Precision medicine in China

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Precision medicine is a lofty goal, put forth as the pinnacle of medical treatment. Boiled down to its essence, precision medicine describes the ability to tailor therapies to a patient's individual needs by examining their particular physiology, genome, and environment. The ideal is that individualized treatment will lead to lower costs, fewer side effects, and better outcomes.

The term “precision” in precision medicine has come to be used in preference to “personalized,” since it is far more difficult (and costly) to arrive at truly personalized treatments for each individual, but is not beyond our reach simply to treat patients—or groups of patients exhibiting similar biomarkers—in a more precise manner.

A central element of precision medicine is accurate, efficient, and effective diagnostic testing. This capability enables clinicians to classify subpopulations of patients who might better respond to certain therapies; much work has been done in the cancer arena to achieve this. Accurate diagnosis can lead to more accurate prognosis and improved monitoring of disease progression or treatment. The development of trustworthy in vitro diagnostic tools and technologies is essential for achieving this goal.

Precision medicine in China, if not in its infancy, is still a long way from reaching maturity. However, it shows significant promise. The country provides unique opportunities for research, including a varied geography and a large population presenting with many different diseases, both common and rare. Furthermore, the government is clearly a strong proponent of precision medicine, supporting it financially through numerous initiatives and also through policy changes at a national level. The first chapter of this supplement outlines this factor in more detail, as well as providing insight into the development of point-of-care testing in China.

The second and third chapters describe how precision medicine is currently being applied in China, more specifically for diagnosis and treatment of cancers (chapter 2) and other diseases (chapter 3). As the population ages and becomes more urbanized, the need for more targeted modalities for diagnosis and treatment is becoming more critical. Next-generation sequencing is the most frequently used methodology for precision medicine applications, although proteomics and metabolomics tests are gaining traction as they improve in accuracy and ease of use.

Only time will tell whether precision medicine will live up to its promise. There is little doubt that China intends to be a leader in this area and is counting on success, investing both money and resources.

Sean Sanders, Ph.D.
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Science/AAAS
Established in 2005 by Dr. Qingwei Ma, Bioyong Technologies has dedicated itself to developing mass spectrometry (MS) technologies for more than a decade. After graduating from the China Agricultural University with his Bachelor’s degree in biology, Ma became interested in global business trends, leading him to join the China Center for Economic Research at Peking University, and eventually to pursue a doctoral degree in medical devices at the Academy for Advanced Interdisciplinary Studies. His diverse background and interests have given Ma a broad understanding of the life science industry, and he has led Bioyong Technologies to become a respected R&D-based innovation pioneer in this area.

The primary field of expertise at Bioyong Technologies is serum peptidomics—the proteomic analysis of polypeptides found in the blood. In order to improve recovery efficiency for low-abundance peptides in serum and other human-fluid samples, Bioyong Technologies developed a nanobead-based peptide extraction kit that has been well received in the research community.

Another technological innovation from the company is the rapid identification of microbes using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. Microorganism identification is usually a tedious procedure for researchers and clinicians alike. The Clin-TOF MALDI-TOF platform allows researchers to develop rapid MS-based microscope identification methods. Through collaborations with prominent Chinese hospitals and academic institutes, Bioyong Technologies has established its own microorganism identification platform and database that has helped physicians identify more than 370 genera, 2,200 species, and 7,900 strains of microorganisms.

Nucleic acid detection by MS is another area of expertise for Bioyong Technologies, for which the company has secured significant intellectual property. Often, knowing the mutation status of a patient’s somatic cells is not enough to provide an accurate diagnosis. The identification, isolation, and analysis of cell-free (cf) and circulating-tumor (ct) DNA can yield important information and more specific indications for precision medicine applications. Mass spectrometric characterization of cfDNA/ctDNA using Bioyong Technologies’ MS equipment is enabling researchers and clinicians to more clearly understand a patient’s condition and improve treatment choices. In fact, the Clin-TOF mass spectrometer was awarded an annual Best Product Award by Frost & Sullivan in 2014, exemplifying the company’s contribution to the in vitro diagnostics (IVD) industry. Recent international conferences have raised awareness among physicians across China regarding the use of MS for IVD.

All of these technologies can and have been applied extensively in the growing field of precision medicine, in an attempt to improve both diagnosis and prognosis for patients with a broad range of diseases. Journalists from the general media in China have become interested in covering this important area and its impact on public health. Reports have been published in the Beijing Daily and the Science and Technology Daily that have helped the average citizen understand and appreciate how new technologies can be applied to improve the delivery of health care.

Bioyong Technologies appreciates the opportunity to sponsor the publication of this supplement on precision medicine, and will continue to devote all of its efforts to creating a healthier future for patients in China and around the world.
Chapter one

Advancing precision medicine in China
Opportunities and advantages for the development of precision medicine in China

Qimin Zhan1,2,3 and Haili Qian2

As science and society evolve, technology has found its way into a wide range of applications in the medical field. The history of medicine shows that technological advances are the key to controlling infectious diseases, achieving breakthroughs in noncommunicable diseases, and developing new applications for clinical treatment. A cornerstone of health care, technological innovation plays a critical role in enhancing disease prevention and control, and improving the response to public health emergencies. The application of new technologies has contributed to better accuracy in diagnosis, improved strategies for care, and the discovery of novel drugs for customized treatments.

Completion of the Human Genome Project has led to extensive advances in research technologies in the fields of genetics and molecular biology, giving rise to the era of precision medicine. Using the technologies of modern genetics, molecular imaging, and bioinformatics, a patient’s genetic background and disease characteristics can be determined and placed into the context of that patient’s living environment, clinical history, and pathology, to achieve both precise classification and diagnosis of diseases and an individualized protocol for prevention and treatment.

At the frontier of medical development

The foundation of precision medicine is built on next-generation sequencing technology, but also goes beyond it to other molecular levels (RNA and proteins) and involves multiple disciplines, such as RNA- and protein-level analysis, molecular imaging, and microrobotics. The essence of precision medicine is twofold: precision diagnosis, and precision intervention and treatment. Precision diagnosis requires a thorough understanding of the disease based on the molecular and morphological changes observed in the context of the patient’s genetic background. Precision intervention and treatment, on the other hand, is required to perform functional and even structural correction at a molecular level, or at least to provide a valid alternative to conventional treatment.

Meeting public health demands

Today, most average citizens have increasing expectations of health and quality of life, and it’s only natural for them to expect that illnesses will be kept at bay, that diseases will be detected early enough to prevent progression, and that a cure will inevitably be available. It is such considerations that push medical science forward. Currently, however, medicine is not always able to meet these expectations. For example, the lack of precise indicators for disease or the technologies to detect them means that certain ailments simply can’t be identified until signs and symptoms become conspicuous, often too late for effective intervention and treatment. Furthermore, efficacy of treatment is evaluated on the basis of sometimes vague macroscopic or molecular indicators, making it impossible to precisely predict the outcome and prognosis of a disease. Lastly, the often small differences between individuals add to the difficulty of properly stratifying patients for optimal treatment, and increase the risk of side effects.

In the current model of clinical medicine, diseases resemble an iceberg: What we know from the patient’s chief complaints, symptoms, and results through physiological, biochemical, immunological, and imaging tests only constitutes the tip of the iceberg, while the bulk of it—the mechanisms and conditions beyond our understanding and capability—remains beneath the water. This means we are very often ignorant or shortsighted in our practices. Therefore, only when the pathogeneses of diseases are understood on a molecular level, when valid and effective molecular biomarkers are

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identified, and when molecular analyses are carried out based on histological and clinical classifications of diseases, can treatments be directed to precise targets.

**A historic opportunity**

The promise of precision medicine has added fresh impetus to medical innovation in China and offers a unique opportunity for China to catch up with those countries leading in medical technology R&D. Currently, China is working at the forefront of research involving methodologies for genomics and proteomics, and is witnessing rapid development in technologies for molecular imaging, drug target discovery, and big data, all of which puts China on an equal footing with the West. Going forward, China needs continuing scientific research, technological development, clinical translation, industrial incubation, and the wide adoption of precision medicine so that the optimal conditions for innovation will be created.

Precision medicine is not only an opportunity for China to revise its medical research model, but is also a test of its capacity to initiate reforms in science and technology. Success in the transition toward precision medicine will provide a solid foundation for China’s approach to the development of science and technology writ large. Precision medicine is the future of health care and offers a historic opportunity to both medical science and science in general. It will bring about a paradigm shift in diagnostics, disease classification, and clinical procedures, standards, and guidelines, and could potentially give rise to a number of emerging industries. Its model for development and operation will have far-reaching implications on other sectors of science and technology. Seizing this opportunity will give China a competitive edge in terms of medical development in the world arena, enhance China’s capacity for innovation, and create incentives for reform.

**China’s advantages in precision medicine**

China is different from the West in many ways, so precision medicine in the Chinese context has some unique features.

**Institutional support**

The Chinese government attaches great importance to precision medicine and has set out measures to facilitate its development. It has hosted strategic consultations with experts and has incorporated precision medicine into the 13th Five-Year National Science and Technology Innovation Plan, with focuses on disease cohort research, disease typing and staging on the molecular level, individualized clinical treatment, and the collecting and mining of big data. Several institutions have been selected to carry out pilot projects for the clinical use of high-throughput sequencing technology in cancer diagnosis and treatment. The National Health and Family Planning Commission has issued Guidelines on Genetic Testing of Drug-Metabolizing Enzymes and Drug Targets (for Trial Implementation) and Guidelines on Genetic Testing for Individualized Tumor Treatment (for Trial Implementation). These measures have laid a solid foundation for the advancement of health care in China, and will help shape its course in the future. Moreover, with support from universities, research institutes, and local governments, several high-caliber centers for precision medicine have already been established.

Through a series of national initiatives, China intends to promote precision medicine to address issues in health and disease control and stimulate growth in related health industries. Apart from the 13th Five-Year Plan mentioned above, multiple government departments also have development plans, and a 15-year outline through 2030 is also expected to be released by the Chinese Ministry of Science and Technology. Precision medicine involves multiple disciplines and government departments and brings up unprecedented legal questions, requiring the mobilization of medical and technological resources around the country and the cooperation and participation of industry. To ensure the success of precision medicine, a synergy has to be created between government, science and technology experts, universities, research institutes, and enterprises.

**Complete disease spectrum**

The Chinese people make up about one-fifth of the world population, and the country boasts a diversity of geographical and cultural environments—two factors that contribute to a wide spectrum of diseases. China therefore has a sufficient number of patients to research both common and rare diseases. Such rich resources will drive the precision medicine movement, enabling China to quickly advance in the medical arena and ultimately contribute to a healthier world.

**Solid foundation in biomedical technologies**

When China proposed its vision for precision medicine, it did not do so just to climb on the
bandwagon; rather, the decision has been a long time in the making. Since the 11th Five-Year Plan, research projects relevant to precision medicine have been carried out under the National High-Tech R&D Program of China (863 Program). These projects cover the genomics of disease, disease classification on a molecular level, individualized treatment, big data, and the building of biodatabases. Currently, China is believed to be a leader in genomics and proteomics research, and is making great strides in research into molecular biomarkers, drug target identification, and big data analysis. With abundant clinical research samples and highly competitive personnel, facilities, and teams, China is well positioned to develop precision medicine on a par with the West. It behooves the country to mobilize its resources and make the best use of the foundation laid by previous efforts.

**Embodiment of precision medicine in traditional Chinese medicine**

The essence of precision medicine is embodied in the philosophy of traditional Chinese medicine, as demonstrated in the ideas of bianzheng, shizhi, tongbingyizhi, and yibingtongzhi. In fact, this approach to precision medicine can even be found in the book *Treatise on Febrile and Miscellaneous Diseases* by Zhang Zhongjing, which dates back over 2,000 years.

In traditional Chinese medicine, bianzheng means to analyze the data collected in all four typical diagnostic techniques (observation, olfaction, inquiry, and palpation) in order to gain a better understanding of the illness in terms of its cause, mechanism, and site. This concept also calls for a close watch on progression of the disease.

Following on from bianzheng, shizhi means to provide the most suitable treatment according to the cause, mechanism, site, and stage of the illness. This idea is still very much alive today in the context of precision medicine.

Tongbingyizhi refers to the fact that treatments for what looks like the same disease may differ because of variation in time and place of onset, stage of the disease, and patient response.

Finally, yibingtongzhi, in contrast to tongbingyizhi, describes how similar treatments can be used for different diseases based on the fact that these diseases may share the same cause or symptoms.

Moreover, traditional Chinese medicine strives to maintain a balance between yin and yang by taking into consideration the patient’s gender and internal and external environments. In practice, this demonstrates a holistic approach that incorporates the concepts of macro- and microenvironments in understanding pathogenesis.

**Targets and priorities of China’s precision medicine program**

**Targets**

In an effort to advance the precision medicine agenda in China, we propose the following targets:

1. Build a world-class research platform and support system for which China maintains ownership of the core technology.
2. Develop novel drugs, vaccines, devices, and equipment with China-owned intellectual property rights.
3. Establish a set of internationally recognized guidelines and standards for clinical interventions to prevent, diagnose, and treat disease.
4. Improve prevention and treatment for major illnesses and stimulate growth in related industries, such as biopharmaceuticals, medical devices, and health services.
5. Contribute to reforming the health care system, revising the current model for medical practice, and successfully delivering the Healthy China 2030 initiative.

**Priorities**

In order to ensure the success of precision medicine programs in China, we believe that the following priorities need to be followed:

1. **Research on technologies and models for precision prevention and control.** Keeping illnesses at bay through prevention should be the ultimate goal of precision medicine. Prospective studies and pilot projects need to be carried out in regions of high disease incidence and with susceptible populations in order to provide individualized disease prevention models that match the unique characteristics of the country and its population.
2. **Identification and application of molecular biomarkers.** Disease-specific biomarkers need to be identified through genomic, epigenomic, transcriptomic, proteomic, and metabolomic research in order to develop methods for early detection, screening, and diagnosis of diseases; to predict patient response, prognosis, and outcome; and to inform treatment choice.
3. **Precision diagnosis based on molecular imaging and pathology.** Precision diagnosis is the
foundation of precision treatment and requires the development of multimodal imaging technology that brings together molecular biomarker-guided magnetic resonance imaging, computed tomography, and ultrasound as well as noninvasive or minimally invasive diagnostic techniques.

4. Precision treatment in clinical settings. Precision treatment is the goal of precision medicine. It takes into account the patient’s molecular characteristics, health record, ‘omics, imaging results, and big data analysis, and employs highly individualized and disease-specific protocols, including targeted molecular therapy, targeted antibody therapy, precision immunotherapy, and individualized cell therapy.

Infrastructure needed for development of precision medicine in China

Development of precision medicine relies heavily on certain technologies, including computer simulation, high-powered computing, and the Internet. As an important part of the infrastructure, a digital biodatabase and platforms for data collection and analysis of genes and proteins will be needed. We believe that the Chinese authorities need to build the following:

1. A national biodatabase. Having a rich database of diverse biological samples will give China a competitive edge in the medical field. The country needs a database that is compatible with international data storage and annotation standards, has complete records containing all relevant clinical information, is ethically and legally sound, and is open and accessible to all.

2. A national center for health data. A central repository is needed to collect, store, analyze, and utilize the massive clinical, ‘omics, structural biology, and pharmaceutical information generated in the precision medicine program. This will aid in the identification of new tumor biomarkers, molecular targets, drugs, and other therapies. In addition, this center will be able to consolidate genomic, transcriptomic, proteomic, and metabolomic data that can be mined for valuable information applicable to biology and medicine.

3. Institutional support. The internal structures of Chinese institutions and the way in which they interact are critical for the success of precision medicine there. Institutional structures should optimize the management and support of staff and remove the silo effect, increasing communication and interaction at all levels. Mechanisms should be in place to protect patient privacy and emphasize informed consent. Efforts also need to be made in terms of legislation and ethics reviews to explore ways to protect information in the biodatabase, while also keeping it accessible.

The precision medicine program in China needs to meet the medical health and welfare demands of the population, making use of both institutional and market advantages in the allocation of resources and finding the best way to work in concert with China’s current health care reform program. A successful program will promote emerging industries, improve the country’s capacity for indigenous innovation, and enable the development of a world-class health care system.

China’s policies regarding next-generation sequencing diagnostic tests

Rui Zhang1,2 and Jinming Li1,2*

Over the past decade, DNA sequencing has become faster and cheaper than ever before, its progress attributable mainly to advances in next-generation sequencing (NGS) technologies. NGS techniques are now rapidly becoming widely adopted in routine clinical practice, including analyses of solid tumors, hematologic malignancies, genetic diseases, and infectious diseases; noninvasive prenatal testing (NIPT); and preimplantation genetic diagnosis (PGD) and/or screening (PGS).

In response to concerns about this rapidly growing field, in February 2014 the China Food and Drug Administration (CFDA) and the National Health and Family Planning Commission (NHFPC)
halted clinical NGS assays (1) and launched a series of regulatory requirements for NGS.

**CFDA's regulatory oversight**

The CFDA requires that all components of an NGS commercial diagnostic system, including the reagents, instruments, and software that are packaged together and sold to laboratories, must be approved before being used in a clinical setting. The first diagnostic products approved by the CFDA (on June 30, 2014) were BGI’s sequencers—BGISEQ-100 and BGISEQ-100—and its diagnostic kits for detecting fetal chromosomal aneuploidy (trisomy 21, trisomy 18, and trisomy 13), using the methods of semiconductor sequencing and joint probe-anchor sequencing (2). NIPT has become one of the most extensively utilized NGS tests in China, following the results of multiple clinical validation studies showing its high sensitivity, specificity, and negative predictive values. To date, CFDA has approved five NGS instruments: BGI's BGISEQ-100 (based on Life Technologies' Ion Torrent sequencer) and BGISEQ-1000 (based on Complete Genomics sequencing technology), Berry Genomics' NextSeq CN500, DaAn's DA8600 (based on Life Technologies instruments), and CapitalBio's BioelectronSeq 4000 (based on Life Technologies instruments), along with their associated noninvasive prenatal assays. However, no commercial NGS kits developed for use in other clinical fields, such as for tumor-associated genetic mutations, genetic evaluations, and PGD/PGS, are currently under an approval process.

**NHFPC’s regulatory oversight**

NHFPC launched a pilot program in April 2014 that grants licenses to NGS laboratories. Thus far, 9 have been approved for NIPT, 6 for PGD/PGS, 26 for oncology, and 18 for genetic diseases. The laboratories in the pilot program must be government-accredited according to the NHFPC regulation document, “Medical Institution Clinical Laboratories Regulation for Amplification-Based Molecular Diagnostics,” and all the clinical tests using NGS technology must be conducted under the corresponding regulations. The laboratories should establish a standard operating procedure (SOP), follow all quality control (QC) metrics, and document the performance of each test. Based on the SOP and QC metrics, the NGS process should be validated to establish the expected performance characteristics within each laboratory. Internal quality control and external quality assessment (EQA) are also essential for the laboratories to verify the reliability of their NGS results.

To assess the proficiency of those laboratories offering NGS-based tests, the National Center for Clinical Laboratories (NCCL) of China launched a pilot EQA in 2014 for the detection of trisomy 21, 18, and 13 by massively parallel sequencing. The pilot showed that the majority of participating laboratories used CFDA-approved reagents and provided reliable diagnostic capacities for NIPT tests (3). In 2015, a nationwide pilot EQA was initiated by NCCL that examined the efficacy of NGS technologies for detecting somatic mutations. The participants included 26 government-licensed laboratories using NGS in oncology, as well as laboratories still undergoing validation for NGS testing. All the tests offered by the participants were laboratory-developed tests. The performance of 51.6% (33/64) of the responding laboratories was deemed to be acceptable, while only 26.6% (17/64) of the laboratories correctly identified all the mutations within the NCCL's panel of DNA samples (4). This result implied an urgent requirement for improved laboratory training in the procedures of NGS for somatic mutation testing in tumors, which is likely due to the complexity of the process.

**Regulatory oversight challenges**

With the adoption of NGS technology, clinical laboratories can perform analyses using disease-targeted gene panels, exome sequencing, and whole-genome sequencing. NGS tests are very different from traditional molecular tests, and create several challenges for regulatory oversight.

For the CFDA, NGS-based tests demand novel approaches to ensure analytical and clinical validity. First, unlike traditional tests that detect only a single or a defined number of factors to diagnose one or several specified conditions, NGS tests can identify an unlimited number of variants in the human genome; thus, it is impractical to evaluate the analytical validity of every possible variant. Although evaluation of representative subsets of variant types might be an effective approach to demonstrate the analytical performance of NGS platforms, it will take time to establish new evaluation checklists for specific NGS tests in different clinical fields. Second, NGS-based tests can identify clinically meaningful variants as well as variants of uncertain significance. Thus, the clinical validation requirements should be different for each
of these claims. Third, the intended use of the NGS test may be specific to certain patient populations, drugs, or diseases. However, according to the U.S. National Cancer Institute’s Molecular Analysis for Therapy Choice (MATCH) program (5), the choice of a therapeutic agent is based on the specific molecular findings obtained using targeted NGS analysis rather than on the type of cancer. With the rapid development of clinical research, the CFDA must reconsider the process for approving applications to expand the intended use of previously authorized reagents.

The advent of NGS technology has also created a great challenge for NHFPC; although the pilot laboratories were required to perform NGS-based testing under the Medical Institution Clinical Laboratories Regulation for Amplification-Based Molecular Diagnostics, as NGS-based clinical tests are much more complex than traditional molecular tests, there is an urgent need to develop new regulatory standards for laboratories offering these tests. In recent years, the U.S. Centers for Disease Control and Prevention (6), the American College of Medical Genetics and Genomics (7), and the College of American Pathologists (8) have defined guidelines for effective NGS method validation, analytical process monitoring, and variant reporting. Nevertheless, it remains a challenge for laboratories to translate these requirements into routine practice.

In conclusion, the Chinese government has initiated regulatory oversight for NGS clinical testing and has made great efforts to standardize NGS diagnostic tests. Proper approaches are being sought with the goal of assuring safety and effectiveness, while enabling innovation in the field and supporting the advancement of precision medicine.

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Quality management for precision medicine clinical applications: A consensus from the China Precision Medicine Clinical Research and Application Association

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The clinical application of precision medicine includes considerations of the safety, effectiveness, and economy of treatment as well as issues of medical ethics, such as the protection of patients’

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2Beijing Anzhen Hospital, Capital Medical University
3Beijing Hospital
4Beijing Obstetrics and Gynecology Hospital, Capital Medical University
5Beijing Xiaotangshan Hospital
6Cancer Hospital of Beijing University
7Jiangsu Provincial People’s Hospital (First Affiliated Hospital of Nanjing Medical University)
8Mount Qianfu Attached Hospital of Shandong University
9Nanfang Hospital
10Ruijin Hospital, Shanghai Jiao Tong University School of Medicine
11Shanghai Centre for Clinical Laboratory
12Shanghai Mental Health Center
13Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University
14The Second Affiliated Hospital of Harbin Medical University
15The First Hospital of Jilin University
16The First Affiliated Hospital of Chongqing Medical University
17Tongji Medical College, Huazhong University of Science and Technology
18West China Hospital, Sichuan University
19The First Affiliated Hospital of Xinjiang Medical University
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23Beijing Friendship Hospital, Capital Medical University
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Quality management of laboratories

Genetic detection results should be trustworthy: Instruments and reagents should be obtained legally, and all genetic detection operations should be accurate.

Participation in an external quality assessment (EQA) for genetic testing provided by a certified capacity verification provider (e.g., Shanghai Center for Clinical Laboratory or the National Center for Clinical Laboratories) is warranted.

Laboratory quality control should be up to EQA standards, and improvements should continuously be implemented. Accuracy rates for detection methods should reach 99%-100%, while EQA results should be perfect.

Samples for genetic testing

Genetic detection should be accurate and meet the needs of treatment in order to avoid partial detection of genes, misdiagnosis, and thus incorrect treatment.

Providing too much genetic information that goes beyond the need for diagnosis and treatment of the disease should be avoided.

Genetic testing for unrelated diseases or treatments is not recommended.

Clinical evidence sources

To find information on drug efficacy, adverse reactions, and treatment protocols, it is recommended that staff refer to the drug package insert.

For the diagnosis and treatment of refractory disease, it is acceptable to use treatments for which evidence is not as strong as for accepted therapies, but only when it can be ensured that the patient will obtain the maximum benefits.

Precision medicine treatment regimen development

A precision medicine treatment regimen should be developed based on the results of the genetic test, and adjustments should be made according to changes in the patient's condition, always ensuring that the benefits gained by the patient outweigh the risks.

The personnel in charge of developing drug treatment regimens should be clinical professionals who are able to provide precision medicine consultation, such as physicians and pharmacists.
Precision medicine consultation on a telemedicine platform

Training is recommended for experts in precision medicine consultation using the National Telemedicine Network, which is organized by CPMCRAA. Trainees should undergo examinations and obtain the relevant certifications.

Only qualified professionals can be registered as an expert in precision medicine consultation using the national telemedicine platform.

Requirements for hospitals using precision medicine

Hospitals are encouraged to adopt standardized technology protocols and treatment regimens so that results from other hospitals can be easily interpreted, patients can be referred to other hospitals, and consultations can be organized between physicians at different hospitals. Hospitals that have joined CPMCRAA are encouraged to provide guidance and consultation to the association’s grassroots medical institutions.

CPMCRAA expert committee

CPMCRAA covers 1,340 Chinese hospitals from which members of the expert committee are drawn. Academician Chen Wang of the Chinese Academy of Engineering is the chairman.

Recommendations on the management and use of POCT in medical institutions (nosocomial)

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General rules

Article 1. Definition. Point-of-care testing (hereafter referred to as “POCT” or “bedside testing”) is a method that uses portable analytical instruments or reagents to test the patient and obtain instantaneous results. When discussing POCT, the test must be clearly defined, whether it involves a medical activity or a piece of equipment, and the similarities and differences between the particular POCT and a clinical laboratory test should also be explained.

Article 2. Applications. POCT in clinical departments needs approval from hospital management. In addition, the following information should be established: who is in charge of POCT within the clinical department, whether the POCT testing results are compatible with the hospital information management system, which test results can be included in the patient’s medical records, the type and number of results needed before a diagnosis can be made, and which results can provide useful information for patient care and monitoring. Most importantly, it needs to be determined which POCT results can form the basis for a diagnosis.

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Article 3. Significance. POCT can reduce the turnaround time for obtaining results, simplify the diagnostic process, provide instant patient information, provide a diagnostic workflow that is easier to follow than current tests, and improve the quality and level of medical care.

Article 4. Management of POCT staff. POCT staff should be properly trained and their work regularly inspected, as well as being properly supervised by the hospital quality control department. They should be required to pass relevant POCT-training examinations before being allowed to perform POCT tasks.

Organization and management

Article 5. The onus is on the hospital to properly integrate POCT into the hospital management system, establishing a strict training, management, and quality control/supervision system, and setting up a POCT management committee or relevant management organizations. POCT offices and committees should be established under the supervision of a medical department, and each department using POCT must have full-time or part-time POCT coordinators. Representatives from relevant departments should participate and together establish a coordinated management system.

The hospital's POCT management committee or corresponding management organization should undertake the following responsibilities:
1. Establish a complete POCT management working system and supervise its implementation according to relevant state regulations;
2. Accept requests for POCT approval from clinical departments;
3. Organize the training and regular evaluation of POCT operators;
4. Organize efficient and sustainable POCT quality control checks; and
5. Handle complaints and suggestions from POCT users, and implement improvements.

Article 6. Appoint point-of-care coordinators (POCCs) under the POCT management organization who are responsible for POCT coordination among different clinical departments.

The following qualifications should be required for POCCs:
1. They must have pre-job training in POCT and hold a relevant work certification. They can be departmental staff from the department of quality management or clinical laboratory, and either full-time or part-time.
2. They must obtain training and education from the administrative section of the Ministry of Health to receive their POCT-related qualifications.
3. They are responsible for implementing the directives from the POCT management organization, including the following responsibilities:
   - Coordinating with different clinical departments to ensure that POCT are being used correctly, including overseeing equipment and reagents that meet clinical standards and ensuring that the quality control requirements for every sample are being met and that results are properly generated and analyzed;
   - Overseeing the establishment of each clinical departments' own quality assurance system; and
   - Supervising theory and skill training for each clinical department and ensuring that staff master the professional knowledge required.

Article 7. The POCT management organization should appoint experienced doctors or technicians to confirm the professional skills and knowledge of POCCs.

Article 8. The majority of POCT operators should be trained doctors, nurses, or inspectors.

Personnel training

Article 9. The management organization should arrange basic training, and related courses should be implemented by the POCC.

Article 10. Content of the training courses should include:
1. The applications and limitations of POCT, as well as interpretation of the results; the justifications for choosing a particular POCT and the most ideal equipment to use, as well as the best applications for that equipment and its reagents; and lessons on the reasons for quality assurance testing.
2. Students should master essential precautions needed for sample collection and factors that may interfere with accurate testing, including clinical factors, diet, and methods of sample procurement.
3. Quality assurance measures, including methods and requirements for internal quality control and comparison, as well as reasons and remedies for errors, should also be addressed.
4. The courses should also include methods for equipment comparison, quality control, maintenance, and troubleshooting.
5. Students should study procedures for preventing exposure to infectious diseases, as discussed in Biological Safety Management Regulations of Pathogenic Microorganism Laboratories, as well as related laws and regulations that are stated in Treatment of Medical Waste, issued by the Chinese State Council.

Article 11. If operators pass a written exam, an operating assessment, and an evaluation, they should be provided with certification, and only certified operators should be authorized to undertake POCT work.

Quality assurance

Article 12. A quality management system should be an integral part of every POCT.

Article 13. Internal quality controls are essential for the implementation of POCT, as they provide a measure of the veracity of the results.

Article 14. Each POCT should develop its own clinical benchmarking system. Second-tier and above hospitals, independent laboratories, and medical examination centers can use internal benchmarking, while hospitals below the second-tier level and community health centers should regularly benchmark with one of the three institution types mentioned above. The latter institutions may also use internal quality assessment in addition to external benchmarking.

Article 15. POCTs should establish a robust system for the management and handling of all related data. There should be specific requirements for sample collection, as well as for the operation, calibration, and maintenance of instruments. The interpretation and reporting of results should also be standardized and properly monitored.

Article 16. Each POCT should establish a quality management network and use standardized quality management systems.

Article 17. Traceable electronic recordings can ensure good quality assurance and are essential to successful POCT implementation and use.

Article 18. As network technology has advanced and societal needs have changed, POCT has shifted towards mobile health care and smart health care. A distinction should be drawn between sports monitoring and specialized instrumentation used for medical testing. All products and systems used at medical institutions should be approved and calibrated.

Point-of-care testing (POCT) is widely used in chronic disease management, antibioterrorism, emergency response and medical rescue, surveillance of infectious diseases, customs inspection and quarantine, food safety, drug control, and other public health fields. Recently, with the rapid development of high technology such as micro- and nanomanufacturing, biotechnology, new materials, and the mobile Internet, POCT continues to develop towards real-time quantification and miniaturization.

The definition of POCT

POCT refers to a means to get results quickly by carrying out testing at the sampling site using portable analytical instruments and related reagents. It also refers to the related equipment found in the medical industry.

In a hospital setting, POCT means testing right at the patient’s bedside, and the test is not always carried out by a clinical docimaster (examiner).

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Characteristics and applications of POCT

**POCT characteristics**

The following characteristics can be expected of POCT:

- Quick results that save turnaround time;
- Small, portable instruments;
- Requirement for small sample volumes;
- Easy to handle—nonprofessionals should require only basic training or written instructions; and
- Low cost.

**POCT applications**

**In the clinic**

POCT medical equipment has been widely used in many hospital departments including emergency, intensive care, respiratory, and cardiology, as well as in operating rooms. This technology complements traditional tests in timeliness and flexibility; it also makes up for insufficient examination resources in some primary hospitals. Taking severe cases as an example, POCT meets the urgent needs of clinicians to find results quickly and accurately in order to make lifesaving clinical decisions.

**Health management**

The widespread use of home blood glucose testing meters is an example of the successful application of POCT technology in the management of chronic disease.

The drive to transform the paradigm for health care has become a powerful impetus for the development of POCT. In contrast to separate family health care, community health care, primary health care, and large, center-level hospital health care models, the new model is disease-centered medical health care. As technology advances and the need for individualized health care grows, this model is morphing into one that focuses on the daily health care of families and communities, which necessitates smaller and simpler devices and testing methods.

**Emergency applications**

Over the years, the function and feasibility of POCT have been demonstrated through global emergencies and disasters. Under emergency conditions like epidemic outbreaks, medical relief of natural disasters, food safety accidents, bioterrorism responses, customs inspection and quarantine, and illegal drug screenings, POCT has played a science-based role in decision-making. POCT provides a basis for the relevant authorities to plan for these emergencies, implement first aid, and provide early warnings to the public. It meets the needs of rapid analysis in a biological emergency scenario for quantitatively testing and identifying a suspicious sample in the shortest possible time, and offers improvement on current abilities and standards for handling biological emergencies.

**Grassroots applications**

Because POCT provides useful results rapidly in a convenient and easy-to-operate format, it is eminently suitable for rural doctors and primary health personnel. This technology can be widely applied to emergency preparedness and to the treatment and management of chronic diseases in remote areas, providing a means to promote grassroots medical technology.

**Trends in POCT**

Emerging POCT technology and its management have been developed and applied worldwide in the clinic, in biological emergencies, in the medical community, and even in family chronic-disease management.

Over the past decade, China has witnessed very rapid development in these areas. But there is still much to learn from more advanced nations in terms of scientific ideas, advanced technology, and market applications and management policies. To promote and support the development of the POCT industry, the State Council of China put in place the Bioindustry Development Plan (2012), the 2011-2013 National 863 Program, and the 12th Five-Year Plan for science and technology projects (2011-2015).

**POCT’s diversity**

POCT has been applied in many different areas using a variety of techniques and instruments; some of these include dry chemistry, multicoating, immunochromatography, filtration (currently the most widely used application), microfluidics, infrared and far-infrared point spectrophotometry (continuous monitoring of hemoglobin and blood sugar through the skin), selective electrodes (used mainly for blood gases and electrolytes),
biosensors and biochips, and small microscopes. Test targets include biochemical indexes, immune indexes, and nucleic acid-level indexes.

**POCT technology— from qualitative to quantitative research**

More precise analysis is the ultimate goal of POCT technology development. With the continual emergence of new materials and the integration of precision manufacturing, biomedicine, automated control, and other new technology elements, the new generation of POCT brings precision and quantitation comparable to larger, fixed equipment. Immunochromatography products, which use colloidal gold as a tracer, are successful examples of POCT qualitative technology, and include the One-Step HCG Pregnancy Test, ovulation kits, and other related products. These products can have a profound impact on human society. In the quantitative technology arena, many luminescence techniques have emerged, including basic fluorescence, time-resolved fluorescence, and electrochemical luminescence.

**POCT networking**

The development of the Internet, especially of mobile wireless technology, has brought unprecedented opportunities to POCT. Large pieces of equipment were previously the testing standard-bearer, but they cannot leave the laboratory or be taken into a family home, to a patients’ bedside, or to the scene of an accident. In contrast, patients can use a portable/wearable POCT to test themselves whenever they need, and to upload the data synchronously to the medical group’s cloud account. Physicians can access these results remotely to diagnose and quickly provide patients with medication and disease management advice.

Data from POCT can be quickly and easily analyzed in the cloud, while the integration of remote data terminals and cloud medical resources can allow for big data analysis, potentially providing an effective health warning system.

**POCT’s plight**

**Lack of administrative management**

Although POCT is generally carried out in the hospital, there is often not any clear management oversight. Experts are calling for the strengthening of POCT hospital administrative management.

**Underdeveloped policies and regulations**

A significant hurdle in the acceptance of POCT is the lack of clear, relevant regulations for the industry, particularly with respect to the fee charged for POCT services and how this compares with standard laboratory tests. It is necessary to consider the access or lack thereof that doctors in rural communities might have to health care systems and technologies, and how new equipment might be developed that is easy to use, convenient, economical, and practical. Improving the quality of diagnosis and treatment of primary care patients through POCT could encourage the expansion of a potentially huge market for these tests, while also improving primary health care for Chinese citizens.

**Quality of user management systems**

Many clinical departments currently have a need to conduct POCT, but quality control and clinical management of these tests are still not perfect. The oversight of equipment maintenance, reagents and consumables, digitization, and information storage and analysis still needs much improvement.

**Nonconformity of product quality and technical requirements**

Both community health service centers in urban settings and health clinics in small towns use blood glucose testing meters more than any other device. At present, these types of rapid-result equipment and their associated reagents (such as immunity analyzers, biochemistry analyzers, hematology analyzers, and blood coagulation instruments) have many varied quality requirements and associated methodologies. This can be a challenge for many primary health care workers, especially in township hospitals and village clinics, as they need to undertake extensive technical training and may need after-sales service that is not easily accessible far from urban centers.

**Suggestions for POCT management**

**POCT certification and management**

Within the hospital environment, the focus should be on strengthening the administration of POCT and improving its development and management, including putting in place a network of POCT coordinators (POCT-Cs) and setting up POCT committees in provincial and city hospitals that are responsible for finding experts to formulate management criteria and regulations, which
would be implemented by a clinical POCT-C. POCT products would need to be approved by the China Food and Drug Administration (CFDA). Manufacturers must be required to comply with the standards and requirements for the production of POCT devices and report any defects found in postmarket equipment. CFDA should also inspect devices sold within the medical equipment market, establish a mechanism to control the quality of medical equipment production, and report any defects in or adverse side effects resulting from use of these devices. Researchers, medical professionals, administrative managers, and business people should come together to develop national POCT policy, quality control standards, guidelines, and training programs.

A standardized POCT nosocomial management approach
Nosocomial POCT development requires the cooperation of many medical departments. Its implementation involves hospital quality management professionals, professional medical engineers, clinical laboratory professionals, nurses, and clinicians, among others. Those carrying out testing should be familiar with the objectives, guidelines, and principles of POCT technology. A nosocomial POCT management committee should be established under the guidance of a supervisor within a medical department, and with the relevant departments providing committee representatives. A POCT-C should be elected from within the medical department or other relevant department who is responsible for the management, evaluation, quality assurance, and future application of POCT.

Implementation of total quality management
The term “total quality management” refers to the concept that the most ideal level of quality should be regarded as the minimal acceptable level when organizing, managing, or using a product or service. For POCT, this means that the requirements for proper clinical diagnosis, monitoring, screening, and prevention are ensured, and that internal quality control of equipment and reagents is guaranteed, as is the safety of patients and medical workers.

Categorizing POCT products
POCT products are complex, and there is currently no clear standard for their classification. Based on the degree to which POCT may be a hazard to public health, the U.S. Food and Drug Administration has divided approval of in vitro diagnostic products into three categories: waived (simple and accurate; negligible chance of erroneous results), moderately complex (minimal expertise needed to administer; minimal interpretation of results required), and highly complex (specialized training and expertise required; extensive interpretation needed). It is recommended that China also put in place a similar POCT classification system to improve management.

POCT monitoring and procedures must minimize the risk of biological hazards to patients and nurses. Additionally, procedures should conform with infection control policies so that the spread of infectious agents can be avoided.

China’s POCT development strategy
China should develop its own POCT by standardizing its application management system and enhancing technical innovation.

Implementation of better management principles for POCT, more discussion of the technical aspects of POCT among experts, and dialogue about the application of POCT in other fields are needed to allow China’s POCT industry to develop on a strong and robust path.

As POCT shifts from qualitative results to more precise quantification, many new technologies from many different disciplines will inevitably come into play, including the use of networking and mobile technology to remotely inspect equipment and receive quality control test results. POCT technology is also shifting from large-scale application within hospitals to applications outside of hospitals, requiring China to formulate policies about how related technologies might be used in this space, while still promoting development of POCT and protecting intellectual property rights.
Chapter two

Application of precision medicine in cancer
Cancer precision medicine in China

Yuankai Shi

Population aging and lifestyle changes have contributed to the rise of cancer incidence in China. In 2015, new cancer cases and cancer-related deaths were estimated at 4.3 million and 2.8 million, respectively (1). Surgery, radiotherapy, and chemotherapy remain standards of care for cancer patients, but molecular targeted therapy has become a promising field in cancer treatment for its ability to discern which patients would benefit the most from a particular treatment.

Precision medicine initiatives in China

The field of precision medicine was proclaimed in 2015 by the United States and China as heralding a new era in health care (2). It has the potential to improve the diagnosis and treatment for complex diseases such as cancer and diabetes mellitus, and thus to keep patients healthier. Based on personal genomics, proteomics, and metabolomics information, precision medicine opens the door for us to analyze and identify specific biomarkers and treatment targets for diseases. The Chinese Ministry of Science and Technology (MOST) held the first national precision medicine strategy conference in March 2015; it announced plans to invest RMB60 billion (US$9 billion) to support precision medicine between 2015 and 2030. Soon thereafter, the National Health and Family Planning Commission (NHFPC) established the first in a series of clinical practice institutions focused on cancer gene sequencing and tasked with the goal of standardizing clinical gene sequencing. The 13th Five-Year Plan for Science and Technology (MOST) held the first national precision medicine strategy conference in March 2015; it announced plans to invest RMB60 billion (US$9 billion) to support precision medicine between 2015 and 2030. Soon thereafter, the National Health and Family Planning Commission (NHFPC) established the first in a series of clinical practice institutions focused on cancer gene sequencing and tasked with the goal of standardizing clinical gene sequencing. The 13th Five-Year Plan for Science and Technology (MOST) held the first national precision medicine strategy conference in March 2015; it announced plans to invest RMB60 billion (US$9 billion) to support precision medicine between 2015 and 2030. Soon thereafter, the National Health and Family Planning Commission (NHFPC) established the first in a series of clinical practice institutions focused on cancer gene sequencing and tasked with the goal of standardizing clinical gene sequencing.

Standardization of molecular biomarker detection occurred prior to the application of precision medicine in China. For example, human epidermal growth factor receptor 2 (HER2) expression levels in breast cancer might determine whether a targeted therapy could be used. A clinical trial that enrolled 3,149 breast cancer patients from 73 hospitals in China compared HER2 levels detected using immunohistochemistry (IHC) or fluorescence in-situ hybridization (FISH) (7). This study helped to establish standard operating procedures (SOPs) for HER2 detection. Many methods are available for biomarker detection, including Sanger sequencing, real-time PCR, the amplification-refractory mutation system (ARMS), and next-generation sequencing (NGS). In China, Sanger sequencing and ARMS are commonly used, and cross-validation can be easily done, if necessary. Circulating tumor DNA (ctDNA) detection is under investigation for application in clinical practice. For example, analyzing blood-derived ctDNA for EGFR mutation status in non-small cell lung cancer (NSCLC) patients could help with the selection of the optimal EGFR-tyrosine kinase inhibitor (TKI) therapy, and ctDNA detection shares a higher specificity compared to mutation analysis performed on tumor samples (8). In February 2015, the China Food and Drug Administration (CFDA) approved gefitinib for from translational research. Yet making precision medicine available to all areas of clinical practice still remains a challenge.

Promotion of cancer precision medicine in China

Recent research into the molecular mechanisms critical to carcinogenesis has led to the development of a series of novel drugs, as well as the use of personalized therapy in clinical practice that targets specific genes. Yet clinical decisions for treatment should take into account patient ethnicities, since genetic differences exist between different populations. For instance, a molecular epidemiology study investigating epidermal growth factor receptor (EGFR) gene mutations showed that EGFR mutation frequency was higher in Asian than Caucasian populations (3, 4). Detection of gene mutation hot spots for colorectal cancer in Chinese patients showed similar KRAS and BRAF gene mutation frequencies compared to Western populations (5), while neuroblastoma RAS viral oncogene homolog (NRAS) mutation rates were higher in Chinese patients (6). Based on these differences, precision medicine research for Chinese patients warrants further investigation.

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use in cases where an EGFR mutation is detected in ctDNA and no tissues samples are available. Additionally, guidelines and expert consensus have been established in China for certain biomarkers, including EGFR, KRAS, anaplastic lymphoma receptor tyrosine kinase (ALK), and HER2 (9–12). Institutes and hospitals involved in molecular biomarker detection participate in external quality control reviews held annually by the Pathology Quality Control Center of NHFPC. These gatherings foster greater data sharing and allow better standardization and normalization of targeted therapies.

Recently, science and technology projects such as the National Science and Technology Major Project for New Drug Innovation have provided significant support for new anticancer drugs and molecular diagnostic reagents. Many domestically developed diagnostic reagents and drugs have emerged, including tests for common biomarkers like EGFR, KRAS, ROS1, ALK, and HER2 (5, 7, 13), while a number of anticancer drugs have been approved by CFDA, such as the EGFR TKI icotinib hydrochloride (14), the subtype-selective histone deacetylase (HDAC) inhibitor chidamide (15), and the vascular endothelial growth factor receptor (VEGFR) TKI apatinib (16).

**The future of cancer precision medicine in China**

NGS is now widely used in laboratories around the world, allowing researchers to detect gene mutations quickly and efficiently, and greatly enhancing genomics research. Previous cancer gene mutation tests were limited to qualitative analysis, but now correlations between gene mutation abundance and treatment efficacy can be used to better tailor treatment to individual patients or tumor types. For example, a study published in 2011 reported a better response to EGFR TKI in patients with a higher abundance of drug-sensitive EGFR mutations (17).

Novel drugs have emerged that can gradually overcome primary and acquired treatment-related resistance. Avitinib, a third-generation EGFR TKI, has been used to overcome EGFR T790M mutation-associated drug resistance in clinical trials (ClinicalTrials.gov Identifier: NCT02274337, NCT02330367). A new ALK inhibitor, CT-707, is also in clinical trials (ClinicalTrials.gov Identifier: NCT02695550). Immunotherapy has become a promising treatment for cancer (18), and clinical trials with domestically developed programmed cell death protein 1 (PD-1) inhibitors have been initiated recently (ClinicalTrials.gov Identifier: NCT02836834).

MOST declared that the 2016 National Major Research Project in cancer precision medicine will focus on the molecular mechanisms involved in the incidence, relapse, and metastasis of lung cancer, gastric cancer, colorectal cancer, and breast cancer. It is also supporting research into cancer prevention and the development of intervention strategies, techniques for clinical diagnosis and treatment, and the improvement of medical treatments through pharmacogenomics. Furthermore, a program has recently been launched that uses big data analysis to improve the diagnosis and prognosis of Chinese cancer patients. All of these initiatives will serve to promote the application of precision medicine in China.

**Conclusions**

In the past decade, cancer molecular subtyping and targeted therapy have become important areas of research and now significantly benefit Chinese patients. Extensive work has been done in the fields of screening and intervention for high-risk cancer populations, as well as the molecular subtyping of cancer and the testing of different diagnostic models. As new technologies are developed and improved, cancer precision medicine will be more widely applied, with the goal of improving diagnosis, stratification, and treatment of cancer patients in China.

**References**

The challenges and prospects of precision oncology

Lun-Xiu Qin

The goal of precision oncology is the selection of a subset of patients with a common biological basis of disease who are most likely to benefit from a drug or other treatment. It includes precision prevention (cancer risk detection and prophylactic intervention), precision diagnosis (early detection and diagnosis, and molecular classification), and precision treatment (targeted molecular therapies, treatment response prediction and monitoring, and precision surgery). Despite its promise, many challenges have to be overcome before precision oncology can become a routine clinical practice.

Advances in understanding the molecular profile of tumor cells have led cancer treatment into a new era in which established clinical-pathological indices, coupled with molecular profiling, are applied to create diagnostic, prognostic, and therapeutic strategies precisely tailored to each patient’s requirements—hence the term “precision medicine” (1, 2). Oncology is arguably the clear choice for enhancing the near-term impact of precision medicine. One obvious reason is the fact that cancer is a genomic disease (3, 4).

The implications of precision oncology

In a narrow sense, precision oncology aims to individualize therapeutic interventions based on ‘omics data (such as genomics, proteomics, and metabolomics profiling) and histopathological insights into tumor type, stage, and grade. In addition, precision surgery, which is based on the combination of visual, cytology, and pathology reviews, as well as molecular profiling assessments, can also be an important part of precision oncology (5).

For those with a significant genetic susceptibility such as a familial cancer, tests for specific genetic markers can confirm the possibility of a higher lifetime risk. In many of these cases, prophylactic interventions are the most effective strategies to reduce cancer risk, such as bilateral prophylactic mastectomy and oophorectomy for patients carrying BRCA 1/2 mutations. Besides prophylactic surgical intervention, chemoprevention may be considered (5, 6).

Predicting the risk of metastatic relapse following surgical treatment is an important factor to consider. Many gene signatures for metastasis risk in solid malignancies have been determined. Some commercial tests, based on a multigene panel of RNA expression, are available for the prediction of recurrence and are beneficial for determining which adjuvant therapies could reduce the risk of relapse (5-10).

Cancer biomarkers are critical when considering which patients to recruit for a particular treatment, as well as for monitoring the success of therapeutic strategies. They can be used to improve the accuracy of clinical diagnosis, to monitor disease progression and therapeutic efficacy, to clarify disease stages and subtypes, and to predict patient outcome (11).

Over the last decade, a steady increase in the number of new targeted therapies has occurred. One example is angiogenesis inhibitors, a class of drugs designed to slow the growth of new blood vessels to tumors, which has proven critical for the successful treatment of many advanced and aggressive cancers (12). More recently, targeting immune checkpoints (such as the PD-1/PD-L1 pathway) have shown promising results against advanced melanoma, kidney, and lung cancers in clinical trials (13, 14).

Predictive biomarkers are used to assess the probability that a patient will benefit from a particular treatment, which significantly influences treatment decision-making. A prime example is breast cancer, in which HER2 gene amplification indicates that the patient would benefit from trastuzumab (Herceptin) treatment. In addition, mutations in the kinase domain of the epidermal growth-factor receptor (EGFR) gene predict the sensitivity of lung cancers to erlotinib or gefitinib treatment; conversely, mutations in the KRAS oncogene indicate that patients with lung cancer will fail to respond to these inhibitors.

A novel paradigm for precision surgery has been proposed that features optimization of therapeutic effectiveness, surgical safety, and minimal invasiveness. Apart from several preoperative and intraoperative assessments of the extent of tumor invasion into surrounding tissue using visual assessment, cytology, and pathology reviews, other newer techniques have recently been employed including radiofrequency spectroscopy, optical coherence tomography, and molecular margin analysis (for example, molecular profiling using iKnife). Using these
modalities in combination provides an ideal means to detect the spread of cancer (5, 6, 15).

**The challenges of precision oncology**

The genetic heterogeneity between different individuals and even different tumors in the same individual is a primary reason why precision oncology can be a powerful technique. Major challenges to accurately applying precision oncology include understanding and characterizing the genetic heterogeneity both within the primary tumor and in its metastases. During cancer progression, tumor cells have increased plasticity, leading to a dynamic phenotype. The genotype of a cancer can also evolve very differently in each patient. The plasticity of cancer cells and their capacity to add new genetic aberrations over time leads to one of the most challenging questions in precision cancer therapy: How many targets in an individual cancer are required to be treated? (1, 4, 12)

Cancer is a complex disease with multiple possible genetic aberrations. A deepening understanding of cancer at the molecular level has begun to influence risk assessment, diagnostic categories, and therapeutic strategies, including the increased use of small-molecule drugs and antibodies designed to counter the influence of specific molecular drivers. Identifying the genetic abnormalities that drive cancer development and growth is at the core of precision oncology, enabling the development of treatments tailored to the genetic makeup of the tumor. Recent massive cancer genomics efforts have provided insight into the underlying biological features of cancer, including the identification of cancer biomarkers and therapeutic targets, and the characterization of factors that predispose certain cells to follow a tumorigenic pathway. The knowledge derived from these studies is expected to have a significant impact on the precision diagnosis and treatment of cancer (12, 15, 16).

A major concern within precision oncology is the need for scrupulous attention to the quality of the genetic and genomic analyses, which must be validated, standardized, and reproducible. Currently, the technology for genetic and genomic analysis is too complex for routine clinical practice, and the bioinformatics and analytical tools are too imprecise. Improvements are required in this area before these tools can be confidently utilized in a clinical setting.

Another challenge is identifying the genetic aberrations that are driving the behavior of individual patients’ cancer(s), and whether the genes or protein products involved in a particular signaling pathway are amenable to therapeutic interventions. Furthermore, the microenvironment in which the immune/inflammatory response to the tumor occurs also plays a critical role in cancer progression and in the success or failure of the treatment (12).

There is a significant gap between cancer genomics and its application in the clinic. In many large-scale genomic sequencing efforts, only the minimum of clinical data, such as tumor type and size, is available. The accuracy of the clinical data is limited, and key clinical information is often missing, such as tumor stage at diagnosis, tumor grade, tissue type, relapse type, and survival time (3). Better collaboration is needed among cancer scientists, pathologists, and radiologists, as well as medical, surgical, and radiotherapy oncologists. Standardization of data from genomic clinical research is required so that it can be more easily cross-referenced with patient medical records (12).

It has been estimated that one individual cancer harbors five or six driver genetic aberrations (12), but these might differ between individuals and between particular tumors. Such complexity in the type and number of driver genetic alterations can be a formidable obstacle to the success of precision oncology. It is widely questioned whether therapies based on targeting a single gene are sufficient to overcome a complex disease like cancer; the benefit from targeting a single driver genetic aberration is almost invariably temporary (16). We currently do not know how many driver genes must be targeted simultaneously or in succession to eradicate all malignant cells in a cancer (16). Using drug cocktails is one way to improve therapeutic efficacy, but this raises further clinical challenges: Which genetic aberrations or pathways should be targeted, and how can increased toxicity to normal cells be avoided (12)?

Furthermore, there is extensive evidence that cancer cells can adapt to and evade targeted drugs, either through mutation of the target genes or by activating alternative compensatory pathways that can bypass the inhibitory effect of the drug. However, few of these theories have been fully characterized or applied in the clinic to counteract drug resistance (12). In addition, the role of the microenvironment in drug sensitivity remains a topic in need of further exploration.

With the development of cancer genomics, it is possible to screen for and identify disease-associated and/or specific genetic alterations (biomarkers) for early detection and diagnosis,
molecular classification, and prognostication of cancers. Although some genes or molecular signatures have been identified, only a limited number of cancer patients have been able to benefit from these biomarkers for monitoring responses and tumor recurrence (3). The ability to select patients whose cancers are likely to respond to a particular targeted therapy has been hampered by the lack of validated genetic and molecular biomarkers (12).

Conclusions
Although genome sequencing is an important aspect of precision oncology, there is more to the field than just this one technology; it also includes prevention, diagnosis, and treatment. The arrival of precision oncology doesn’t indicate the end of evidence-based medicine. On the contrary, it is hoped that precision oncology will aid in the design of better clinical trials, improved clinical diagnosis, and more precise treatment.

The ultimate goal of precision oncology, as stated earlier, is the selection of a subset of patients with a common biological basis of disease who are most likely to benefit from a particular drug or other type of treatment. Understanding cancer at the molecular level is necessary for the effective practice of precision oncology—and essential if it is to become a standard, valuable part of routine clinical decision-making and practice.

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Precision medicine for nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is a complex disease involving environmental factors, human genetic heterogeneity, and Epstein-Barr virus (EBV) infection, and remains endemic in southern China, with a peak annual incidence approaching 30 per 100,000 persons (1). Overall prognosis has dramatically improved over the past three decades because of advances in management, including more accurate disease staging, improvement in radiotherapy technology, and broader application of chemotherapy. Although overall survival exceeding 75% at five years can now be achieved, 20%–30% of patients will develop local or systemic relapse. Clinical TNM (tumor-nodes-metastasis) staging is still fundamental for prognosis and treatment decisions. There is currently no effective biomarker to tailor treatment to individual needs. The genetic landscape of NPC shows that typical tumors are characterized as having comparatively low mutation rates (2). Among the rare mutations, PIK3CA is considered to be a therapeutic target. Recently, several fusion genes have been identified in NPC, but the targetable fusion gene FGFR3-TACC3 was detected in only 4 of 159 (2.5%) NPC patients in a 2014 study (3). Nevertheless, 95% of NPC in the endemic areas in China is associated with EBV, and has a type II EBV latency pattern with expression of EBNA1, LMP1, LMP2, EBERs, BART, and BARF1 microRNAs. Interestingly, most of these genes are consistently expressed and were reported oncogenic in NPC (Figure 1). Therefore, to reach the ideal of precision medicine for NPC patients, better prevention, screening, and treatment targets need to be explored.

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lowered the rate of infectious mononucleosis, but failed to prevent EBV infection (4). EBV glycoproteins gH/gL and gB, but not gp350, are essential for EBV infection of epithelial cells, suggesting that vaccination using gH/gL or gB might yield better results. In addition, virus-like particles (VLPs) assembled from the EBV capsid may also provide a promising vaccine (5).

**Precision prevention of NPC**

Considering the importance of EBV infection in the development of NPC, EBV prophylactic vaccines may have the potential to prevent this deadly disease. Several clinical studies have shown that vaccination with glycoprotein 350 (gp350), the most abundant glycoprotein in the envelope of EBV virions, induced EBV-neutralizing antibodies and lowered the rate of infectious mononucleosis, but failed to prevent EBV infection (4). EBV glycoproteins gH/gL and gB, but not gp350, are essential for EBV infection of epithelial cells, suggesting that vaccination using gH/gL or gB might yield better results. In addition, virus-like particles (VLPs) assembled from the EBV capsid may also provide a promising vaccine (5).

**FIGURE 1. Schematic of Epstein-Barr virus (EBV)-regulated pathways and immune responses in nasopharyngeal carcinoma (NPC).** EBV exists in NPC with a type II latency pattern and predominantly expresses latent genes EBERs, EBNA1, LMP1, LMP2, BARF1, and BART microRNAs (miRNAs). LMP1 and LMP2 activate the NF-κB, JNK/SAPK, PI3-K/Akt, ERK-MAPK, PLC/PKC, and JAK/STAT signaling pathways. EBNA1 is required for the maintenance of EBV genomes, destabilization of p53, and activation of I kappa B kinases (IKKs); EBERs block protein kinase R (PKR) and type I interferons (IFNs); BART miRNAs inhibit apoptosis of NPC cells and activation of EBV lytic cycles; and excreted BARF1 possibly modulates the immune system by inhibiting macrophage colony-stimulating factor (MCSF). NPC cells harboring these latent genes can be recognized by immune cells such as CD4+ T cells, CD8+ T cells, dendritic cells (DCs), and macrophages. EBV infection of NPC cells might be blocked by the EBV glycoprotein gp350 or gH/gL-neutralizing antibodies.
**Precision screening of NPC**

The importance of precise screening cannot be overemphasized in precision oncology—not only do patients with early disease detection have significantly better outcomes (6), but they can also be spared the financial burden and the toxicities associated with additional chemotherapy treatments. EBV serology and the presence of EBV DNA are meaningful risk indicators for NPC. Several large-scale prospective screening programs to assess optimal early detection strategies for NPC in progress. Additionally, a microarray capable of screening for single nucleotide polymorphisms (SNPs) in NPC susceptibility genes and for EBV subtypes was recently evaluated to predict the NPC risk for a given individual in the endemic area (7).

**Precision treatment of NPC**

Chemoradiotherapy with/without adjuvant chemotherapy is the standard treatment regimen for advanced-stage NPC patients. Irrespective of the availability of modern treatments, 20%-30% of patients with locoregionally advanced NPC still die from complications caused by distant metastasis. This is in part due to the current lack of effective biomarkers to guide individualized treatment. Therefore, precise treatment is a tough clinical dilemma that we need to address. Circulating EBV DNA is an independent prognostic biomarker for patients with NPC and currently considered to be the most attractive potential biomarker to complement TNM classification (8-10). Several clinical trials will incorporate serum EBV DNA as a biomarker to guide precise treatment for patients with NPC (11, 12). However, the drawback of plasma EBV DNA as a biomarker at present is that its measurement has yet to be globally standardized.

The benefits gained from concurrent chemotherapy are set to plateau, and new treatments should be explored to improve the prognosis for NPC patients. In view of the consistent presence of EBV in NPC, it is possible to exploit molecular agents, specific antibodies, therapeutic virus vaccines, and immunotherapies to target EBV antigens or EBV-regulated pathways.

The extracellular loop domains of EBV latent membrane proteins (LMP1 and LMP2) may serve as therapeutic targets. An antibody against the TES1 domain of LMP1 inhibited the proliferation of NPC cells in a dose-dependent manner (13). Recently, agents targeting viral DNA replication protein EBNA1 and LMP1 have been explored. A new approach targeting EBNA1 proposed a targeted expression vector pMC.BESPX-oriP (origin of plasmid replication) that could carry diversified therapeutic genes for EBV-positive NPC (14).

Regarding the EBV-regulated pathways, other treatment approaches may be available. Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) are upregulated by LMP1 in NPC, and clinical trials of their inhibitors, such as cetuximab and sorafenib against EGFR and VEGF respectively, show efficacy and tolerance for NPC treatment (15, 16). DZ1 is an LMP1-targeted DNAzyme that downregulates the expression of LMP1, and potentially inhibits proliferation of and increases radiosensitivity of NPC cells (17).

Another promising targeted therapeutic strategy combining induction of EBV lytic cycles and antiviral drugs has been proposed. Representative substances that activate the lytic cycle of EBV include drugs affecting host DNA methylation and histone deacetylation. Nucleoside analogs, such as acyclovir and ganciclovir, can eliminate EBV effectively. As several clinical studies have shown, combining arginine butyrate with ganciclovir and sodium valproate, an inducer of DNA demethylation, had therapeutic benefits for EBV-associated malignancies (18, 19). Further phase 3 clinical trials are needed to assess the clinical safety and efficacy of these strategies.

Immunotherapies targeting EBV provide a highly promising strategy to cure NPC. NPC cells harboring EBNA1, LMP1, and LMP2 can be recognized by cytotoxic T cells, suggesting the potential to produce EBV-specific therapeutic vaccines by inoculating patients with EBV-associated latent antigens. Several clinical trials have reported that infusions of EBV-specific cytotoxic T lymphocytes (CTLs) into advanced NPC patients resulted in remissions (20). Combining conventional therapies with EBV-CTLs in patients with advanced NPC achieved positive survival outcomes (21). Chimeric antigen receptor T cell (CAR-T) and T-cell receptor T cell (TCR-T) immunotherapies are also potentially promising strategies. Immune checkpoint modulators, such as PD-1, CTLA4, CD160, TIM3, and LAG3, are reported to be upregulated in exhausted cytotoxic T cells in tumor and chronic virus infection models (22). PD-L1 is reported to associate with NPC and is regulated by the LMP1 and interferon (IFN)-γ pathways (23). The anti PD-1 drugs pembrolizumab and nivolumab have been applied to cancer patients and achieved a certain durable objective (complete or partial)
response rate of 31%-44% for advanced melanoma, 19%-20% for non-small cell lung cancer, 22%-25% for renal cell carcinoma and 14%-25% for head and neck cancer (24, 25). Targeting a combination of two checkpoints, such as PD-1 and LAG3, could theoretically improve NPC outcomes.

In summary, as more low-frequency mutations are identified by whole-genome sequencing, integration of panomics data may help to explore the predictive biomarkers that will enable selection of patients for a specific therapy. Nevertheless, strategies targeting EBV will be of great value.

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CAR T-cell–based therapeutic modality in solid tumors: How to achieve precision

Yang Liu, Hanren Dai, Yao Wang, Zhiqiang Wu, Yi Zhang, and Weidong Han

The clinical successes of CD19-targeted chimeric antigen receptor (CAR) T-cell treatment in B-cell lineage hematopoietic malignancies in recent years (1), especially in acute lymphocytic leukemia (ALL), have shown that tumor cells can be precisely and efficiently targeted and eliminated by tumor-associated, antigen-rediredicted immune cells. However, consideration of the significant differences between liquid and solid tumors is needed to ascertain rational, feasible, and efficient CAR T-cell–based therapeutic modalities in solid tumors. How to precisely instruct transfused CAR T cells to attack tumor cells growing outside of normal circulation is still a great challenge. The following considerations and opinions may help move us to this end.

Safety
Unlike CD19, which is expressed solely in B-cell lineages, tumor-associated antigens identified thus far are not strictly limited to solid tumors. These antigens can always be detected in comparatively lower levels in several normal cell types and may even be essential for maintaining cell homeostasis. Toxicities derived from off-tumor effects—also known as the “bystander effect”—have to be anticipated when using increasing dosages of CAR T-cell infusions. The complete clearance of cells expressing the target antigen can possibly lead to severe, and even lethal, events. To avoid this, one strategy is to modify CAR T cells to recognize only cells that express two aberrant antigens (2). Alternatively, fine tuning the cytoidal activities of CAR T cells so that less of a bystander effect occurs may broaden tumor-associated antigen targets (3).
Target selection

Contrary to CD19 expression in nearly all B-cell lineage malignant cells, tumor-associated antigens identified so far are highly heterogeneous in solid tumors. Any one antigen cannot cover all tumor cells existing within one single tumor mass, let alone those in adjacent and distal metastatic lesions. Theoretically, the high heterogeneity inherent in solid tumors would inevitably result in tumor recurrence if just one antigen was selected as a target for CAR T cells. Analysis of CAR T-cell infusions reveals a clinical response rate of less than 20%, and the effect is always transient. As we reported recently, only 2 out of 10 patients with refractory and advanced non-small cell lung cancer experienced partial remission lasting 2 to 10 months following epidermal growth factor receptor (EGFR)-redirected CAR T-cell infusion. This was after we demonstrated via tumor biopsies that the EGFR-positive tumor cells should largely be cleared after cell therapy (4). To target >90% of tumor cells, we believe that at least two antigens need to be targeted. Recent unpublished results from immunohistochemical screening of cholangiocarcinoma and pancreatic carcinoma samples suggest that approximately 50% of patients with epithelium-derived tumors would be suitable for the combinatorial targeting of EGFR and EGFR2 using the CAR T-cell system.

There is evidence that a tumor-initiating cell, or tumor stem cell, is involved in the processes of tumorigenesis, metastasis, progression, and potential treatment resistance that occur in most solid tumors (5). Prior research has shown that most tumor cells in the tumor mass are descendants of rare and senescent stem-like tumor cells. Many molecular pathways and signaling events impacting the behavior of tumor stem cells have been characterized through research on stem cell-enriched tumor cells isolated using differential surface markers such as CD44, CD133, and ABCG2 (6). Targeting dormant tumor stem cells using CAR T-cell-based therapies has produced promising results (7). CD133 is a particularly attractive target because it is the preferred marker for epithelium-derived tumor stem cell isolation (6), and is also a marker of circulating epithelial progenitor cells (EPCs), which are thought to be the cell type that forms the premetastatic niche at distant sites in the body (8). A clinical trial utilizing a “double-hit” (cancer stem cells and EPCs) CD133-specific CAR T-cell treatment in refractory epithelium-derived tumors is currently underway.

Considering the requirement of periodic CAR T-cell infusions in solid tumors and the development of antimurine single-chain fragment variable (scFv) immunity in patients, we have proposed a promising treatment regimen involving the sequential administration of CAR T cells targeting multiple antigens (Figure 1) (1, 4, 8, 9).

Trafficking and persistence

Trafficking into the tumor mass and proliferation of CAR T cells in situ is a rate-limiting step in this therapy. Only CAR T cells migrating outside of the circulatory system have the chance to squeeze into the solid tumor mass. Modification of CAR T cells that focuses on how to improve their trafficking efficacy has been explored using experimental animal models (9). One promising strategy to at least partially solve the low efficiency of CAR T-cell trafficking is to combine this therapy with other approved agents or approaches, such as chemotherapies, radiotherapies, immune checkpoint inhibitors or activators, and small molecule compounds.

Some chemotherapies serve not only as cytotoxic agents but also as immune adjuvants (10). Nab-paclitaxel, an accepted tumor stroma targeting agent, may help CAR T-cell homing to the tumor mass, and cyclophosphamide can deplete endogenous lymphocytes including immune-inhibitory cells within the tumor bed, to make room for CAR T-cell expansion. In our current trial, EGFR-, HER2-, CD133-, and mesothelin-directed CAR T, together with Nab-paclitaxel and cyclophosphamide, were administered before cell infusion as a preferred neo-adjuvant conditioning regimen. In addition, fludarabine/cyclophosphamide, a commonly used conditioning regimen in hematopoietic malignancies, can also be effective as a part of this treatment plan.

Local radiation can induce an antitumor immune response by promoting lymphocyte infiltration, and in rare cases, trigger an abscopal effect possibly mediated by enhancing antigen presentation and tumor immunogenicity, increasing production of cytokines and altering the tumor microenvironment though a cancer-immunity cycle mechanism (11). We have postulated that the trafficking and functional activity of CAR T cells would be greatly enhanced by using combination therapies that include radiation.

Alternative assembly

Some laboratories are upgrading to “armed” or “robust” CAR T cells (9): To enhance and amplify the antitumor efficacy of CAR T cells, an alternative “weapon”—such as interleukin (IL)-12 or an oncolytic
virus—can be assembled in CAR T cells and released in an antigen/CAR T-cell interaction-dependent manner. Additionally, CAR T cells can be made more robust through a genetic engineering strategy that switches inhibitory receptor signaling to activating transduction (12).

CAR T cells generated from T cells enriched with tumor neoantigen-specific T-cell receptor (TCR) are of great interest, because the antitumor activity of T cells produced by this strategy will be greatly extended, at least theoretically. So far, the epigenetic alterations of tumor cells have been well linked to the manifestation of tumor antigens including neoantigens and the subsequent antitumor immune response of the host (13). Our previous work also showed that the TCR repertoire of peripheral blood in patients treated with low-dose decitabine, a DNA methyltransferase inhibitor, can be extensively broadened (14), further raising the possibility that more powerful CAR T cells could be produced in patients treated with epigenetic agents.

Future perspectives

Although several barriers need to be overcome, current trials of CAR T-cell treatments in solid tumors show promise. Rational and precise integration of CAR T-cell treatment into other approved approaches is an important treatment option in the clinic, coupled with careful engineering of CAR T cells in the laboratory. Given the heterogeneity, hierarchy, antigen shift, and immunosuppressive microenvironment seen in solid tumors, we have proposed a novel CAR T-cell–based therapeutic modality (Figure 1), termed a “neoadjuvant conditioning-primed CAR T-cell cocktail and combinatorial modality.” We are hopeful that using the precision of CAR T-cell therapy in this new way will enhance the landscape of cancer treatment and patient care in the future.

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Breast cancer research in the era of precision medicine

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As next-generation sequencing continues to reveal new information about the genetic background of different cancers, novel cancer-associated genetic alterations are being identified and their functional consequences investigated. It is therefore an ideal time to implement precision medicine; but despite its exciting potential, there are only a few selected diseases and molecular subtypes for which effective therapies have been shown. One example is anti-human epidermal growth factor receptor 2 (HER2)-targeted therapy for HER2-positive breast cancer (1).

Breast cancer is the most common cancer diagnosed in women, and approximately one in eight women living in the United States has a lifetime risk of developing the disease (2). Breast cancer is also one of the most studied solid tumors and has the potential for being manageable with a precision medicine approach. It has been well established that these tumors are extremely heterogeneous (3, 4). According to gene expression profiles, breast cancer can be divided into several molecular subtypes, including luminal breast cancer, HER2-positive breast cancer, and triple-negative breast cancer (TNBC) (3, 5). For each subtype, there are unique tumor biology characteristics and treatment strategies. Intrinsic subtypes are associated with different gene expression and mutation profiles as well as different prognoses and responses to therapies (6). In the era of precision medicine, therapies are being developed using the framework of molecular subtyping (7). Here, we summarize the major challenges and possible solutions of treating so-called “refractory” breast cancer.

Late recurrence in luminal breast cancer

Luminal breast cancer can be further divided into the Luminal A and Luminal B subtypes. Both subtypes are associated with high expression of the estrogen receptor (ER); they are typically responsive to endocrine therapy but are less responsive to chemotherapy. Recurrently mutated genes in both subtypes include PIK3CA, CDH1, MAP3K1, GATA3, MAP2K4, FOXA1, TP53, PTEN, and RUNX1 (6). The Luminal A subtype is characterized by a low pathological grade, high differentiation, and low proliferation rates. This subtype has the highest number of recurrently mutated genes and the lowest total number of mutations for each tumor, indicating that these alterations are likely to be driver mutations. There are lower progesterone receptor (PR) and higher Ki-67 expression levels in the Luminal B subtype than the Luminal A subtype. Luminal B tumors also have higher complete remission rates in response to neoadjuvant chemotherapy, and they are possibly more sensitive to adjuvant chemotherapy than Luminal A tumors (8).

Luminal breast cancer accounts for approximately two-thirds of all cases (9). Compared to TNBC and HER2-positive breast cancers, luminal breast cancer has more therapeutic options, including hormonal therapy and HER2-targeted therapy. However, after undergoing 5 years of adjuvant hormonal therapy, patients with luminal breast cancer have a sustained risk of disease recurrence and death for at least 15 years after diagnosis (10). Luminal breast cancer includes a group of highly heterogeneous diseases, and the prognosis of each patient is highly variable. Therefore, it is important to identify patients with a high risk of late recurrence and poor prognosis so that further treatments can be applied. The combination of molecular biomarkers and traditional clinicopathological characteristics may offer alternative routes for further classifying luminal breast cancer into subclasses showing different endocrine sensitivity and therapeutic outcomes.

Resistance to targeted therapy in HER2-positive breast cancer

HER2-positive breast cancer is characterized by high levels of HER2 amplification and low levels of ER or PR expression. It also has the highest single nucleotide mutation rate and a short list of recurrently mutated genes (of which TP53 and PIK3CA are most frequently mutated) compared to other subtypes (6). These tumors generally have a high pathological grade and a high rate of brain metastases (11). They are sensitive to adjuvant

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anthracyclines and taxanes, and they are typically responsive to the (neo)adjuvant trastuzumab (HER2-targeted therapy) in combination with chemotherapy (7).

While HER2-targeted therapies, such as trastuzumab and lapatinib, have considerable efficacy in treating HER2-positive breast cancer patients (12-14), clinicians and researchers alike have increasingly observed primary and acquired drug resistance in HER2-positive breast cancer patients, undermining the clinical value of these HER2-targeted drugs (15, 16). Extensive research has been conducted on the molecular mechanisms contributing to trastuzumab and lapatinib resistance (14), including the overexpression or hyperexpression of other HER family receptors and their ligands, PI3KCA mutations, AKT mutations or amplifications, and loss of phosphatase and tensin homolog (PTEN) expression, all of which result in amplification of the PI3K/Akt/mTOR pathway. Recently, we revealed that mutations in the HER2 kinase domain might be a key mechanism of resistance to HER2-targeted therapy, and that irreversible tyrosine kinase inhibitors, such as neratinib, may offer alternative treatment options. Using panomics solutions, researchers are able to further identify comprehensive drug resistance mechanisms and determine ways to overcome resistance to HER2-targeted therapies.

**Heterogeneity and lack of druggable targets in TNBC**

TNBC, which lacks ER and PR expression as well as HER2 amplification, accounts for 10%-20% of all breast cancers. TNBCs occur more frequently in younger patients and in general, the tumors are larger in size, have a higher grade, are more likely to show lymph node involvement at diagnosis, and are biologically more aggressive (17, 18). This breast cancer subtype is associated with BRCA1 germline mutations, and TP53 is the only recurrently mutated gene (6). Chemotherapy is the primary treatment
for TNBC. Despite their increased rates of clinical response to neoadjuvant chemotherapy, women with TNBC have a higher rate of distant metastasis and a poorer prognosis than women with other breast cancer subtypes.

According to the Lehmann/Pietenpol classification, TNBCs can be further subdivided into seven types that display unique gene expression patterns and ontologies (19). A challenge to TNBC treatment might be the disease’s heterogeneity and the absence of well-defined molecular targets. Similar chemotherapy strategies evoke diverse responses and clinical outcomes in TNBC patients. At present, no method exists for categorizing differences between TNBC patients at the time of diagnosis. Thus, further classifying this aggressive disease subtype in order to better tailor patient treatment is a top priority. Meanwhile, identifying effective biomarkers and druggable targets specific to TNBC patients would significantly enhance personalized treatments. Substantial parallel sequencing and other ‘omics technologies have identified potentially actionable molecular features in some TNBCs, such as germline BRCA1/2 mutations, high expression of the androgen receptor, and several rare genomic alterations (20). Recently, using comprehensive transcriptome profiling, we have been able to develop a novel TNBC classification system and also identify subtype-specific long noncoding RNAs that may be useful biomarkers and therapeutic targets (21).

The challenges noted above are the most problematic in the current treatment of breast cancer. The implementation of multiomics-driven cancer medicine may help to overcome them (Figure 1). First, a high-quality, representative patient cohort should be established. Second, the panomics data profile of the patients’ tumors should be characterized using state-of-the-art technologies. Third, the ‘omics data should be filtered using a knowledge base of existing and emerging anticancer drugs to identify potential targets and predictive/prognostic biomarkers. Finally, an annotated list that can be incorporated into clinical decision-making should be presented to the treating oncologist. Future clinical research and patient treatment with targeted agents are likely to be more informative when using genetic studies performed on specimens and with precise preselection of patients.

We are entering an exciting era in which it is possible to provide breast cancer patients with precision therapy based on the factors driving their tumors and using the emerging drug toolkit. This therapy may provide the greatest opportunity for combating breast cancer to date. With the continued progress in developing new biomarker-based therapies and tests for earlier cancer detection, we can anticipate giant steps forward in improving patient outcomes in the future.

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Gene expression profiling–guided clinical precision treatment for patients with endometrial carcinoma

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Endometrial carcinoma (EC), a common gynecologic cancer, is the main component of uterine corpus malignant tumors (1). In China, uterine corpus malignant tumor incidence in 2014 was 5.84 per 100,000 individuals, and the disease composition ratio was 1.54% (2). The incidence trend has been increasing, recently measured at 6.46 per 100,000 individuals in 2015 (3). Traditionally, the histological grading of EC has been based on Bokhman’s classification, which divides it into type I and type II. Personalized therapy strategies have been based on age of onset, International Federation of Gynecology and Obstetrics (FIGO) staging, histology types, intraperitoneal features, or patient preference (1, 4). Most EC patients, especially those with early-stage endometrioid endometrial adenocarcinoma (EEC), will have a good prognosis (5). However, a 2014 study showed that similar staging and histology results in early-stage EC patients had completely different prognoses postoperatively, with EEC recurring in more than 8% of patients (6). Moreover, 14.5% of high-risk patients, including those with poorly differentiated EEC or uterine papillary serous carcinoma (UPSC) survived for more than five years after surgery in our clinical practice (7).

In a previous study, we found that differential surgical management does not contribute to these diverse prognoses, and that even similar clinical management may result in quite different outcomes (5). These results suggest that EC is a highly heterogeneous disease (8), and that different genetic alterations may exist in tumors exhibiting the same histological phenotype, resulting in different biological behavior as well as different sensitivities to treatment. Additionally, different clinicopathological characteristics of molecular subtypes may exist in the same histology types of EC, which also suggests that some flaws exist in the current therapy strategy based on the clinical pathological diagnosis and Bokhman’s classification. It is therefore necessary to perform tumor genotyping in EC patients to properly understand and predict treatment responses (9, 10).

We performed a series of gene expression profiling experiments using EC specimens to identify the molecular subtypes present in each sample and ascertain the individual’s clinical characteristics and prognosis. Our aim was to establish a gene expression profile–guided and clinically precise treatment for EC patients (11–13).

In a previous study of EC, we measured the expression levels of several routine biomarkers in tumor specimens to assess prognosis after surgery. These biomarkers included the estrogen receptor (ER), progesterone receptor (PR), tumor protein p53, phosphatase and tensin homolog (PTEN), and the Ki-67 proliferation marker. The results showed that only PR expression could be used as an independent marker of poor prognosis, and that the incidence of PR-negative tumors in the Chinese population was 14.5% (14). These results suggest the importance of establishing a more comprehensive estimation system (including biomarker panel typing, age of onset, FIGO staging, histology types, and intraperitoneal features, among other factors) to predict outcomes of EC patients based on intertumor heterogeneity.

UPSC vs. EEC

We selected 492 EC-related genes from the National Center for Biotechnology Information (NCBI) database, and oligoprobes against these genes were then designed using the UniGene Database and the Reference Sequence (RefSeq) Database to create a 492-gene microarray (11). Figure 1 shows the workflow for gene expression profiling of these putatively EC-related genes (11–13). The gene expression profiles of 37 EC samples were tested on this microarray; samples were collected from hysterectomies performed at

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the Department of Obstetrics and Gynecology at Peking University People’s Hospital from October 1, 2007, to December 31, 2008. A hierarchical cluster algorithm was used to sort tumor samples according to the expression patterns of the analyzed genes (11). Four distinct tumor clusters (clusters 1–4) were present among the 37 patients, and a clear correlation existed between the histological features and clinical characteristics of the tumors (11). UPSC samples exhibited distinct gene expression profiles (cluster 1) compared to those of EEC (clusters 2–4) (12). Using significance analysis of microarrays (SAM), 46 genes that could highly discriminate UPSC from EEC were identified. These gene expression patterns were further evaluated by an immunohistochemistry assay to validate the results, indicating that the expression levels of several genes were significantly different between EEC and UPSC, including mitotic checkpoint serine/threonine-protein kinase BUB1 (BUB1), fucosyltransferase 8 (FUT8), kinetochore protein Hec1 (NDC80), annexin A4 (ANXA4), and bcl-2 binding component 3 (BBC3) (12).

EEC subtypes

Based on hierarchical cluster analysis, EEC cases could be divided into three subtypes (clusters 2–4) (13). Distinct clinicopathological features appeared in each cluster. However, in a pairwise analysis, the differences in clinicopathological features between cluster 3 and cluster 4 were not significant. The distribution of clinicopathological parameters among these EEC clusters was also examined, and more prognosis-related high-risk factors (including high grade, deep myometrial invasion, and PR-negative expression status) were observed in cluster 2. To identify EC prognosis-related genes, the genes that were differentially expressed between cluster 2 and clusters 3 and 4 were identified by the SAM method; 47 differential genes were selected and defined as the primary EC prognosis-related gene candidates. Based on our previous retrospective study (14), the grade and PR status were independent risk factors for EC outcomes. The 37 EC samples were therefore divided into two groups according to their PR expression status and grade. SAM indicated 21 differentially expressed genes (Figure 1), and these genes were defined as secondary EC prognosis-related gene candidates. When these two sets were compared and combined with the 46 genes differentially expressed in UPSC and EEC samples, 21 overlapping genes were identified. These were regarded as EC prognosis-related genes.

Unsupervised clustering in 33 EC cases was performed with the 21 EC prognosis-related genes (including 24 FIGO stage I EEC cases, four FIGO stage III EEC cases, and five UPSC cases). The 33 cases were divided into two clusters (clusters 5 and 6), with the high-risk prognosis-related factors distributed as follows (see Figure 2): (1) Nearly all of the recurrent and deceased cases were found to be in cluster 6, and only one deceased case was present in cluster 5; (2) the only two nonrecurrent stage III cases were in cluster 5; (3) all of the ER-negative, PR-negative, or UPSC cases fell into
cluster 6; and (4) three of 15 cases with grade 3 and/or deep myometrial invasion were found in cluster 5. These results suggested that EC cases were successfully classified into two clusters in which outcomes segregated together with defined EC prognosis-related genes. These 21 genes may therefore be useful in the future for predicting outcomes in EC patients and for guiding clinical management (15).

To further confirm the correlation between the 21 EC prognosis-related genes and EC progression, the genes and their molecular mechanisms were individually analyzed and verified through in vivo and in vitro experiments. For example, dual-specificity phosphatase 1 (DUSP1) was overexpressed in the early stage and nonrecurrent stage of the EC samples, while it showed little expression in recurrent-stage EC samples (Figure 3) (16). A stable knockdown of DUSP1 increased human EC cells migration and proliferation and activated the MAPK/ERK pathway in the Ishikawa endometrial adenocarcinoma cell line. Medroxyprogesterone acetate upregulated DUSP1 expression and inhibited the MAPK/ERK pathway as

FIGURE 2. Hierarchical clustering analysis of 21 EC prognosis-related genes in 33 EC samples. The upper table shows high-risk prognosis-related factors in each case. Recurrence indicates that the patient experienced recurrence within an average of 64 months. The “Dead” label indicates that the patient died, and that death was attributable to EC, within an average of 64 months following treatment. UPSC, uterine papillary serous carcinoma; H. Stage, FIGO stage III or IV; H. Grade, grade 3; D.MI, myometrial invasion ≥2; ER (-), estrogen receptor negative; PR (-), progesterone receptor negative. The lower panel shows hierarchical clustering. Red indicates gene expression above the median; green indicates expression below the median; black indicates expression equal to the median. Gray indicates inadequate or missing data.

FIGURE 3. DUSP1 expression in nonrecurrent and recurrent stage Ia EEC tissues (100×). Scale bars: 100 μm. EEC, endometrioid endometrial adenocarcinoma.
These results indicate that DUSP1 may be a target for precision treatment and for predicting EEC outcomes.

Recently, we committed to developing a biomarker panel utilizing these 21 EC prognosis-related genes with the goal of predicting outcomes for Chinese EC patients. Using this panel, we hope to be able to determine the precise postoperative adjuvant therapy that should be administered to each EC patient to prevent progression of the disease, whether that be chemotherapy, endocrine therapy, molecular targeted therapy, radiotherapy, or a combination of these.

Furthermore, by combining studies of inter- and intratumor heterogeneity with the practice of precision therapy in gynecological oncology, we believe that a targeted drug development program can be created.

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Glycomics and its application potential in precision medicine

The human genome encodes approximately 40,000 proteins (1), with posttranslational modifications (PTMs) such as phosphorylation, ubiquitination, acetylation, lipidation, and glycosylation increasing protein complexity by several orders of magnitude. Of the numerous protein PTMs, glycosylation is the most complex and predominant (2). Over half of all human proteins are glycosylated (3). Glycosylation is a ubiquitous process in which proteins are modified by linear or branched carbohydrates known as glycans (4). Glycans are structurally diverse, and several glycans can bind to the same protein on different sites, resulting in multiple different glycoforms (5, 6). Glycans are further diversified by acetylation, sulfation, and phosphorylation (7). The study of the complete set of these complex cellular biomolecules in a given tissue or cell is termed “glycomics” (8), and entails glycan structure characterization, large-scale analysis of glycan-structure relationships, and glycan-protein interactions and their functions in human physiology (9).

Glycans are involved in molecular trafficking, protein folding, clearance of misfolded proteins, intercellular adhesion and cell–cell recognition, and mediating protein function when linked to proteins such as growth factors, immune receptors, cytokines, and enzymes localized in the cell-extracellular interphase (10). As glycans are large and bulky, they alter the chemophysical properties of proteins, including solubility and stability.

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turnover, and immunogenicity (6). Glycans also form a critical part of integrins, regulating adhesion to the basement membrane, triggering cell migration, and preventing apoptosis (11). The addition of N-acetylneuraminic acid (sialic acid) to the terminal end of charged glycans can regulate the half-life of many glycoproteins, making them useful for improving protein-based drugs (12).

Glycosylation is altered with changing environmental conditions, aging, and the presence of disease (3, 13, 14). When triggered by an external or biological stimulus, glycans undergo structural changes that can be exploited as biomarkers to predict disease onset, progress, or response to therapy. Glycomics thus has the potential to be applied in precision medicine.

The mammalian cell contains a repertoire of glycans (2). These glycans comprise chains of monosaccharide units that are linked by α and β-glycosidic bonds (6, 7). Three main types of glycans exist: N-glycans, O-glycans, and glycosaminoglycans (GAGs). These glycans differ in their core structure, whether they are branching or not, and the recognition sequence, if any, by which they attach to proteins (15).

N-glycans are the most common and well-understood, with an estimated 50%-90% of plasma proteins being N-glycosylated (6, 16). Figure 1 shows the different glycan types in the Golgi apparatus—where many glycosylation reactions take place—including N-glycans, O-glycans, two examples of proteoglycans (heparan sulfate and chondroitin sulfate), and membrane-bound glycans such as glycosphingolipids, and glycosylphosphatidylinositol (GPI) anchors (4, 15).

**Analytical high-throughput methods in glycomics**

Mass spectrometry (MS) is a sensitive tool for analyzing glycoforms (8, 17). MS allows the ionization of macromolecules such as proteins, glycans, and lipids for accurate molecular weight determination and structure analysis (18). Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, tandem MS/MS (16), electrospray ionization MS, ion trap MALDI-TOF MS, and Fourier transform ion cyclotron resonance MS are commonly utilized for glycan structure elucidation (19). A limitation of MS methods is that monosaccharide components are difficult to assign using these techniques, and intersaccharide linkages in the glycan structure often yield poor spectral intensity, so the information obtained is often not adequate for complete glycan characterization (10, 20). MS-based methods, therefore, require additional analytical techniques for complete structural characterization (20). Nuclear magnetic resonance (NMR) is another useful tool for glycan profiling that elucidates structures based on their chemical shifts and coupling constants (21). NMR provides information about the glycan molecules’ stereochemistry, the orientation of anomeric atoms, and the intersaccharide bonds. However, adequate structural elucidation of glycans by NMR is dependent on the amount and purity of the sample (≥95% pure sample is needed) (21). Glycans are polar macromolecules, hence they need to be separated by high-performance liquid chromatography (HPLC) with fluorescence detection (3), high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (22), or weak anion-
exchange HPLC (17). HPLC has become automated as hydrophilic interaction chromatography and ultra-high performance liquid chromatography, both having high resolution and the ability to separate N-glycans with precision and efficiency. These techniques are suitable for glycan analysis because they can separate both charged and neutral glycans in a single column (23); but their disadvantage is the inability to fully distinguish different glycan isomers, leading to a partial derivation of complex samples. Additionally, coelution of other glycans may occur, thereby preventing good quantitation. This limitation seems to be overcome by HPAEC-PAD, but at the expense of poor sensitivity and a longer analysis time. Capillary electrophoresis can quantify and resolve glycan structures with high selectivity, separation efficiency, and reproducibility, making it a powerful tool for determining glycan isomers, but it has a low throughput (24). Fluorophore-assisted carbohydrate electrophoresis is another technique for separating fluorophore-labeled glycans on polyacrylamide gel. It does not require extensive expertise, making it a widely used approach for glycan analysis (25).

**Glycomics and precision medicine**

Although glycosylation is a nontemplate-driven, biosynthetic process, it is regulated by “glyco-genes” (5) encoding specific enzymes that promote the glycosylation process (26). Mutations can change the orientation of the enzyme at glycosylation sites, thereby preventing glycosylation of the given polypeptide chains and affecting glycoprotein capabilities (27). Thus, an alteration in the genetic and molecular machinery can lead to human diseases, generally termed “congenital disorders of glycosylation” (CDGs) (28).

The N-glycosylation of the immunoglobulin G (IgG) Fc (fragment crystallizable) domain mediates the effector function of IgG, and hence IgG glycosylation is implicated in inflammatory and autoimmune diseases (29, 30). Analysis of systemic lupus erythematosus (SLE) patients showed a decrease in siaylation and galactosylation of plasma IgG among sufferers. Similarly, these patients have a decrease in core fucose and increased bisecting plasma GlcNAc (29). An investigation of the plasma IgG glycome composition in patients with ulcerative colitis (UC) and Crohn’s disease (CD) showed that both diseases are associated with significantly decreased IgG galactosylation and a significant decrease in the proportion of sialylated structures in CD (30). A TwinsUK registry study showed the association between IgG glycans and renal function (31). Our case-control study found that IgG glycans have potential as a putative biomarker for rheumatoid arthritis (RA), and that differences in glycan composition can be seen in RA active and remission states (32).

Cancer, Alzheimer’s disease, diabetes, metabolic syndromes, RA, SLE, and hypertension have been directly linked to N-glycosylation (3, 29, 32-36). Because genetic polymorphisms are distant from the phenotypes, and the nature of gene-gene interactions can be complex, glycans may be intermediate/dynamic biomarkers for risk stratification, diagnosis, and prognosis of disease (36).

**Outlook**

As interesting as N-glycan profiling is, it is not devoid of challenges. Most analytical methods are unable to detect the concentration of glycans on a microscale level (16). The requirement for high-purity samples is another challenge. Heterogeneity and the complexity of glycan structures makes N-glycan analysis difficult, warranting the need for new streamlined and automated glycobioinformatic resources (6). Additionally, only a few laboratories with advanced tools and expertise are able to analyze glycan structures, posing a challenge for those new to the field.

Despite these challenges, the scope of glycomics is broadening, and it is now becoming possible to integrate glycomics with other ‘omics. This new idea has led to the publication of the first “genomics meets glycomics” study (37). Validation of N-glycan profiling in different populations is vital for further understanding the influence of population-specific and gene-environment interactions on the dynamics of N-glycan structures. Currently, our team is focused on building upon this novel research by applying it to disease states in African, Australian, and Chinese populations, in order to establish N-glycans as biomarkers for chronic diseases.

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Urgent need for implementation of precision medicine in gastric cancer in China

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It was estimated that more than half a million people in China died from gastric cancer in 2015 (1). Early diagnosis, accurate classification, novel therapeutic targets, and personalized therapy, as well as effective control strategies for gastric cancer, are urgently needed. As the precision medicine initiative and Cancer Moonshot program for the exploration of novel cancer treatments get underway in the United States, the war against cancer is also ongoing in China.

Despite the continuous decline in both incidence and mortality in the past decades, gastric cancer remains the fifth most prevalent malignancy and the third leading cause of cancer death worldwide, with an estimated 1 million new cases and 723,000 deaths in 2012, almost half of which occurred in China (2, 3). The decline in cancer deaths is slow in China, due likely to the aging population, increased environmental threats (e.g., air pollution), and lifestyle (e.g., chronic infection, smoking, drinking, lack of exercise, and high-calorie diets) (4). According to a national survey, the number of new gastric cancer cases in China in 2015 was 679,100, with 498,000 mortalities, making it the second leading cause of cancer death after lung cancer (1). Here, we summarize the current status of gastric cancer-related public health issues and treatment options as well as government policies on fighting this disease.

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Anatomic, histologic, and molecular classifications of gastric cancer

Several classifications of gastric cancer based on anatomical and histological criteria have been established. The Laurén classification, which is widely accepted in clinical practice, groups gastric cancer into three subtypes: intestinal (well differentiated, comprising 74% of total gastric cancer patients), diffuse (undifferentiated, 16%), and other (10%) (5). The intestinal subtype is considered to have a better prognosis than the other subtypes (6, 7).

However, histological classification alone is unable to provide sufficient guidance for the selection of optimal chemotherapy regimens.

Taking advantage of improvements in our knowledge of cancer genetics and advances in genomic sequencing technologies, multiple studies have demonstrated the potential for molecular classification of gastric cancer for personalized treatments. In 2014, based on a comprehensive analysis of genetic, epigenetic, proteomic, and histological features of 295 primary gastric adenocarcinomas, The Cancer Genome Atlas (TCGA) project introduced a new molecular classification of gastric cancer (8) (Table 1), which is expected to facilitate personalized cancer treatments based on an individual’s molecular profiling.

<table>
<thead>
<tr>
<th>Molecular subtypes of gastric cancer</th>
<th>Molecular features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epstein-Barr virus (EBV) positive</td>
<td>PIK3CA mutation; PD-L1/2 overexpression; CDKN2A silencing; EBV-CpG island methylator phenotype (CIMP); and immune cell signaling</td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td>Hypermutation; gastric-CIMP; MLH1 silencing; and mitotic pathways</td>
</tr>
<tr>
<td>Genomically stable tumors</td>
<td>Diffuse histology; CDH1 and RHOA mutations; CLDN18-ARHGAP fusion; and cell adhesion</td>
</tr>
<tr>
<td>Tumors with chromosomal instability</td>
<td>Intestinal histology; TP53 mutation; and RTK-Ras activation</td>
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Precision medicine treatment of gastric cancer in China

In March 2015, the Chinese Ministry of Science and Technology launched a nationwide precision medicine-oriented health program with an estimated investment of US$9.2 billion over the next 15 years (2016–2030). The short-term goal is to bring the concept of precision medicine into the standard treatment of complicated diseases including cancer, metabolic diseases, and cardiovascular and cerebrovascular diseases within the first 5 years (2016–2020).

The ultimate goal is to improve disease diagnosis and to develop better-targeted therapeutics based on updated translational research for a better outcome in public health management. A total of 20 top public cancer hospitals and third-party medical laboratories were selected as pilot centers to conduct high-throughput sequencing for the identification of novel biomarkers that will eventually provide more accurate diagnosis and guide subsequent therapies. It is anticipated that this large population study will facilitate the collection of important data that can be applied in the development of new therapeutic strategies.

Currently, new adjuvant and postoperative chemotherapies are considered to be standard procedures following D2 lymphadenectomy for advanced gastric cancer patients in Eastern Asia, and laboratory tests are available to predict the chemosensitivity, toxicity, and prognosis of gastric cancer patients (9–11). To further standardize treatments, the Clinical Research and Application Alliance of Precision Medicine in China, established in late 2015, is focusing on the implementation of clinical pharmacogenomics in clinical practice. Also, the “Manual for Chinese Precision Medicine Medications” will soon be released, which focuses on 110 medication types listed in PharmGKB, a pharmacogenomics knowledge base, detailing criteria for genetic testing in clinical applications, including routine gastric cancer tests.

Comprehensive genomic studies have established the molecular foundation for applying trastuzumab in combination with chemotherapy as the first-line treatment of HER2-positive metastatic and locally advanced gastric cancers (12, 13). Trastuzumab, approved by the China Food and Drug Administration in 2012, is currently the only targeted therapy for HER2-positive gastric cancer in China. HER2 gene amplification and protein overexpression occur in 13%-16% of Chinese gastric cancer patients, with higher rates found in the intestinal subtype (14–17). Patients with this subtype, showing either well-differentiated or poorly differentiated gastric cancers without lymph node metastasis, may represent suitable candidates for receiving trastuzumab (17).

In addition to HER2 abnormalities, other molecular biomarkers have been identified in gastric cancer,
providing more potential options for personalized therapies. These include alternations in epidermal growth factor receptor (EGFR, 8%), fibroblast growth factor receptor 2 (FGFR2, 9%), mesenchymal-epithelial transition factor (MET) amplifications (4%), and V-Ki-Ras2 Kirsten rat sarcoma (KRAS) viral oncogene homolog amplifications (9%) (18, 19). A few novel molecular markers have been recently discovered in the Chinese population and still need to be validated by suitable clinical studies (20).

Next-generation sequencing utilizing a panel of actionable cancer genes has been widely applied in cancer hospitals to aid in tumor classification and to guide targeted therapies. Gene panels, which range from several genes to hundreds of genes, can analyze circulating tumor DNA (ctDNA), snap-frozen tissue, or formalin-fixed, paraffin-embedded samples. Tissue from advanced or metastatic gastric cancer patients can therefore be used to search for diagnostic and actionable drug targets (21, 22). Isolation and testing of ctDNA could serve as a convenient and noninvasive approach for preoperation evaluation and real-time monitoring of cancer progression/regression/relapse following treatment (23, 24).

The majority of gastric cancer cases are associated with pathogen infection, including the bacterium Helicobacter pylori (25). In addition, based on TCGA’s molecular classification, 9% of patients are Epstein-Barr virus (EBV)-positive, and 22% of them have high microsatellite instability (8). Thus almost one-third of patients are expressing foreign or mutated antigens that can be exploited for T-cell-based immune targeting. With this in mind, the initial success from the KEYNOTE-012 trial, a multicenter phase 1b study utilizing the anti-PD1 antibody pembrolizumab to treat 39 PD-L1-positive gastric cancer patients, is not surprising, indicating that 22% of patients in this population had positive responses to the antibody. This result is especially exciting because the cohort tested included East Asian patients from Japan, South Korea, and Taiwan (26). A more extensive study with a larger cohort and more Chinese patients would certainly yield some interesting data.

**Challenges for precision medicine in China**

The urgency to implement cancer precision medicine in China to provide better patient care and outcomes is now being recognized and driven by physicians. Concerted effort is needed in translating ‘omics information to the clinic. The challenges to reaching this goal include issues of tissue quality, higher demands on bioinformatics capacity, and the difficulty of communicating complex biological interpretation of ‘omics data to patients. Due to the heterogeneity of gastric cancer and the lack of targeted therapeutics, more innovative clinical trials need to be designed that explore treatments based on each tumor’s molecular profile, such as the U.S. National Cancer Institute Molecular Analysis for Therapy Choice (NCI-MATCH) precision medicine clinical trial (27). Physicians and researchers need to work closely to drive the application of precision medicine for the benefit of both Chinese patients and all those impacted by gastric cancer.

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Opportunities and challenges for precision medicine in pancreatic cancer prevention and treatment

Chengfeng Wang

The incidence of pancreatic cancer has been increasing year by year, and it is now one of the fastest-growing cancer types worldwide (1). Data from Europe shows that in 2014, pancreatic cancer was the only cancer causing increasing mortality (2). By 2030, the estimated mortality rate of pancreatic cancer will be second only to lung cancer (3). The American Cancer Society reported that in nearly 40 years, the 5-year survival rate for patients with malignant tumors overall has risen from 49% to 68% in the United States, while the 5-year survival rate for those with pancreatic cancer rose from 2% to only 7% in the same period (4).

Current prevention and treatment

Pancreatic cancer lacks effective treatments, making prevention a more critical objective. The direct cause of pancreatic cancer is unknown, although smoking, obesity, diabetes, alcoholism, and family history are known to be risk factors. However, in 50% of patients, the risk factors are unclear (4). The 5-year survival rate of pancreatic cancer patients carrying tumors with a ≤1-cm diameter is 100% after surgery. The 3- and 5-year survival rates of patients with tumor diameters of 1 cm to 2 cm and no lymph node metastasis or local invasion are 88.7% and 59.1%, respectively. The median survival time (MST) of patients with a tumor diameter of 2 cm to 3 cm, confined within the pancreas and without lymph node metastasis is 32 months, and the 5-year survival rate is 40%. However, the overall 5-year survival rate of pancreatic cancer is only 5% (5).

Early diagnosis of pancreatic cancer is important, but the rate of early diagnosis is just 5%. The reasons for this low rate include the following factors: (1) There is no way to determine who in the general population will benefit from early diagnosis screening; (2) existing pancreatic cancer screens are unsatisfactory; (3) the required frequency of screening is unknown; (4) the current treatments for preinvasive lesions are still controversial; (5) there are no effective, simple, and safe ways to identify early lesions that require treatment; and (6) no scientifically validated, efficient, economical, and convenient screening protocols exist (6).

Surgical resection is the only available means to effectively treat pancreatic cancer and achieve complete remission. The 2-year local recurrence rate after surgery is 80%, but the 5-year survival rate is only 20% to 30% (5). Research into new chemotherapy drugs, drug delivery systems, and radiotherapies has improved treatment success, but the efficacy of these modalities is still unsatisfactory (5).

Precision medicine: A golden opportunity

Precision medicine provides possibly the only means to overcome the current bottleneck in the treatment and prevention of pancreatic cancer. Melo et al. found that the protein glypican-1 in pancreatic cancer patients could be used to distinguish pancreatic cancer tissue from normal tissue, benign disease, and benign tumors; furthermore, the specificity and accuracy for diagnosis of early-, middle-, and late-stage pancreatic cancer were all 100%. This breakthrough has provided some hope for the early diagnosis of pancreatic cancer (7).

Wu et al. (8) used gene sequencing technology and found five unique genetic variations in the Chinese population; individuals carrying these variations had a sixfold higher risk of receiving a positive diagnosis of pancreatic cancer than noncarriers. Waddell et al. (9) identified four subtypes of pancreatic cancer using gene sequencing and found that different subtypes showed different types of DNA damage and therefore different drug targets. For example, poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors were effective on the unstable subtype of pancreatic cancer. As new gene sequencing technologies are developed that are more efficient, higher throughput, higher precision, and lower cost than previous technologies, this method of subtyping tumors will undoubtedly improve in precision (9).
Zheng et al. (10) found that suppression of the epithelial–mesenchymal transition—a critical step in tumor metastasis—could slow the proliferation of cancer cells and contribute to enhanced sensitivity to gemcitabine treatment. Another study found that knocking out genes that encode vitamin D-binding proteins could also increase the efficacy of gemcitabine. This result suggested that drugs that inactivate the vitamin D receptor could selectively kill pancreatic cancer cells without affecting healthy cells (11).

Using integrated genomic analysis, Bailey et al. (12) identified four pancreatic cancer subtypes: squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine/exocrine. Among these subtypes, the squamous form had the worst prognosis, with only a 4-month median survival, half that of the other subtypes. The data provided by this research presents many new opportunities for the prevention and treatment of pancreatic cancer.

Challenges for precision medicine
The efficacy of diagnosis and treatment of pancreatic cancer has reached a plateau; the application of precision medicine, however, provides some hope for advancement. A major challenge in prevention and treatment is the heterogeneous nature of pancreatic cancer. Different subtypes of the same tumor, the primary tumor and its metastases, and primary and recurrent tumors may all have different gene expression profiles. Yachida reported that about two-thirds of gene mutations in pancreatic cancer occur in the primary tumor, and a further one-third in the metastatic tumor. Present clinical diagnostic techniques are not able to achieve definite detection and diagnosis of tumors, and dynamic monitoring of treatment, particularly using noninvasive tests such as blood tests, is not yet a reality (13).

Those working to defeat pancreatic cancer face enormous challenges, such as early diagnosis and prevention, some of which can be addressed through the application of precision medicine methodologies. More research is clearly required before a cure for this cancer can be said to be within reach.

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ing the great burden of health care for Chinese women with breast cancer, early detection and precise treatment are vitally important for them.

Precision medicine is an approach to the prevention and treatment of disease that takes into account individual variation in genes, environment, and lifestyle. The recent development of next-generation sequencing technology has enabled a rapid and dramatic increase in our understanding of individual genetic variations and their impact on disease. This genetic information is helpful for the clinicians when identifying the right patients to receive a particular treatment, and to spare the patients who may not respond to such treatment.

Approximately 10% of Chinese women with breast cancer have a family history of the disease and are defined as having familial breast cancer (3). A subset of familial breast cancer patients may harbor a deleterious BRCA1/2 germline mutation. Breast cancer patients with this mutation exhibit different clinicopathological characteristics than those with sporadic breast cancer (4). The genetic makeup of BRCA1/2 patients could therefore allow them to benefit from precision medicine.

**Prevalence and breast cancer risk in Chinese women with BRCA1/2 germline mutations**

BRCA1 and BRCA2 are the most critical breast cancer susceptibility genes currently known to us; mutations in these genes lead to an increased risk for developing breast cancer. Genetic testing for BRCA1/2 mutations is widely applied to high-risk women (e.g., those with a family history of breast cancer). In order to understand the prevalence of BRCA1/2 germline mutations in Chinese women with breast cancer, we recently screened for these mutations in 5,931 unselected Chinese women with breast cancer. To our knowledge, this is the largest study of BRCA1/2 mutation prevalence in Chinese women with breast cancer to date. The overall BRCA1/2 mutation rate was 3.9% across the entire cohort. However, the BRCA1/2 mutation rate was as high as 16.9% in women with familial breast cancer in this group. The mean age of breast cancer onset in BRCA1/2 mutation carriers is significantly younger than noncarriers in Chinese women with familial breast cancer (BRCA1 carrier vs. noncarriers, 43 vs. 50; BRCA2 carriers vs. noncarriers, 46 vs. 50, respectively), suggesting that BRCA1/2 mutations play a crucial role in the development of breast cancer (4).

The prevalence of BRCA1/2 mutations in familial breast cancer patients in our cohort is similar to that in other ethnic populations. In addition, we observed that approximately 41% of BRCA1/2 mutations found were specific for Chinese women and have not previously been reported in the Breast Cancer Information Core database of the U.S. National Institutes of Health’s National Human Genome Research Institute (4). This discrepancy between Chinese and Caucasian women in the spectrum of BRCA1/2 mutations indicates that clinical relevance and breast cancer risk may differ among different ethnic groups. We therefore conducted a kin-cohort study to investigate the estimated cumulative risk in Chinese women who carry a deleterious BRCA1 or BRCA2 mutation. A total of 1,816 unselected breast cancer patients were enrolled in this study, in which we screened for BRCA1/2 mutations and compared the history of breast cancer in first-degree female relatives of BRCA1/2 carriers and noncarriers. We found that the estimated cumulative risk of breast cancer by age 70 is 37.9% [95% confidence interval (CI), 24.1%-54.4 %] for BRCA1 mutation carriers and 36.5% (95% CI, 26.7%-51.8%) for BRCA2 mutation carriers in Chinese women (5). Although the risk of breast cancer in Chinese BRCA1/2 mutation carriers is lower than in Caucasian partners, the over 30% lifetime breast cancer risk approaches the threshold used when considering prophylactic mastectomy. Apart from the BRCA1/2 genotypes, environmental and lifestyle factors may also influence the overall breast cancer risk for an individual, making early detection and preventative measures essential for these individuals and a topic worthy of further study.

**Treatment strategies for Chinese women carrying tumors with BRCA1/2 mutations**

Breast cancers exhibiting BRCA1/2 mutations differ from those with sporadic mutations in a range of clinicopathological characteristics, including early onset, high tumor grade, presence of secondary primary breast cancers, and a high incidence of contralateral breast cancers (4). Therefore, the treatment strategies for patients with BRCA1/2 mutations should differ from those with sporadic breast cancer.

Breast-conserving therapy is a standard approach for early-stage breast cancer patients; patients who received breast-conserving therapy had a good cosmetic outcome as well as similar survival rates compared to those who received
mastectomies (6). Whether BRCA1/2 mutation carriers should be treated with breast-conserving therapy or undergo more aggressive treatment is a topic of continued debate. Although data from the retrospective studies are inconclusive, many studies support the notion that breast cancer patients with a BRCA1/2 mutation should not undergo breast-conserving therapy because of the relatively high risk of cancer recurrence in the ipsilateral breast (the 5-year and 10-year recurrence risks were 5.8% and 12.9%, respectively) (7). In fact, the guidelines for cancer treatment published by the National Comprehensive Cancer Network—a not-for-profit alliance of 27 leading cancer centers around the world—suggest that breast-conserving therapy is not recommended for patients with a BRCA1/2 mutation. Women with a BRCA1/2 mutation have a high risk of contralateral cancer following the initial diagnosis (8). Therefore, preventative contralateral mastectomy is often a good option for these women.

Neoadjuvant chemotherapy is widely used in cases of operable primary breast cancers and provides a platform to quickly evaluate the efficacy of such treatment regimens. Both BRCA1/2 genes play a role in the repair of double-stranded DNA (dsDNA) breaks through the homologous recombination (HR) pathway. In vitro experiments suggested that cells without functional BRCA1/2 proteins are sensitive to agents causing dsDNA breaks (e.g., adriamycin and cisplatin). Therefore, one would expect breast cancer patients with a BRCA1/2 mutation to respond well to treatment with these compounds. Several studies have demonstrated that breast cancer patients with a BRCA1 mutation are more sensitive to cisplatin monotherapy than noncarriers in the neoadjuvant setting, showing a higher pathologic complete response (pCR) rate (range: 61%–75%) (9). The response to neoadjuvant chemotherapy in Chinese women with a BRCA1/2 mutation shows similar results (10). We investigated the associations between the presence of BRCA1 mutations and response of tumors to neoadjuvant anthracycline-based or taxane-based chemotherapy in Chinese women with BRCA1/2 mutated triple-negative (estrogen receptor-negative, progesterone receptor-negative, and HER2-negative) breast cancers. We found that BRCA1 mutation carriers are more sensitive to anthracycline-based neoadjuvant regimens than noncarriers (pCR rate: 57.1% vs. 29.0%), but this is not the case for taxane-based neoadjuvant regimens (10). These studies suggested that BRCA1/2 mutation carriers may be more likely to benefit from cisplatin- or anthracycline-based neoadjuvant regimens, but not from taxane-based regimens. These findings raise the possibility that BRCA1/2 mutation status is not only a marker for evaluating breast cancer risk, but can also inform the optimal neoadjuvant regimen. Randomized prospective clinical trials are warranted to confirm these findings before these results can be translated to the clinic.

**Targeted Therapy**

It is well documented that breast cancer patients with a BRCA1/2 mutation are deficient in the repair of dsDNA breaks via the HR pathway, and rely on other pathways to repair DNA damage, such as base excision repair (BER) (11). Poly(adenosine diphosphate-ribose) polymerase (PARP) plays a key role in the repair of single-stranded DNA breaks through the BER pathway. Tumor cells with a BRCA1/2 deficiency being blocked by PARP inhibitors show the accumulation of dsDNA breaks and eventually succumb to synthetic lethality. This effect is restricted only to BRCA1/2 deficiency tumor cells and is not seen in normal cells, making PARP inhibitors an optimal targeted therapy for BRCA1/2 mutated cancers (12, 13).

Olaparib, an orally active PARP inhibitor, induced synthetic lethality in BRCA1/2-deficient cells. It has shown promising results in early phase 1 and 2 clinical trials with advanced breast and ovarian cancer patients carrying a BRCA1/2 mutation (14). Although olaparib was halted in phase 3 trials in advanced breast cancer, it was successful in the treatment of advanced ovarian cancers with a BRCA1/2 mutation (15). Olaparib is the first targeted drug to be licensed for the treatment of recurrent ovarian cancer harboring deleterious BRCA1/2 germline mutations.

Currently, at least six PARP inhibitors are being tested in more than 20 phase 3 clinical trials, with the majority being used as monotherapy in BRCA1/2-mutated tumors (11). PARP inhibitors in combination with adjuvant chemotherapy may improve the efficacy of treatment for BRCA1/2-mutated tumors; therefore, selecting the optimal chemotherapy regimens and the right patients are the primary challenges for future clinical trials.

Chinese women with advanced breast cancer carrying a BRCA1/2 mutation have also been recruited in the international phase 3 clinical trial of
olaparib, with results from these trials anticipated in two to three years.

**Future directions**

BRCA1/2 germline mutations are found in approximately 17% of Chinese women with familial breast cancer (4). Other breast cancer susceptibility genes may exist in patients who have a familial history of breast cancer but are negative for BRCA1/2 mutations. Whole-genome or whole-exome sequencing assays provide a means to search for such genes. Using whole-exome sequencing to screen nine early onset familial breast cancer patients without BRCA1/2 mutations, we found a novel breast cancer susceptibility gene, RecQ helicase-like (RECQL) (16), which plays a crucial role in the maintenance of genome stability; approximately 2% of BRCA-negative familial breast cancer patients carry a RECQL mutation (16). We speculate that there are still other potential breast cancer susceptibility genes to be discovered.

In recent years, multigene panels of 25–30 genes have been commonly used for routine genetic testing. More importantly, the panels usually contain many other high or moderate breast cancer susceptibility genes, such as TP53, PALB2, PTEN, ATM, and CHEK2. A subset of women may carry a germline mutation in these susceptibility genes even though the BRCA1/2 genes remain unmutated. However, the clinical impact and breast cancer risk are largely unknown for mutations in these additional genes. Thus, the management of treatment for women carrying a mutation in other genes besides BRCA1/2 is a great challenge for clinicians. The authors encourage collaborations to study large numbers of patients that will provide the data needed to better assess the impact of these susceptibility genes on breast cancer prognosis and treatment.

**References**

Chapter three

Other applications of precision medicine
Recent progress in autism spectrum disorder research in China

Jinchen Li¹,², Lin Wang¹, Kun Zhang², Leisheng Shi², Yan Wang², Kun Xia¹, and Zhongsheng Sun²,³*

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized mainly by impaired social communication, repetitive behavior, and limited interests or activities. ASD has a prevalence of 1%–2% in the population, with a strong male bias and high frequency of comorbidities such as intellectual disability, microcephaly, macrocephaly, and epilepsy. Over the last few years, there have been unprecedented advances in understanding ASD in Western countries. Studies have also shown a significant increase of ASD incidence in China, leading to growing attention and research among the country’s scientists, clinicians, and government. Increased ASD research funding has been allocated by government and private agencies into its epidemiology, clinical features, and underlying genetic mechanisms. This review focuses on recent progress in clinical assessment and treatment intervention for ASD, and in the understanding of the genetic makeup of ASD in China. It also discusses the challenges and opportunities for future research into this disorder.

Clinical assessment

Since child psychiatrist Leo Kanner first reported on 11 ASD patients in 1943 (1), this disorder has become a major public health concern in the Western world. According to a report from the U.S. Centers for Disease Control and Prevention, ASD prevalence in 2012 was 14.6 per 1,000 (1 in 68) American children aged 8 years, and affected about 4.5 times more males than females (1 in 42 males and 1 in 189 females) (2). In 1982, Guotai Tao described the first Chinese ASD patients (3), and in recent years, ASD has been recognized as a major disorder in China.

No nationwide epidemiological surveys on ASD in China have been conducted, but there have been several reports indicating prevalence rates of 0.28–2.95 per 1,000 individuals, based on local surveys in Beijing, Tianjin, Jiangsu, Guizhou, and Fujian (4). The ASD prevalence in China is much lower than in the United States; this may be due to the lack of public awareness about ASD or the use of different diagnostic tools in China that focus primarily on autistic disorders rather than all ASD clinical subtypes (5) (Table 1). Screening methods such as the Autism Behavior Checklist have commonly been used in China, even though the Autism Diagnostic Observation Schedule (ADOS), the Autism Diagnostic Interview-Revised (ADI-R), and the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) have now been more extensively adopted (6). Supported by the Special Research Program of the National Health and Family Planning Commission of China, nationwide epidemiological surveys of ~120,000 children at 12 centers and the systematic evaluation of various diagnostic tools are currently underway. We expect that through these research efforts, Chinese-specific diagnostic tools with high sensitivity and specificity will emerge, along with more accurate reports of ASD prevalence in China.

ASD displays a broad range of clinical manifestations and comorbidities, such as intellectual disability and social anxiety disorder (Table 1) (7). Several clinical features, such as elevated serum brain-derived neurotrophic factor (BDNF) (8–10), decreased serum superoxide dismutase levels (11), and decreased serum 25-hydroxyvitamin D levels (12), have been observed in ASD patients in both China and Western countries. Additionally, broad abnormalities in brain structures and various risk-conferring factors are shared between the Han Chinese and Caucasian populations.

Genetic architecture

Twin studies have shown that ASD is highly heritable in both European (79) and Chinese populations (80). Several genome-wide associated studies (GWAS) have attempted to detect specific risk-conferring single nucleotide polymorphisms (SNPs) for ASD, including two studies from China that focused on the Han Chinese population (36, 37). The first study, by Xia and colleagues, examined 2,150 ASD cases in mainland China and identified three SNPs in 1p13.2 (P = 3.26 × 10⁻⁸ for rs6537835, P = 4.49 × 10⁻⁸ for rs936938, and P = 8.70 × 10⁻⁸ for rs1877455) that significantly associated with ASD (36). Another study detected two candidate genes associated with ASD, OR2M4 (P = 3.4 × 10⁻⁵) and MNT (P = 8.0 × 10⁻⁴), based on 315 Han Chinese patients in Taiwan and 1,115 controls (37). Beyond that,
Clinical Features
Prevalence
Risk Factors Maternal depression (SNPs)
Rare Variations
62, 63
Interventions Vitamin D3 supplementation (CNVs)
variations (CNVs) (6)
international collaborations reported that subjects of European ancestry (Table 1). For example, of these large-sample studies primarily investigated effects on ASD than common SNPs. However, most several studies have identified some risk-related SNPs (Table 1). Although the effect of each SNP is weak, a combination of several SNPs may account for most narrow-sense heritabilities (81).
Whole-exome sequencing (WES) and whole-genome sequencing (WGS) studies have found that rare mutations, including inherited mutations (82, 83), de novo mutations (DNMs) (84–91), and copy-number variations (CNVs) (92–94), have larger penetrance and effects on ASD than common SNPs. However, most of these large-sample studies primarily investigated subjects of European ancestry (Table 1). For example, international collaborations reported that ANK3 mutations are associated with ASD susceptibility based on WES of a cohort of 20 Caucasian patients (95). To promote further collaboration and data sharing in ASD research, Chinese scientists have established a consortium known as “Autism Clinical and Genetic Resources in China” (ACGC) (96).
Several WES and WGS studies of ASD in the Chinese population are currently ongoing. For example, 189 ASD risk genes in 1,543 ASD probands of Han Chinese were sequenced, and 10 genes with recurrent DNMs in Chinese cohorts were identified, including SCN2A, which was found in 1.1% of patients (96). In another project, microcephaly- and macrocephaly-associated genes in 536 ASD probands were sequenced, and it was found that risk genes for ASD and abnormal brain size were involved in chromatin modification, mitotic cell cycle, and synaptic transmission. Moreover, WGS was used on 32 trios of Chinese ASD to detect functional mutations in both coding and regulatory regions of the genome. Several genes harboring DNMs or CNVs in both Han Chinese and Caucasian patients were found, suggesting shared genetic burdens among these different populations. Shared clinical symptoms were observed among patients with ASD, schizophrenia, intellectual disability, and epileptic encephalopathy. Recently, we cataloged all publicly available DNMs of these four disorders in the NPdenovo database (www.wzgenomics.cn/NPdenovo) and characterized both the unique genetic components of each disorder and their shared patterns (97).

### Intervention
Because of the pathogenic complexity of ASD, personalized intervention is considered an effective clinical approach to treat patients. Recently, several drugs and behavioral approaches have been used to improve social skills and communication in Chinese ASD patients (Table 1). In addition, supplementation with vitamin D3 (75), vitamin A, and carotene have been recently recommended for ASD patients in China; however, their effectiveness needs to be evaluated in clinical trials. Moreover, structured teaching, social communication training, and mind-body exercise (76, 77) have been applied to some Chinese ASD patients, which may provide some fundamental data for ASD interventions in China and Western countries.

### Genotype–phenotype correlations for autism
Correlating the relationship between phenotypes and genotypes is a key step in ASD precision medicine. To address it, we launched the Genotype-Phenotype Correlation for Autism (GPCA) project. Briefly, GPCA incorporates two major procedures: correlating from genotypes to phenotypes (Figure 1A) and from

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**TABLE 1. Summary of findings from ASD studies of Han Chinese populations.**

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<td>Prevalence (per 1,000)</td>
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phenotypes to genotypes (Figure 1B). GCPA is currently recruiting large cohorts of ASD patients (including unaffected parents) and collecting detailed clinical data, with plans to recruit at least 10,000 ASD trios of Han Chinese descent within three years. Thus far, it has recruited approximately 3,000 trios.

For the genotypes-to-phenotypes procedure, we will first detect pathogenic variations in known-risk genes by targeted sequencing of large cohorts—these steps are expected to diagnose about 10%-20% of ASD patients (Figure 1A). We will then prioritize novel risk genes by applying WES/WGS in samples without pathogenic mutations of known genes, and detect more pathogenic variations of novel risk genes by targeted or Sanger sequencing. Finally, we will correlate the genotypes to the phenotypes of patients harboring pathogenic mutations in the same risk genes to define shared phenotypes in ASD patients.

For the phenotypes-to-genotypes procedure, we expect that sequencing of ASD samples based on clear classification of clinical phenotypes will be the next step in defining subtypes and deciphering etiology. In doing so, we have compiled detailed clinical data on all ASD and subtype patients, collecting those with similar clinical phenotypes into groups in order to identify common genetic components (Figure 1B). We will employ trio-based WES/WGS to prioritize candidate risk genes, and targeted sequencing and/or Sanger sequencing to detect additional pathogenic risk genes. Moreover, we will investigate their convergent functional pathways by bioinformatics analysis (such as protein–protein interaction and coexpression analysis), and validate their neurobiological mechanisms based on in vitro and in vivo models.

**Future studies**

Unprecedented progress has been made in understanding ASD etiology; however, several fundamental issues remain, including (1) deciphering the genetic architecture of ASD, including the influence of polygenic models or major gene models; (2) investigating the biological basis of ASD, such as male bias in ASD prevalence, female protective effects, and X-linked variations in males; (3) elucidating the neurobiological mechanisms involved in ASD, such as the link between specific gene sets and certain neurons and/or brain regions (98); (4) sequencing more patients and prioritizing new risk genes by developing new bioinformatics tools such as mirTrios (99); (5) building bridges between clinical features and genotypes of risk genes, such as CHD8 (100); and (6) elucidating the interactions between genetic burden and environmental factors in ASD probands.

**Conclusions**

In China, ASD research is still in its early stages. More research is needed, including the establishment of systematic methods for clinical diagnosis and intervention, and the sequencing of large cohorts of Chinese samples for identifying novel risk genes. Although there are many hurdles to overcome, with the collaborative efforts of research communities, financial support from administrative bodies and funding agencies, and public awareness and participation, we are likely to see significant scientific advances in understanding ASD.

**References**

Acknowledgments

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Diagnosis and treatment of inherited metabolic diseases in China

Zhi-Chun Feng*, Yan Wang, and Yao Yang

Inherited metabolic diseases (IMD), also called “inborn errors of metabolism,” are a series of hereditary disorders associated with genetic abnormalities that affect organs and systems. These diseases mainly present after birth, during the neonatal period or in infancy. There has been a growing body of evidence that suggests the incidence of these diseases is very low, approximately 1 in 1,500 to 1 in 5,000 live births, while their clinical presentations vary widely. Due to the lack of specified instruments or specialized measurements, diagnoses of these diseases have not become accessible for Chinese pediatricians until just the past decade. Despite popularization and promotion of analytical methods, including mass spectroscopy and enzymatic and genetic analyses, it remains difficult to obtain an accurate overview of the history and current status of IMD in China. Progress in analytical techniques, appropriate communication, and professional training have contributed to a moderate increase in the accurate diagnosis of IMD in the country. Consistently, clinicians have obtained better knowledge and awareness of these diseases.

Neonatal screening

Screening for phenylketonuria (PKU) and congenital hypothyroidism (CH) was first conducted in Shanghai in 1981. Since 1989, screening for PKU and CH has been routinely performed in several provinces/regions, including Beijing, Shanghai, Guangzhou, and Tianjin. Neonatal screening is now done even in medium-sized and smaller cities. From 1992 to 1993, the Chinese Ministry of Health and the World Health Organization (WHO) jointly conducted neonatal screening in the country, also founding the National Neonatal Screening Cooperative Group. Maternal and infant health care legislation was introduced in China in October 1994, which legalized neonatal disease screening and genetic health care for disease prevention. Over 200 neonatal screening laboratories had been founded by 2013. The number of children screened annually increased from around 50,000 in 1985 to over 11 million in 2013. Areas covered by neonatal screening exceed 84.9% in the central and eastern areas of the country, including Beijing, Shanghai, Guangdong, Zhejiang, and Hebei. However, the coverage of relatively poor western areas (including Sichuan, Shanxi, Gansu, Tibet, and Sinkiang) is still disproportionately low—below 20% (1).

Currently, neonatal screening in China covers mainly PKU and CH. In addition, testing for glucose-6-phosphate dehydrogenase deficiency (G6PD) is frequently performed in parts of southern China. Congenital adrenal hyperplasia (CAH) is also screened for in Nanjing and a small number of other areas. A 2013 study showed that the incidence rates of PKU, CH, G6PD, and CAH were 1:12,189, 1:2,281, 1:44, and 1:6,084, respectively, and the screening rates of these four diseases were 86.3%–87.5%, 87.90%–89.1%, 24.0%–25.0%, and 18.9%–19.9%, respectively (2).

Fluorescence analyses, quantitative enzymatic analyses, and bacterial inhibition assays are the most commonly used neonatal screening methods for PKU in China. The use of bacterial inhibition assays decreased from 35.4% about 10 years ago to 4.6% today. Fluorescence analyses are accurate, sensitive, and less time-consuming; they have now become the mainstream technique for phenylalanine (Phe) measurement. Time-resolved fluoroimmunoassays (TRFIAs), enzyme-linked immunosorbent assays (ELISAs), and fluorescence enzyme immunoassays are the most commonly used methods for CH screenings. Fluorescence spot tests (FSTs), recommended by the International Committee for Standardization in Hematology, are used for G6PD screening in southern China. The diagnosis of G6PD deficiency depends mainly on the quantitative assay ratio of G6PD:6-phosphogluconate dehydrogenase (6PGD) and genetic analyses.

In addition to the increase in the number of children undergoing neonatal screenings, since 1988 the Clinical Laboratory Center of the Chinese Ministry of Health has initiated a nationwide evaluation of screening practices across different laboratories engaged in Phe and thyroid-stimulating hormone
(TSH) screening. The evaluation is conducted three times a year and performed according to the guidelines of the International Organization for Standardization (ISO). Laboratories are increasingly subjected to quality evaluation, to inculcate the awareness of stringent quality control measures all across China (3, 4). The acceptable rates for quantitative testing of Phe and TSH were 94.48% and 98.31% in 2014, respectively (3).

Shanghai Xinhua Hospital and the Overseas Chinese Hospital of Jinan University first introduced liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based next-generation screening technology in 2003, and have since expanded the coverage of neonatal screening to 35 inherited metabolic diseases, including disorders of amino acids, organic acids, and fatty acid metabolism. In the past few decades, LC-MS/MS technology has been introduced in more than 10 hospitals in Beijing, Shanghai, Guangzhou, and Zhejiang. The positive rate of screening tests for inherited metabolic disorders by LC-MS/MS ranges from 1 in 8,304 to 1 in 1,578 (5, 6). Those IMD that are most prevalent include methylmalonic acidemia (MMA), PKU, glutaric acidemia (GA), primary carnitine deficiency (PCD), maple syrup urine disease (MSUD), multiple carboxylase deficiency (MCD), transcarboxylase deficiency (OTCD), and propionic acidemia (PA) (7). Among these diseases, MMA and MCD have relatively high incidence (21.3% and 3.6% of all confirmed cases, respectively), and prevention and treatment prior to the onset of these diseases could effectively improve the prognosis and quality of life of the patients (8). Therefore, MMA and MCD should be considered as “priority diseases” in the expanded neonatal screening of IMD.

Over the past 20 years, neonatal screening in China has advanced greatly, and has evolved from a reactive response to proactive systematic planning and organization. However, several limitations still exist. First, only a limited number of diseases are covered in neonatal screening, and the availability of testing technologies varies considerably across the country. Second, next-generation screening lacks qualified technical personnel, experimental setups and analysis of results from MS biomarker screening differ considerably between laboratories, and professional standards for confirmation of data veracity are still lacking. Third, epidemiological data on the prevalence of multiple IMD in China and across different ethnicities are not available. We suggest that the government should increase its investment in IMD or seek the assistance of charitable organizations to expand the coverage of disease testing, establish standard protocols or guidelines for neonatal screening, create a comprehensive database of positive cases, and conduct unified patient management.

**High-risk screening and diagnosis**

In recent years, several biochemical techniques, including LC-MS/MS and gas chromatography-mass spectrometry (GC-MS), have been used for high-risk screening and have increased the diagnosis rate of small molecular IMD, such as amino acid disorders (PKU, tyrosinemia, MSUD) and organic acid disorders (MMA, PA, isovaleric acidemia). Specialized testing laboratories have been established in all the provincial capitals of China. However, the majority of these laboratories, with the exception of a few in Shanghai and Zhejiang, perform only high-risk screening of IMD. Operational reports revealed that the small molecular IMD in high-risk patients encompassed mainly metabolic disorders of amino acids and organic acids, in addition to a few fatty acid metabolic disorders. Common diseases included PKU, citrin deficiency (NICCD), OTCD, MSUD, MMA, PA, and multiple acyl-coenzyme A-dehydrogenase deficiency (MADD). The incidence of PKU in northwest China is higher than in southern China, while NICCD is prevalent in southern China (8-10).

Lysosomal storage diseases (LSD) are another group of IMD that are diagnosed mostly using assays for lysosomal enzymes in peripheral leukocytes or dermal fibroblasts. Technologies for multiple enzyme analyses have already been developed in Beijing, Shanghai, Guangzhou, and Wuhan, for the diagnosis and classification of LSD and glycosogen storage disease. The most common LSD in China include mucopolysaccharidosis (MPS, mainly MPS I and II), Fabry disease, Gaucher disease, and Pompe disease (11-14).

With the development of molecular screening technologies, genetic analyses have become more widely used in clinical practice. They play a key role in the diagnoses of IMD and the identification of carriers. Further studies investigating genetic diagnoses of PKU, glycosogen storage disease, MPS, neonatal intrahepatic cholestasis, hepatolenticular degeneration, MMA, and Niemann-Pick disease (NPD) have been conducted in recent years and have improved our understanding of diseases associated with hotspot mutations in Chinese patients. Several rare IMD, such as Krabbe disease, were also diagnosed.
genetically. Next-generation sequencing technologies can be used for the simultaneous detection of multiple genes, improving the diagnostic efficiency for IMD (15-17).

Biochemical, enzymatic, and genetic analyses are essential for the screening and diagnoses of IMD; they not only complement MS assays, but also provide key evidence essential for proper diagnoses. Currently, few laboratories in China engaged in screening for IMD use such technologies—these diagnostic technologies should be universally applied to improve the diagnosis of IMD.

**Prenatal diagnoses of IMD**

Metabolites of amino acids and organic acids can be detected in amniotic fluid supernatant during the prenatal diagnoses of organic aciduria, which are disorders of amino acid metabolism. Molecular biological techniques, including polymerase chain reaction performed on DNA from cord blood, amniotic fluid cells, or villus cell cultures, have been used for prenatal diagnosis of IMD. Prenatal diagnosis of MPS, MMA, and NPD in Beijing and Shanghai effectively reduced the number of neonates with IMD (18-20). However, the role of gene mutations in IMD is still unclear, which limits the utility of prenatal testing for mutations. Further investigation into the etiologies of IMD is needed to improve prenatal diagnosis.

**Treatment of IMD**

Comprehensive treatments for IMD in China encompass both acute phase and long-term interventions. In recent years, the publications “Expert Consensus on Diagnosing and Treating Gaucher Disease in China,” “Consensus on Diagnosing and Treating Congenital Hypothyroidism,” “Expert Consensus on Diagnosing and Treating Type II Glycogen Storage Disease,” and “Consensus on Diagnosing and Treating Hyperphenylalaninemia” have been issued by the Subspecialty Group of Endocrinology, Hereditary and Metabolic Diseases, the Society of Pediatrics, and the Chinese Medical Association. However, the treatment options available for severe IMD are still severely limited. Substantial gaps exist in enzyme replacement and genetic therapies for IMD in China compared with developed countries. Only very few hospitals, mostly in Beijing, Shanghai, and Guangzhou, have successfully treated patients with Gaucher or Pompe disease using enzyme replacement therapies donated by international pharmaceutical companies. Most orphan drugs for treating IMD authorized in other countries have not been launched in China. In addition, Chinese pharmaceutical companies currently do not manufacture such drugs.

The government, pharmaceutical companies, health care workers, and society as a whole should focus on the diagnosis, treatment, and management of rare diseases. Treatments could include foods and drugs that are not batch-produced or widely available commercially. Custom production of specialty foods and drugs by manufacturing companies for limited prescription and delivery to patients based on screening results is recommended.

**References**

A brief overview of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) applications in clinical microbiology in China

Meng Xiao¹, Timothy Kudinha², Fanrong Kong³, Ying-Chun Xu⁴, and the Chinese MALDI-TOF MS Clinical Microbiology Consensus Working Group†

Clinical microbiology plays a crucial role in the diagnosis of infectious diseases, antimicrobial therapy guidance, nosocomial infection control, and antimicrobial stewardship—especially in this era of increasing antimicrobial resistance and emerging infectious diseases. However, for a variety of reasons, the development of the clinical microbiology field in China has lagged behind other laboratory diagnostic areas, which poses great challenges for health care staff, patients, and the nation as a whole.

In response to the global call for joint efforts in combating infectious diseases and antimicrobial drug resistance in the last decade (1–3), the Chinese government has gradually paid more attention to the further development of the diagnostic capacity of clinical microbiology laboratories, including infrastructure, human resource development, and laboratory quality assurance. At the same time, the introduction of novel techniques, especially matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), has revolutionized laboratory diagnosis of infectious diseases, making the process more accurate, time- and labor-efficient, and cost effective (4), which has contributed significantly to the development of precision medicine.

In China, since the introduction of the first MALDI-TOF MS systems to clinical microbiology laboratories in early 2010, over 50 hospitals have been equipped with these instruments, and many more hospitals are considering acquiring them. However, as a novel technique in the diagnosis of infectious diseases, the standardization and quality assurance for routine application of MALDI-TOF MS assays present challenges for many laboratories.

To address these problems, the Chinese MALDI-TOF MS Clinical Microbiology Consensus Working Group, led by Peking Union Medical College Hospital, was set up in early 2015, consisting of 27 experts from 7 tertiary hospitals, 3 experts from 2 basic research institutions, 2 from 2 universities, and 3 from 2 mass spectrometry manufacturers in China. In January 2016, the first preliminary draft of the publication “Chinese MALDI-TOF MS Clinical Microbiology Consensus Working Group—Chinese Expert Consensus on Application of Matrix-Assisted Laser Desorption Ionization/Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in Clinical Microbiology” was created. After many revisions, the final consensus document was published in the Chinese Journal of Nosocomiology (5).

The consensus version comprised three chapters, the contents of which are summarized below.

Chapter I. The fundamental principles, operation, and maintenance of MALDI-TOF MS

This chapter is divided into three sections, providing a comprehensive introduction to MALDI-TOF MS.

Section 1. Brief introduction and fundamental principles of MALDI-TOF MS

Considering that many laboratory researchers in China are still not familiar with this new technique, this section offers a detailed explanation of the instrument’s design principles and functionality, including a brief historical review of MALDI-TOF MS development, the hardware components, and the reagents used.

Section 2. Key performance indices of MALDI-TOF MS

In this section, a comprehensive introduction of the main performance indices of MALDI-TOF MS is given, taking into consideration the following aspects: resolution ratio, range of the detector, sensitivity, precision, repeatability, and accuracy. Knowledge of these key physical indices can help...
users gain a better understanding of the routine application, management, and maintenance of the machine.

**Section 3. Operation and maintenance of MALDI-TOF MS equipment**

It is important to carry out mass calibration of the instrument prior to use, and also to include relevant quality control procedures essential for monitoring and validating the instrument’s working status. In addition, as the MALDI-TOF MS is a precision instrument, it requires users to pay extra attention to its maintenance. These critical issues, which might be ignored by laboratory staff, are covered in this section.

**Chapter II. MALDI-TOF MS applications in the identification of clinical isolates**

This chapter comprises three sections in which the most recognized application of MALDI-TOF MS in clinical microbiology laboratories to date—the use of the assay in the identification of cultured clinical isolates—is introduced and discussed.

**Section 1. Applications in the identification of cultured isolates**

Three subsections are included: (1) identification of common bacteria species, (2) identification of common yeast species, and (3) other microorganisms that need specific sample preparation. The first two subsections cover the significance of using a mass spectral database for identification of isolates and the pros and cons of using direct smear vs. crude pre-extraction methods for samples. The last subsection discusses protein extraction procedures for microorganisms requiring specific sample preparation, such as mycobacteria and filamentous fungi.

**Section 2. Factors influencing the identification capacity of MALDI-TOF MS**

Although it has been widely accepted that MALDI-TOF MS techniques are highly accurate and reproducible in the identification of microorganisms, users must be aware of several factors in the testing process that may affect the accuracy of the results. Potential confounding factors are systematically reviewed in this section, including those affecting the preanalytic and analytic phases. The key factors discussed include culture conditions (type of media, temperature, time, and O₂/CO₂ levels, among others), sample processing methods, number of samples, sample storage, mass spectra acquisition, interpretation of results, and handling samples from mixed infections.

**Section 3. Setup and quality assurance of MALDI-TOF MS in-house database**

The capacity of MALDI-TOF MS to identify microorganisms is greatly dependent on the quality of the spectral database used. Many organisms are not identified because they are absent from the current databases. Setting up a local, in-house database can overcome some of these deficiencies. However, to ensure identification accuracy in routine laboratory work, the process needs a robust quality assurance program. The procedures for setting up a MALDI-TOF MS in-house database and recommendations on establishing quality assurance are given in this section.

**Chapter III. Other developing applications of MALDI-TOF MS**

Apart from the identification of microorganisms directly from cultured colonies, this expert consensus describes other developing applications of MALDI-TOF MS assays that may be applied to routine laboratory work in the future. This chapter comprises the following four sections:

**Section 1. Direct identification of microorganisms in clinical samples**

It is well known that invasive infections, for example, bloodstream infections, are associated with high morbidity and mortality, and hence require rapid identification of the causative pathogens. MALDI-TOF MS, being a sensitive analytical method, has great potential in this area. We have therefore included an up-to-date description of its applications in the direct detection and identification of organisms in positive blood cultures, urine, and other sterile body fluids.

**Section 2. Determination of antimicrobial susceptibilities**

In addition to the accurate identification of bacteria in clinical material, another critical responsibility of a clinical microbiology laboratory is to determine antibiotic susceptibilities of pathogenic organisms and to report these findings to both physicians and pharmacists for the effective application of targeted antimicrobial therapy. This section describes the application of MALDI-TOF
MS in antibiotic susceptibility testing, including the detection of carbapenem-resistant organisms, extended spectrum beta-lactamase producers, and methicillin-resistant isolates of Staphylococcus aureus (MRSA).

**Sections 3 and 4. Typing of pathogenic isolates and determination of virulence factors**

This section gives a brief review of current research findings regarding the use of MALDI-TOF MS in the typing of pathogens and the study of their virulence. These are two promising fields in which it can be of significant use in the future. Although there is currently insufficient data on this potential application, further investigations are ongoing.

**Conclusions**

Although MALDI-TOF MS has received widespread acceptance to date, the methodology itself is still evolving. Efforts are being made to expand the clinical species database for this system; overcome deficiencies in recognizing closely related species, e.g. *Escherichia coli*, *Shigella*, and *viridans* streptococci; optimize and standardize the direct identification of microorganisms in clinical samples; and further investigate strain typing and antimicrobial susceptibility testing. The MALDI-TOF MS working group has plans to continually update the expert consensus to keep up with further development of techniques and technologies. As the first expert consensus on the application of MALDI-TOF MS in clinical microbiology in China, we are open to and constantly seeking international collaborations in this field.

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**References**

Application of MALDI-TOF mass spectrometry for identifying clinical microorganisms

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is used in clinical diagnostic microbiology laboratories under routine conditions as a rapid, accurate, and cost-effective method of microbial identification. The use of MS for bacterial identification was first described by Anhalt and Fenselau in 1975 (1). MALDI-TOF MS was developed in the late 1980s and is now commonly used for analyzing peptides and proteins for bacterial profiling. This technique gained acceptance and recognition after the 2002 Nobel Prize in Chemistry was awarded to Koichi Tanaka and John B. Fenn for “their development of soft desorption ionization methods for mass spectrometric analysis of biological macromolecules” (2).

In order to identify unknown microorganisms, a database containing reference mass spectra of many characterized microorganisms is used. The database is produced by combining the results of thousands of individual spectra of a given sample. By comparing the protein expression profiles obtained from unknown organisms to the database, the genus, species, or even subspecies of those organisms can be quickly determined (3, 4).

In recent years, MALDI-TOF MS has been applied to the routine identification of microorganisms in a clinical microbiology laboratory setting (5, 6). This technology can be used to detect bacteria (especially mycobacteria) and yeast in blood cultures and urine samples (7). MALDI-TOF MS has potential advantages over other identification methods in that results can be obtained rapidly and sample preparation is simple; it also offers standardized and automated procedures for data acquisition (8–10). Furthermore, it is the most cost-effective method for bacterial identification. Several studies have shown that sample identification by MALDI-TOF MS can be performed for only 17%–32% of the cost of conventional identification methods (11–13).

Three commercial MALDI-TOF systems are available for bacterial identification: the MALDI Biotyper System (Bruker Daltonics, Germany), the Vitek MS system (bioMérieux, France), and the Clin-ToF MS system (Bioyong Technologies, China). The accuracy rate of MALDI-TOF-MS in identifying bacteria depends on the sample pretreatment, the particular MALDI instrument, the reference database, and the scoring algorithms used.

Sample pretreatment is an essential step to properly characterize those microorganism proteins that can be used for successful identification. Currently, there are two common pretreatment methods: the intact cell method (ICM) and the protein extraction method (PEM). For the ICM method, a single colony of the microorganism is directly smeared on the sample target plate using an inoculation loop or other sterile tool, then covered with a matrix that can be analyzed directly following drying. For PEM, proteins are extracted by chemical solvents and then analyzed.

The pretreatment process is carried out using either a customized protocol or a commercial kit. Kits are generally simpler and more convenient than processes that require making reagents, and they also provide more consistent results. Most pretreatment methods can be used in various brands of MALDI-TOF MS instruments and are suitable for samples prepared by both ICM and PEM. Furthermore, they can often be used to process fungal specimens.

Many factors can directly affect identification results—inconsistent reference databases, insufficient spectra from reference strains, errors in the reference maps, and/or missing/incomplete maps of reference strains. Using the Clin-ToF MS instrument, Bioyong Technologies has established a database called MicroDetect that contains information from approximately 370 genera, 2,200 species, and 7,900 strains of microorganisms, including common clinical pathogens, foodborne pathogens, and veterinary and environmental microorganisms. This database has come about in the past four years through collaborations between the U.S. Centers for Disease Control and Prevention, the Academy of Military

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With the development of shotgun proteomics technologies, many database search-and-score algorithms for the identification of microbial peptides from mass spectra have improved significantly, particularly in areas such as cross-correlation (15), hypergeometric distributions (16), Poisson distributions (17), Mowse scores (18), and Bayesian statistics (19). The limitation of these methods is that they rely mainly on the mass-to-charge (m/z) ratio measured by MS, without considering the intensity of the matched peaks. Furthermore, many of these algorithms are not suitable for clinical applications due to their low accuracy.

In order to identify microbes using the MicroDetect database, Clin-ToF data from a test sample is preprocessed using three steps: smoothing, baseline correction, and peak detection (20). We developed a method of processing preprocessed peak data to normalized-intensity peak data that is compatible with the MicroDetect database. After preprocessing, the peaks are ordered by decreasing intensity. If the number of peaks is greater than 100, the 100 peaks with the highest intensity are retained. The total intensity of all retained peaks is defined as T1. Next, for each peak, a “judge score” (cumulative intensity) is calculated and divided by T1, generating a value between 0 and 1. We set “judge score” cutoffs of 0.5, 0.7, and 1.0, and divide all remaining peaks into three classes based on these cutoffs. We reset the intensity of peaks in each class to 3, 2, and 1, which represent high-intensity, medium-intensity, and low-intensity peaks, respectively, allowing for the generation of normalized intensity peaks. Finally, each data point is compared to the MicroDetect database and a probability score calculated using the following formula:

\[ P(y_1 = y_1, y_2 = y_2, \ldots, y_k = y_k) = \frac{\binom{m}{y_1} \binom{m}{y_2} \cdots \binom{m}{y_k}}{\binom{m}{n}}, (y_1, y_2, \ldots, y_k) \in N^k \]

where \( y \) denotes the number of peaks in each judge score range \((i = 1, 2, 3, \ldots)\), \( m \) denotes the matched number of peaks of the same range, \( n \) denotes the total number of peaks, and \( m \) denotes the total number of matched peaks. Here, \( k \) is equal to 3. We then define the matched score as:

\[ \text{score} = -\ln P() \]

The species in the MicroDetect database that have the highest matched score are considered as the best matches. If the score is above 25, the result is considered to be a reasonable match. The database search algorithm for MicroDetect has robust performance with clinical data; the overall precision is above 95%, adequate for clinical applications.

MALDI-TOF MS has become an important rapid microbial identification approach in many fields, including food safety, medicine, biosafety, and veterinary medicine. Many clinical microbiology laboratories already use it for clinical pathogen identification. Whether MALDI-TOF MS could be used extensively depends on its speed and accuracy (17).

Time plays a key role in food processing, clinical testing, and other fields. It takes less than an hour for testing and identification of each microbial sample (without subculture) using MALDI-TOF MS. Additionally, MALDI-TOF MS has a reported accuracy rate of 90%–97% (21–23). Therefore, this technology could meet the need for rapid identification of microorganisms in various fields, providing a significant improvement over conventional methods (7, 24).

The Clin-ToF MS system was able to accurately identify Gram-negative bacteria. In an evaluation study using 1,025 Gram-negative strains of 32 genera and 56 species or species complexes, 98.05% (1,005/1,025) were correctly identified at the species or species-complex level. By comparison, 99.22% (1,017/1,025) were correctly identified by the Bruker MS system (unpublished data).

In addition, different MS systems have unique advantages when identifying certain pathogens; this is likely due to differences in their reference databases. In these MS systems, the Clin-ToF MS system has a high sensitivity and concordance rate compared to 16S ribosomal RNA (rRNA)-based sequencing for bacteria isolated from saliva. One study collected saliva samples from 120 subjects with dental caries, isolated and cultured these bacteria, and had each isolate identified by the Clin-ToF MS system and by 16S rRNA sequencing. The results obtained on the Clin-ToF MS system were concordant at the genus level with those of conventional 16S rRNA-based sequencing for 88.6% of lacticobacili (62/70) and 95.5% of non-lacticobacili (21/22) (25). A second study collected saliva from 90 carious patients and tested these samples using the Clin-ToF MS system and 16S rRNA-based sequencing. It indicated that the sensitivity of the Clin-ToF MS system was 96.0%, and the concordance rate compared with 16S rRNA sequencing was as high as 98.7% (26).
The overall identification rate of microorganisms is a crucial factor in obtaining fast and accurate diagnostic results from clinical microbiology laboratories. Improving efficiencies in this area will benefit both clinicians and patients.

References


Oral precision medicine: Identification of microbes from saliva by mass spectrometry

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Microorganisms are responsible for many infectious diseases—including foodborne illnesses, sepsis, subacute infective endocarditis, meningitis, and pyogenic infections—most of which could become life-threatening if early identification of causal agents and subsequent appropriate antimicrobial treatments are not available. However, conventional identification of an isolated culture is routinely based on phenotypic tests, including Gram staining, as well as determining culture, growth, and biochemical characteristics, which sometimes require expensive molecular techniques. Such time-consuming processes need to be replaced by more rapid, accurate, and cost-effective identification methods.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a procedure based on the comparison of specific mass spectra of cellular components (mainly proteins and peptides), has proven to be an effective tool in clinical microbiology laboratories (1). In previous tests, it has identified isolates correctly in septic patients with a 91.7% success rate (2), and showed 100% concordance with genotyping at the species level in the rapid identification of 10 different species of Viridans streptococci from clinical cultures such as blood, abscess tissue, and wound swabs, among others (3). Our previous studies with the Bioyong (Bioyong, Beijing, China) MALDI-TOF MS system in the identification of caries-related Lactobacillus isolates from the saliva of adult carious patients have also confirmed the validity of the system. These studies showed 88.6% concordance for lactobacilli and 95.5% concordance for non-lactobacilli at the genus level with results using conventional 16S
Precision medicine is a novel area of medical science that promises to bring about a better understanding of healthy body function and the nature of disease by combining modern technology with conventional medicine, and by maximizing individual and social health benefits using the safest, most economical, and most efficient health services. At present, precision medicine is entering its golden age. The size of the global market was around US$40 billion in 2015, and is expected to grow to over US$80 billion by 2023. It is estimated to grow at an annual rate of more than 10% over that period (1).

China has attached great importance to precision medicine. It is the new frontier of modern medical science, according to Huaijin Qin, head of the science and education department of the National Health and Family Planning Commission. In 2015, the first National Precision Medicine Strategic Conference, called by the Ministry of Science and Technology, was held in Beijing, at which commitments were made to invest significant funds in precision medicine over the next 15 years. An initiative supporting R&D into technologies for precision medicine or personalized therapy will be launched when the government’s 13th Five-Year Plan is implemented (2016–2020). Several hospitals and medical centers have already been selected for precision medicine clinical trials by the government, and this number is expected to increase.

As the national center for biotechnology innovation, Beijing possesses the most abundant scientific and technology resources for clinical R&D. The scale of its biomedical industry is now over RMB100 billion (US$14.8 billion), and precision medicine is under rapid development in seven key areas including genomics, proteomics, stem cells and regeneration, vaccines and antibodies, biotherapies, novel personalized diagnosis and treatment technologies, and medical instruments and bioinformatics. In recent years, numerous innovative companies focusing on genetic testing for inherited birth defects and cancer have been created. These companies’ fresh business models have accelerated the clinical transformation of scientific achievements and brought quality-of-life benefits to many people.

Departments within the Beijing municipal government have contributed to precision medicine by supporting pilot units and improving corresponding policies on industry standards, mutual recognition systems, medical supervision, price approvals, and market access.

With the rapid development of the biomedical industry and the precision medicine field, the

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**References**

Beijing Pharma and Biotech Center (BPBC) is taking a leading role in the development of the biomedical industry in Beijing. Following the maxim of “Virtue, Dedication, Innovation, and Practicality,” BPBC sees itself as a thought leader in this industry and as a window to the world for the Beijing biomedical industry. In 2005, the Alliance of Bio-Box Outsourcing (ABO), the nation’s first organization dedicated to domestic biotech R&D outsourcing services, was created in Beijing. ABO was established with the aim of serving the needs of the biopharmaceuticals and precision medicine industries. With a decade of dedicated focus on technological and institutional innovation, ABO has grown into an alliance of 38 members with a revenue of over RMB2 billion (US$394 million) in 2013 (2). Its business encompasses a variety of technology service platforms, including high-throughput sequencing, animal model generation, and clinical evaluation, among others.

References

Expert consensus on inborn errors of metabolism screening
Chunhua Zhang

Awareness of inborn errors of metabolism (IEM) has been growing since A. E. Garrod first described alkaptonuria in 1902 (1, 2). Currently, approximately 600 IEM have been described (3). Although the overall number of IEM cases is large, each disease alone has a relatively low incidence rate and often lacks a specific clinical phenotype. This makes the diagnosis of IEM heavily dependent on information from special laboratory tests. Metabolic diseases can be caused by a range of genetic errors that impact various metabolic pathways. A method that can identify changes in the levels of a broad range of metabolites can be valuable in pinpointing where a metabolic pathway is being affected, allowing subsequent confirmation of the enzyme or coenzyme deficiency or surplus. Since a method of this type is not yet available, the accurate diagnosis of IEM has been challenging.

Since the early 1960s, chromatography and mass spectrometry (MS) techniques have been used to analyze metabolites, including blood and urinary amino acids, organic acids, sugars, sugar-alcohols, and nucleic acid bases (2, 4). MS has since proven to be the better technology, enabling rapid and simple diagnosis of more than 140 IEM using body fluid samples (5–11). MS has also allowed for the successful diagnosis of difficult and rare cases and has been used in China for more than 15 years. Below, we review the use of MS for IEM screening and discuss strategies for standardization of MS analysis.

Screening using mass spectrometry

Screening for specific target metabolites, or so-called “target screening,” falls into two categories: newborn screening and high-risk screening. Newborn screening is a preventive practice that screens infants for specific metabolic diseases. Using tandem MS (MS/MS) to detect acylcarnitines (involved in fatty acid metabolism) and amino acids in dried blood samples, between 20 and 40 inherited metabolic diseases can be identified in a single test.

High-risk screening is a clinical strategy that attempts to identify clinical patients who might have undiagnosed metabolic diseases. More than 400 metabolites in urine can be detected via gas chromatography-mass spectrometry (GC-MS), an effective and economical screening tool. GC-MS testing for acylcarnitine and amino acid levels in blood allows for the identification of approximately 140 types of inherited metabolism diseases including organic acidemia and disorders involving carbohydrate metabolism, fatty acid oxidation, purine metabolism, and aberrant neurotransmitter function.

Combining liquid chromatography (LC) with MS/MS, even very similar compounds can be quickly and accurately identified. LC-MS/MS is used mainly...
when testing acylcarnitines and amino acid levels in serum or dried blood spots. This method has several advantages, including simple sample preparation, short testing time (only 2 minutes is needed per sample), easy detection of aberrations in the data change (12-14), and quick identification of the suspected disease. Disadvantages include potential interference from extraneous factors—such as high levels of hydroxyl-isovaleroyl-carnitine caused by 3-methylglutaconic aciduria, the presence of 3-methylcrotonylglycinemia, multiple carboxylase deficiency, 3-methyl-3-hydroxyglutaric acidemia, beta-ketothiolase deficiency, 2-methyl-3-hydroxybutyric acidemia, or high levels of isovaleryl-carnitine—that might cause a high false-positive rate. For more accurate diagnoses, either GC-MS analysis of urine, or other, more accurate enzyme activity assays and genetic mutation analysis are often needed (15-17).

GC-MS is adept at the quantitative and qualitative detection of gassifiable metabolites and, in conjunction with other methods, can aid in the discovery of novel IEM. Its disadvantages include sample preparation that is about 1 to 2 hours longer, different sample preparation methods needed for different sample types, occasional inconsistency in intralaboratory results, and longer testing times than LC-MS (about 20 minutes to 60 minutes per sample). Additionally, highly trained professionals are needed who have knowledge of the underlying theory of metabolic disorders and are able to recognize abnormalities in MS data pointing to metabolic disorders. Of course, this applies equally to other MS modalities.

In China, MS analysis of IEM was first used at the end of the 1990s (8-9). Today, hundreds of registered laboratories use this technology. An existing challenge is that laboratories have their own equipment, sample preparation methods, and methods for data analysis. This can result in inconsistent data analysis between laboratories, potentially confusing patients, their families, and even their doctors. Standardization for MS analysis of IEM is sorely needed. Below we provide some recommendations.

**Collection, storage, and delivery of MS samples**

The sample types most frequently screened for metabolic diseases are blood and urine. Proper handling of samples during collection, storage, and transfer is important in obtaining accurate test results. MS analysis should show the patient’s metabolic status (metabolite identity and levels) at the time of collection. Snap-freezing followed by -20°C storage is currently the optimal sample storage method, as it stops any enzymatic reactions and fixes the status of all metabolites. When transported, sample-to-sample cross-contamination as well as environmental contamination must be avoided. Samples need to be securely stored to prevent infection of handlers, and constant temperatures must be maintained to avoid thawing. When accepting samples, testers must confirm that they have been correctly handled.

**Sample preparation**

Sample preparation for MS depends on the type of sample and the particular analysis method being used. Blood samples, which can be either serum or dried blood spots, analyzed by LC-MS/MS for detection of acylcarnitines and amino acids, are prepared as follows: (1) Collect a serum sample or a 3-mm diameter puncture of a dried blood spot (approximately 3 µL of blood); (2) add methanol containing internal amino acid and acylcarnitine standards to extract metabolites; (3) the sample can then go through a derivatization process before being injected into the LC-MS/MS machine for analysis, or can be analyzed in its nonderivatized form. Derivatization involves a chemical treatment that breaks down the sample in an attempt to improve the ease and quality of the analysis.

Commercial reagents are available for derivatization, such as NSK-A and NSK-B from Cambridge Isotope Laboratories and the NeoBase kit from Perkin-Elmer. The former requires additional preparation of the mobile phase lyase, while the latter provides it ready-made. The decision whether to derivatize a sample depends on the sensitivity of the mass spectrometer; the derivatization method improves the detection sensitivity for microconstituents. As new MS models are released (see Table 1) it may become easier to use nonderivatized samples, making operation quicker and cheaper. For the older MS models, derivatization is generally required.

When analyzing urine by GC-MS, two sample types are generally used: fresh urine or dried urine on filter paper. Either organic solvent extraction and/or urease treatment can be applied to these samples (Table 2). Organic solvent extraction uses three rounds of acidified diethylacetic acid treatment to extract organic acids; after drying and trimethylsilyl derivatization, the prepared sample is injected into the GC-MS. Urease treatment decomposes the urea, followed by drying and trimethylsilyl derivatization for compounds containing hydroxyl, amino, and carboxyl groups, and then injection into the GC-MS. It should be noted that the organic solvent method can extract only the carboxylic
acid in samples. Thus, this method is useful in the diagnosis of organic acidurias. The urease treatment method can analyze organic acids as well as amino acids, carbohydrates, and nucleic acids, making it widely used in the diagnosis of metabolic disorders.

Currently, the main suppliers of LC-MS/MS devices are AB Sciex, Waters, and Shimadzu. Models such as the AB Sciex API 4000, the Waters TQD series, and the Shimadzu LCMS-8040 or more advanced models, are able to meet the requirement for blood acylcarnitine and amino acid analysis. GC-MS producers include Agilent, JEOL, and Shimadzu. See Table 1 for more details. MS makers also provide data analysis software to help in the quantitative and qualitative analysis of metabolites. When choosing an MS technology, whether for LC-MS/MS or GC-MS, it is important to ensure that the analysis software is compatible with IEM screening.

When performing LC-MS/MS, the instrument settings on different machines are likely to be very similar. However, different instruments or laboratories may use slightly different methods based on the metabolites they are targeting. For example, although precise detection of certain basic amino acids such as lysine, arginine (and its derivatives, ornithine and citrulline), and histidine is challenging, it is very helpful in the diagnosis of certain metabolic disorders and therefore important to some laboratories. Similarly, detection of long-chained acylcarnitines aids in the diagnosis of metabolic disorders involving long-chained or very long-chained fatty acids.

With respect to GC-MS, the temperature during sample preparation and the columns used can significantly impact the results. For example, when using a 30 m × 0.25 mm × 0.25 mm Ultra ALLOY metal capillary column, the temperature range should be 60°C-350°C, the sampling method should be split flow, electron ionization should be used for MS, and a scan mode should be used for detection. Utilizing these conditions, more comprehensive metabolomic information will be obtained.

**Peak identification**

Peak identification is a critical step in MS analysis. Most metabolites lack standard compounds against which they can be measured, and the mass spectra libraries found in analysis software are often incomplete. Furthermore, identifying each peak in a mass spectrum can be tedious, often requiring the input of professional technicians.

Proper identification and interpretation of peaks in the mass spectra is essential for ascertaining the metabolic status of a patient and determining whether their metabolic profile is within normal parameters, or if unconventional metabolites are detected and the ratio of marker metabolites is abnormal. To make a clear clinical diagnosis of IEM, a solid theoretical knowledge of human metabolomics and metabolic pathways is required, as well as an understanding of the biochemical role of each metabolite.

Following metabolome analysis, a report should

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**TABLE 1. Types of mass spectrometers.**

<table>
<thead>
<tr>
<th>Instrument type</th>
<th>Producer</th>
<th>Model</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS/MS</td>
<td>AB Sciex</td>
<td>API 4000</td>
<td>Blood acylcarnitine and amino acid analysis</td>
</tr>
<tr>
<td></td>
<td>Waters</td>
<td>TQD series</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shimadzu</td>
<td>LCMS-8040</td>
<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td>Agilent</td>
<td>6890A series</td>
<td>Urine metabolomics analysis</td>
</tr>
<tr>
<td></td>
<td>JEOL</td>
<td>JMS-Q1050GC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shimadzu</td>
<td>GCMS-QP2010 Ultra</td>
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</tr>
</tbody>
</table>

**TABLE 2. Comparison of organic solvent extraction and urease treatment.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Detectable compounds</th>
<th>Target metabolic diseases range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic solvent extraction</td>
<td>Organic acids</td>
<td>Methylmalonic acidemia, propionic acidemia, maple syrup urine disease, isovaleric acidemia, glutaric acidemia I/II, and others</td>
</tr>
<tr>
<td>Urease treatment</td>
<td>Organic acids</td>
<td>Methylmalonic acidemia, propionic acidemia, maple syrup urine disease, isovaleric acidemia, glutaric acidemia I/II, and others</td>
</tr>
<tr>
<td></td>
<td>Amino acids</td>
<td>Urea cycle disorders, phenylketonuria, sarcosinemia, hyperprolinemia, lysine intolerance, citrin deficiency, and others</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>Galactosemia, fructose-1,6-diphosphatase deficiency, citrin deficiency, and others</td>
</tr>
<tr>
<td></td>
<td>Nucleic acids</td>
<td>Lesch-Nyhan syndrome, xanthinuria, thymine-uraciluria, and others</td>
</tr>
<tr>
<td></td>
<td>Neurotransmitters</td>
<td>Neuroblastoma, Canavan disease, 4-hydroxybutyric aciduria, and others</td>
</tr>
</tbody>
</table>
be issued to the treating clinician. Since these doctors do not often encounter IEM, they may have little experience in the therapy or treatment of the resulting diseases. The test report should therefore inform doctors not only what the abnormality is, but also provide possible reasons for the abnormality, what possible diseases may result, the manifestations of such diseases, and suggested therapies and follow-up treatments.

Quality control
All steps in the analysis of a sample by MS should be standardized using clear quality assurance and quality control criteria, from sample collection through spectrum analysis to generation of the final report. However, there is currently no such standardized quality control system; each laboratory uses its own protocols and standards. Clinical test laboratories in China can now maintain a high-quality MS analysis service and reduce instrument error by having their devices inspected annually by the General Administration of Quality Supervision, Inspection and Quarantine. Furthermore, in 2013, the clinical laboratory center of the Chinese Ministry of Public Health began an external quality assessment (EQA) of newborn MS/MS screening methods used to analyze amino acids and acylcarnitine from dried blood-spot samples. This program is still maturing and more data on different methodologies needs to be collected before conclusions can be drawn as to its efficacy. Most international laboratories participate in an EQA system developed by the U.S. Centers for Disease Control and Prevention (CDC). However, since most of the CDC’s quality control samples are prepared in the laboratory rather than being true patient samples, this systems has been deemed less than satisfactory for use in China. The European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM) has been the authority for EQAs in this area for the past decade, comprising the world’s leading experts on metabolic diseases. ERNDIM has a large database of metabolic-disease patient information, and its quality control samples are from diagnosed patients. Hundreds of laboratories screen for metabolic diseases using the ERNDIM EQA system, including the metabolic disease laboratory at the Matsumoto Institute of Life Sciences (MILS) in Japan, which has been participating for 10 years. There are currently 75 types of Asian IEM patient samples in the ERNDIM database. MILS has an extensive database of Asian IEM patient samples that makes it possible to compare EQAs from different laboratories in China, as well as compare and combine the results from the same patient sample processed in different Asian laboratories, just as the ERNDIM system can do.

Globalization of the IEM screening model
IEM screening laboratories require technicians and clinical experts, as well as delicate instruments. Since doctors are highly dependent on accurate results, there can be little tolerance for errors in the process, as this can lead to incorrect diagnoses and treatments. To create a reliable IEM detection workflow using MS, MILS has established an international IEM screening laboratory network consisting of trained MS technicians and IEM medical experts (Figure 1). The goal of this network is to regulate the setup of IEM testing laboratories, sample treatment methods, detection conditions, and data reporting, as well as to ensure reliable technical support for equipment. Following the generation of MS data, results are first delivered to a network data analysis center where data analysis experts carry out peak identification and examine the output spectra, creating a final report for interpretation by IEM experts. Utilizing rapid online communication, IEM experts can analyze the
data and rapidly submit a diagnostic report to the local laboratory. Finally, this report can be passed from local laboratories to the clinicians, and the results can be shared with the patient.

**Joining the screening network**

Any laboratory that plans to carry out IEM screening is welcome to join the MILS network. Participating groups will be required to equip their laboratory and prepare and test samples under strict guidelines. Test results will be delivered through online submissions to the analysis center. The data analysis center will provide a report, and participating laboratories must then transmit these results to the doctors in a timely fashion. This system is intended to be efficient and flexible. MS technicians need minimal training, and onsite metabolic diseases experts are not required, as professional support from international experts within the network is available. Using this network, IEM MS screening in China can rapidly reach international standards.

**References**


**Cardiovascular precision medicine in China**

Lei Song1 and Rutai Hui2*

Traditional Chinese medicine has long proposed the concept of “treating different diseases with the same method, the same disease with different treatments,” which emphasizes the concept of individual treatments. Precision is the standard, while personalization is the ultimate goal.

**Background and policy**

In China, cardiovascular and cerebrovascular disease hospitalization expenses have increased rapidly since 2004, with the growth in costs exceeding the national gross domestic product growth rate (1). One of the reasons for this high expense is that the traditional medical model treats all patients with the same disease using the same method, resulting in low treatment efficacy and high medical expenditure. Precise and individualized medical models undoubtedly reduce costs, as evidenced by the practice in east China’s coastal city of Qingdao. Since 2011, Qingdao has piloted an insurance-supported precision medicine program, covering precise target treatment for tumors, pulmonary vascular diseases, blood diseases, immune diseases, and diabetes. After the new initiative was implemented, the average number of per-patient hospitalization days decreased significantly, from 15.57 days in 2010 to 9.74 days by the end of 2015. The percentage of medical expenses covered by patients themselves has dropped by 39.5%, while the probability of catastrophic health expenditure—defined by the World Health Organization (WHO) as when “the personal health expenditure in cash of a

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family reaches or exceeds 40% of its total spending in a certain period (usually a year)—has dropped by 76.6%.

**New initiatives and organizations to promote precision medicine**

In 2014, the National Health and Family Planning Commission assigned the first high-throughput sequencing technology pilot units for clinical applications in three areas: genetic disease diagnosis, prenatal screening and diagnosis, and preimplantation embryo genetic diagnosis. The Chinese central government launched a precision medicine initiative on July 29, 2015. A plan to construct a pilot center to demonstrate the application of gene detection technology was approved by 27 Chinese provinces in April 2016.

Under the auspices of the Chinese Society of Cardiology (CSC), the Cardiovascular Precision Medicine Group (CPMG) was founded by scientists from different fields involved in precision medicine, including cardiovascular disease, cerebrovascular disease, molecular imaging, reproductive medicine, medical statistics, bioinformatics, and big data analysis. They will establish research cohorts to study monogenic cardiovascular disease and cardiovascular disease pharmogenomics in China. The goals of CPMG are: (1) to establish a precision diagnosis system for clinical decision-making; (2) to seek genetic markers, risk factors, and targets to improve clinical outcomes, minimize or avoid adverse effects, and guide drug treatment; (3) to train personnel and formulate industry standards, norms, and guidelines for Chinese cardiovascular precision medicine; and, (4) to construct a nationwide network to promote the clinical transformation and application of precision medicine scientific research production.

**Precision medicine in monogenetic diseases**

Even though monogenetic cardiovascular diseases are rare, the number of patients is significant in China due to its population of 1.4 billion. For example, in 2004, the prevalence of hypertrophic cardiomyopathy (HCM) was 0.16% in adults aged 18–74 (2). That equates to almost 2 million HCM patients. HCM in almost 60% of patients has a known genetic origin (3, 4). Genetic determination of HCM can benefit both genetically affected and unaffected familial members in terms of obtaining an early diagnosis, better predicting risk, and choosing treatment options. On the other hand, monogenic cardiovascular disease can be masked by a host of other conditions that may lead to misdiagnosis. Gene diagnosis is considered the gold standard of diagnosis for these cases.

Since publication of the “Chinese Experts Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies” in 2011 (5), molecular detection of monogenic cardiovascular diseases, including a variety of cardiomyopathies and channelopathies (e.g., long QT syndrome), monogenic pathogenic hypertension (e.g., Liddle’s syndrome), inherited lipid metabolic abnormalities, familial pulmonary hypertension, and hereditary disease of the aorta (e.g., Marfan syndrome) has been widely carried out by hospitals, institutions, and commercial companies in China. At present, gene diagnosis techniques based on preimplantation genetic screening have been successfully applied to clinical practice.

However, with most Chinese genotype-phenotype studies conducted in individual centers, the results are often difficult to repeat and even conflict with each other. Thus, it is difficult to apply these genetic discoveries directly in clinical practice (6). Moreover, the determination of heterogeneity of genetic and clinical manifestation needs an accurate genotype-phenotype relationship analysis system. A recent case of a Chinese family carrying a new mutation in the beta-myosin heavy chain (MHC-β) gene, MYH7, and exhibiting compound characteristics of hypertrophic cardiomyopathy and restrictive cardiomyopathy, exemplifies why the comprehensive evaluation of phenotype and genotype is critical (7). Multicenter, large-scale, and long-term follow-up genetic and clinical research can provide high-quality evidence for precise medical diagnosis and treatment.

Native Chinese exhibit a different genetic mutation profile compared with Western populations. For example, the codon 403 mutation in the MHC-β gene, which is a known cause of HCM, was reported as a hot spot and malignant mutation among Caucasian HCM patients (8, 9) but was not identified in 529 Chinese patients (10). The cardiac troponin (cTnT) mutation was identified in 10% of patients with HCM of European ancestry, but only <1% of Chinese HCM patients (11, 12). In addition, researchers now know that much of the variation may come from individual environmental adaptation to pathogens, climate, altitude, diet, and possibly cognitive challenges (13). China has 56 ethnic groups with different geographic environments, lifestyles, diets, and cultures. Therefore, genomic data of transethnic origin need to be evaluated in the Chinese population before being applied in the clinic.

European and American countries have large population genetic databases, such as the 1000 Genomes Project, the Exome Sequencing Project 6500 (ESP6500), and the Exome Aggregation Consortium (ExAC), which
are useful in interpreting the clinical significance of variations. China’s gene database has yet to be built.

Application of pharmacogenomics and pharmacogenetics

Data gathered from pharmacogenomics and pharmacogenetics studies are important for guiding targeted treatments in precision medicine, so that drug treatments can be precisely optimized with minimal side effects and at an affordable cost. Molecular detection of warfarin resistance, clopidogrel resistance, and homocysteine metabolic enzyme genotyping (14), as well as antihypertension medication receptor gene tests, angiotensin-converting enzyme genotyping, and statin side effects have been used widely in the field of pharmacogenomics and pharmacogenetics in China.

It has been reported that the required warfarin dose could vary by up to 20 times in order to maintain therapeutic international normalized ratio (INR) values in different individuals (15). Chinese patients are more sensitive to warfarin, with dosing being 40%-50% lower in patients of Chinese ancestry compared with that in Europeans (16). Cytochrome P450 2C9 (CYP2C9) is an important genetic factor affecting response to warfarin. The presence of the CYP2C9*2 or CYP2C9*3 alleles reduces warfarin metabolic enzyme activity by 30% and 80%, respectively. Among the Chinese, the CYP2C9*3 allele is most common, while CYP2C9*2 is rare (17). The heritability of platelet response to clopidogrel has also been found to be highly divergent among individuals (18, 19).

As for monogenic diseases, the transethnic verification of variants and the heterogeneity of genotype-phenotype relationships are still key issues when studying genetic markers associated with drug responses in clinical practice. Pharmacogenomic traits need to be verified across samples of other ethnic minorities—in addition to Han Chinese—requiring multicenter and large-scale cohort studies.

Challenges ahead for precision medicine

Current genetic data applied to clinical practice are mostly limited to variants in the protein-coding region of single genes and mostly derived from Western population studies. Chinese medical and biomedical societies need to collaborate with the international community to clinically evaluate these genetic markers and develop novel markers for the Chinese population. This should also include the effects of nonprotein coding genetic variants, multiple gene variants, and variant-variant interactions.

China still predominantly uses third parties for genetic testing and interpreting results. However, the techniques used by these companies can differ, making direct comparisons difficult. Additionally, interpretation of the results can be subjective and there can be deviations between the testing results and the clinical phenotype. The expense of genetic testing is still too high for patients to pay, and it may still be years before China’s health insurance and commercial insurance cover these tests.

In the era of precision medicine, cardiovascular specialists have much to learn regarding the use of biomedical approaches (20) to diagnose disease and to guide the choice of clinical treatment. For clinicians, genomic analysis is dauntingly complex and the amount of data is huge. The accuracy of the analysis itself is also uncertain. These issues may discourage some doctors from even trying to apply precision medicine-based diagnoses and treatments. Up to now, there is no national institution to formally conduct training, certification, and assessment for clinicians in this field. Thus, precision medicine application for cardiovascular disease in China has a long way to go.

Thanks to collaborations among clinicians, scientists, patients, and their families, as well as government and industry, the hope that the hype from precision medicine is that it will reduce the incidence, mortality, and medical costs of cardiovascular disease, bring new opportunities for early warning and intervention in cardiovascular disease, improve patients’ quality of life, and extend their life expectancy.

References

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精准检验：临床质谱时代，Clin-TOF系列

Clin-TOF I
Clin-TOF II

应用范围

微生物鉴定
基因检测
多肽组指纹图谱

应用示范单位

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