persist, as the tumor cells can develop resistance to drugs by

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**FIGURE 1. Factors that allow for tumor development.** MHC, major histocompatibility complex; CTL, cytotoxic T-lymphocyte; Treg, regulatory T cell; Breg, regulatory B cell; NKreg, regulatory natural killer (NK) cell; DCre, regulatory dendritic cell; MDSC, myeloid-derived suppressor cell; APC, antigen-presenting cell.
the upregulation of a partially inhibited pathway, a mutation of the target gene, or an activation of alternative pathways, for example. Many patients will experience drug resistance, cancer progression, and ensuing metastasis.

The immune system can recognize mutant cells and eliminate many early malignancies. However, tumors have a variety of means to evade immune attack. They can decrease the expression of major histocompatibility complex class I molecules to escape natural killer (NK)-cell cytotoxicity, and increase the expression of coinhibitory molecules to inhibit T-cell activation and function. A tumor may also establish an immunosuppressive state in the tumor microenvironment by producing regulatory cytokines, including indoleamine 2,3-dioxygenase, vascular endothelial growth factor, and TGF-β, and by recruiting myeloid-derived suppressor cells and regulatory T cells. Overcoming these obstacles and strengthening the immune response are the keys to successful immunotherapy.

The current state of ACT

Immunotherapy can be classified into two broad categories. The first is nonspecific immune stimulation, and includes cytokine immunotherapy (which acts to stimulate the immune system to fight the cancer), oncolytic virus immunotherapy (which selectively kills tumor cells while also stimulating antitumor immunity), Toll-like receptor agonist therapy (which activates both innate and adaptive immune responses), and some forms of ACT (such as the transfer of natural killer cells into the patient), among others. The second category is specific immunotherapy, including dendritic cell (DC) vaccines, other kinds of ACT (for example, the transfer of T cells), and immune checkpoint inhibitors.

Among these approaches, ACT may be the most complicated. As eluded to above, it encompasses both nonspecific and specific immune stimulation. The cells are produced in one of two ways. In the first strategy, the doctor may isolate specific immune cells from a patient’s tumor (such as tumor-infiltrating lymphocytes) or from surrounding blood, culture them in the lab to expand their numbers, and then administer them back into the patient. In the second strategy, T cells or NK cells collected from the patient are engineered with new receptors to recognize specific antigens on the surface of cancer cells, generating so-called CAR-T cells or CAR-NK cells. The recombinant cells are then infused back into the patient following expansion in culture. ACT therefore uses immune cells to either promote the immune response or to destroy the cancer cells that carry specific antigens.

CAR-T is currently the most promising strategy. It has demonstrated clinical benefits in some leukemias (1–4). However, because few specific tumor antigens have been identified, the effect of CAR-T on solid tumors is still marginal. In addition, the side effects of CAR-T can be dangerous or even life threatening (4–6).

Less attention is being paid to nonspecific ACT therapies (NK or DC therapy). This may be because the effect of these therapies is hard to assess using the present evaluation system. The transferred cells often trigger inflammation inside the tumor, leading to an apparently larger tumor in the short term. Another key factor is the timing of ACT. Some advanced tumor patients have tried various other treatments before they choose cellular therapy. It is therefore difficult to isolate the specific effects of the ACT treatment and evaluate what its real clinical benefits might be if used at the optimal time. Furthermore, ACT therapy must be adjusted depending on the patient’s condition, the type of cell transferred, the number of effective cells, and the frequency of treatment, making it complicated to administer.

The future of ACT

The complexities of tumors, including their heterogeneity and their ability to suppress an immune response, are at least partly responsible for therapeutic failures. There has been great progress made with traditional cancer treatments, including surgery, chemotherapy, radiation therapy, and targeted therapy. However, these modalities often cannot completely eradicate tumor cells. And sometimes micrometastases are occurring even as the tumor is diagnosed. Scientists face the question of how to build a new strategy that preserves the benefits of traditional treatments but overcomes their limitations and provides long-term remission or even a cure.

Combining multiple treatments offers a potentially powerful approach. The innate and adaptive parts of the immune system work together to sustain a state of equilibrium in the body. If we can understand more deeply the antitumor mechanisms of the immune system, design ACT therapies accordingly, and use them in combination with traditional cancer treatments, tumor cells could be eradicated and active immunosurveillance reinstated. Thus, in addition
to killing tumor cells, one of the most important effects of ACT is to reverse a tumor's ability to suppress the immune response. A strengthened immune system can work as a policeman so that tumor cells that are not killed by traditional cancer treatment can be either eradicated or controlled by the immune system. We predict that this type of combination therapy will lead to the elimination of many cancers, or at least to longer survival with no worsening of the symptoms (Figure 2).

In the battle against tumors, surgery can be considered the vanguard, decreasing the tumor burden and eradicating most tumor cells. Chemotherapy, radiation therapy, and targeted therapy are the troops sent in to attack inoperable cancers. And immunotherapy, especially ACT, becomes the rear guard, killing escaped tumor cells and creating a microenvironment in which metastasis cannot occur. A "special forces" corps—for example, more recently identified cell subsets that are strongly cytotoxic to tumors—would be a major help to the body in its battle against cancer. Each patient is different, and winning this battle requires a personalized regimen in which ACT therapy with broad-spectrum antitumor effects may be a critical factor.

Following this principal, we treated cancer patients with personalized combination treatment protocols. Based on our finding that genetically dissimilar DCs can induce the expansion of a specific subset of natural killer T (NKT) cells that are more strongly cytotoxic to tumor cells, we carried out a clinical trial of ACT with NKT cells taken from patients with solid tumors. We used NKT cells cultured in vitro to treat patients after surgery to decrease the tumor burden, or after chemotherapy or radiotherapy to kill most of the tumor cells. We observed in this study that many recipients of targeted therapy took longer to develop drug resistance when their therapy was combined with adoptive NKT transfer. The preliminary results of the study are quite promising, and some of the patients have experienced a complete remission of their cancer (Figure 3, next page) (unpublished observations). Final results are still pending.

Our strategy suggests that well-designed, personalized combination cancer treatments, especially those using adoptive transfer of cells...
with strong cytotoxicity against tumors, might provide enhanced clinical benefit for cancer patients. Further investigation is needed to optimize the strategy, and to clarify the effects and mechanisms underlying the combination of targeted therapy with ACT. We believe we are on the right track and that patients will benefit from this strategy.

References

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**FIGURE 3.** The clinical benefits of adoptive novel NKT cell transfer in two patients. A gastric cancer patient with lung metastasis (upper panel) and a pancreatic cancer patient with liver metastasis (lower panel) were treated with adoptive transfer of NKT cells after surgery. The images show that the tumors in both cases shrink and eventually disappear completely.
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