Predictive Value of MRD in Postoperative Prognosis and Adjuvant Therapy for NSCLC Patients

MGI's DNBSEQ Sequencing Platform Empowers MRD Detection and Research in Lung Cancer Solid Tumors

With the assistance of DNBSEQ sequencing platform of MGI, a research team led by professor Wu Yilong from the Guangdong Provincial People's Hospital and Guangdong Lung Cancer Institute investigated the value of MRD detection in the postoperative prognosis and adjuvant therapy for NSCLC patients.

This study was published as a cover article in a top international journal *Cancer Discovery* (IF: 39.397) in 2022, titled with "Longitudinal Undetectable Molecular Residual Disease Defines Potentially Cured Population in Localized Non–Small Cell Lung Cancer"[1].

Recommended application: Cancer Genomics (Lung Cancer)

Recommended models: DNBSEQ-T7RS, DNBSEQ-G400RS

- ### Facilitating MRD detection in lung cancer

  The DNBSEQ sequencing platform can provide support for highly sensitive MRD detection for ctDNA, and help prospective study in early- and mid-stage resectable NSCLC.

- ### Perfect compatibility with DNBSEQ sequencing platform

  MGIEasy Universal Library Conversion kit enables Third-party targeted enrichment solution perfectly work on DNBSEQ sequencing platform.

- ### Data output is efficient and high-quality

  DNBSEQ sequencing technology has significant features such as high accuracy, low repeat sequence rate, and low index hopping rate, etc.

- ### Offer a complete product combination for experimental procedures

  Based on independently developed automatic solutions and analysis software, MGI provides a complete set of product combination from sample to result output.

Background

Minimal Residual Disease (MRD) is also known as molecular residual disease, and related detection technology can be applied in the diagnosis and therapy for patients with cancers. Many cancer patients still have small lesions or residual cancer cells after cancer treatment, but the amount of these cells may be quite low and there won't be any sign or symptoms. In this situation, residual lesions are undetectable utilizing imaging techniques alone. Tumor progression or recurrence and metastasis are most likely to happen if no medical intervention is perferomed[2].

Recently developed techniques for minimal residual disease (MRD) detection in non-small cell lung cancer (NSCLC) mainly depend on tracking ultra-low frequency cellular tumor mutations in cell-free DNA (cfDNA)[3-6]. MRD detection has significant value for the prognosis of NSCLC patients after definitive surgery. Increasing evidence suggests that if molecular-level tumor residual lesions remain in the body after radical treatment in solid tumor patients, it may lead to tumor recurrence and thus produce adverse prognosis[7]. Figure 1 provides a panoramic view of the entire process of tumor load and disease development[8].

This study confirmed the prognostic value of ctDNA-based MRD detection for patients with NSCLC after radical resection. It emphasized the value of undetected MRD, which can be used to define potentially cured populations in localized NSCLC. In addition, subgroup analysis suggested that adjuvant therapy may not be necessary for patients with undetected MRD.

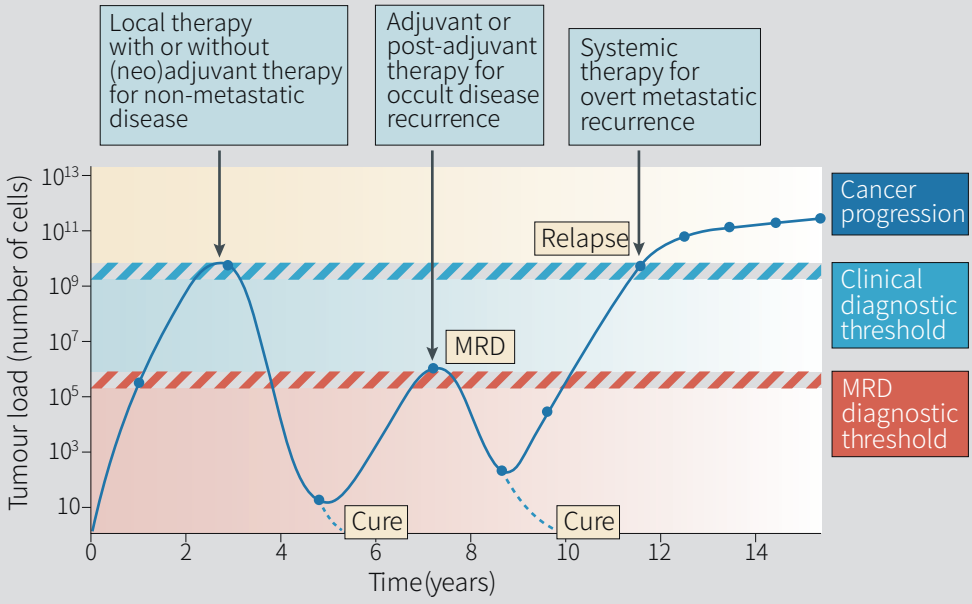

Figure 1. The development of therapeutic strategy based on the dynamic changes of tumor load in cancer patients[8]

Materials and Methods

Sample Collection and DNA Extraction

Samples from 261 patients with stage I to stage III NSCLC (tumor diameter≥2 cm) were collected for this prospective observational study between March 2019 and January 2021 at the Guangdong Provincial People's Hospital and Guangdong Lung Cancer Institute. Please check the relevant literature for more information on experimental design procedures.

For patients who have not received adjuvant therapy, those who have received adjuvant chemotherapy, and those who have received long-term EGFR inhibitors or ICI adjuvant therapy, two 10ml peripheral blood samples were collected in Streck tubes at predetermined time points before and after surgery and corresponding sample processing was performed. Subsequently, circulating cfDNA was extracted from plasma and germline genomic DNA was extracted from peripheral blood lymphocytes. The corresponding tumor DNA was extracted from fresh tumor tissue or FFPE tumor tissue specimens. To ensure that the lung tumors had not already been shed before surgery, 5 to 10 ml of blood were collected from the residual pulmonary vein stump of the resected lung lobe in another 11 patients with stage I NSCLC.

Library Preparation and Sequencing

a.cfDNA Panel Design

This study designed a 338-gene panel based on an idea comparable to CAPP-seq[3]. This panel comprehensively considered lung cancer, colorectal cancer, and hepatocellular carcinoma, and could cover a 550-Kbp genome. In the panel design process: First, the most prevalent driver mutations among these three cancer types were selected; Then, sensitive and resistant mutations that are actionable, and gene alterations pertinent to the effects of immunotherapy were added; Finally, the regions with high-frequency mutation were selected based on the data from 48,353 Geneplus-sequenced patients with cancer and other open databases.

b.Library Preparation, Target Region Capture and Sequencing

For germline genomic DNA and matched tumor DNA, 400-800 ng of DNA were sheared into fragments with the peak at 200-250 bp using an ultrasonicator. The NEBNext Ultra DNA Library Prep Kit (NEB) was then used to prepare sequencing libraries. In addition, cfDNA was utilized for library preparation, and unique identifiers (UIDs) were labeled on each double-stranded DNA. These UIDs served the purpose of distinguishing between authentic somatic mutations and artifacts, thereby improving the accuracy in tracking individual plasma molecules.

Hybridization enrichment was performed using a custom-designed panel that targeted 1021 cancer-related genes, covering approximately 1.5 Mbp of the genome, for both tumor genomics and their corresponding germline DNA libraries, as previously reported. Similarly, for plasma DNA and its matched genomic germline DNA libraries, the cfDNA panel specifically designed for this study was utilized for hybridization enrichment.

Sequencing of the prepared DNA libraries was carried out on either the DNBSEQ-T7RS genetic sequencer from MGI or the Gene+seq-2000 sequencer from Geneplus with the 100 bp paired-end reads (PE100) sequencing recipe.

For peripheral blood lymphocytes, plasma, and fresh/FFPE specimens, 5, 40, and 5Gb of data were generated, respectively.

Data Analysis

Following the removal of adaptor and low-quality reads, the sequenced reads underwent mapping to the reference human genome (GRCh37) using default parameters in BWA version 0.6.2. For tumor and germline genomic DNA, the MarkDuplicates tool in Picard was utilized to mark and remove duplicated reads. For cfDNA, duplication identification was carried out using UID and the position of template fragments to eliminate errors caused by PCR or sequencing using realSeq. GATK was employed for local realignment around SNVs and indels, as well as quality control assessment.

SNVs and small indels in tumor somatic cells were analyzed with realDcaller and TNscope. Copy number variations were detected with CNVKit. Structural variations were analyzed using the NCsv algorithm. Following these analyses, ctDNA-MRD detection and statistical analysis were conducted.

Sample collection	Library preparation	Bioinformatics analysis	Result analysis
Samples collected from 261 patients with stage I to stage III NSCLC (tumor diameter≥2 cm)	Third-party library preparation kit +Targeted hybridization panel capture  MGIEasy Universal Library Conversion Kit (App-A)  DNBSEQ-T7RS Genetic Sequencer	BWA Picard GATK realDcaller TNscope CNVKit NCsv	Exploring the prognostic value of ctDNA-based MRD detection in non-small cell lung cancer patients after radical resection

Results

Negative predictive value has significant importance in MRD detection, and continuous negative can be defined as potential cure.

In this study, single-node (Landmark) MRD detection was conducted on postoperative patients, and the results revealed that patients with negative test results had a significantly better prognosis compared to those with positive results (Figure 2). To enhance the accuracy of prediction, dynamic MRD detection was performed on patients, yielding a negative predictive value (NPV) of 96.8% in the test results. This data indicates that 96.8% of the persistently negative population did not experience tumor recurrence. Consequently, this finding can be used as an indicator to identify the persistently negative population as a potentially cured population (Figure 2).

MRD detection can provide beneficial value for predicting adjuvant therapy

The findings of the study reveal that among patients with positive postoperative MRD detection, receiving adjuvant therapy significantly improves Disease-Free Survival (DFS). Conversely, for patients with negative MRD detection results, there is no observed benefit from adjuvant therapy (Figure 3). This suggests that the tumor load in the MRD-negative population is extremely low, indicating a potential state of being close to a cure. As a result, adjuvant therapy may not be necessary for this group (Figure 3). The persistently MRD-negative population can be identified as a potentially cured population, characterized by an exceptionally low tumor load, which may not require adjuvant therapy (Figure 3).

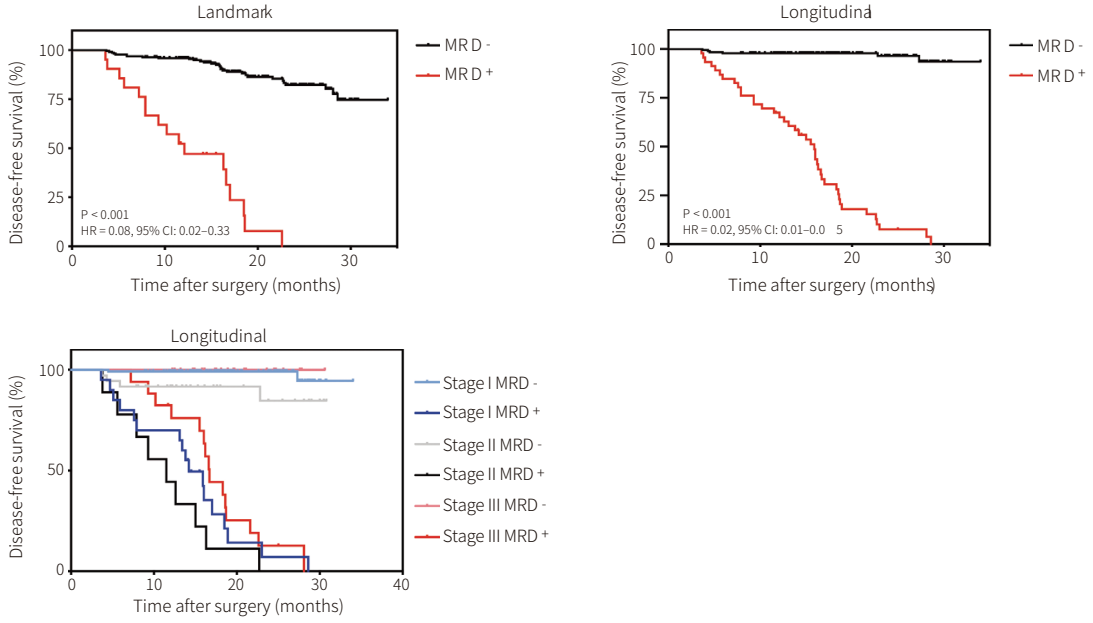

Figure 2. Persistently negative MRD detection after surgery defined potentially cured population

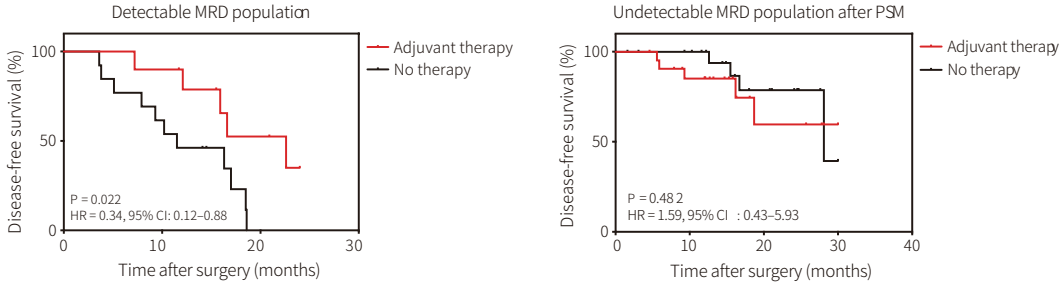

Figure 3. Postoperative MRD-negative patients cannot benefit from adjuvant therapy

Nonshedding tumors affect lung cancer MRD detection

Nonshedding tumors refer to the tumors of which ctDNA-MRD detection is negative before surgery, and no minimal residual lesions are found before surgery. In the study, 11 patients with stage I NSCLC who underwent lobectomy were selected, and ctDNA tests were performed on their venous residual blood and peripheral blood. Among them, 6 patients' samples showed undetectable ctDNA in their venous residual blood and peripheral blood, indicating the inevitable existence of Nonshedding tumors, and various factors affecting ctDNA release (Figure 4).

In addition, postoperative MRD detection was performed on 14 patients who were preoperative ctDNA negative and had recurrence after surgery. In all 14 patients, MRD could be accurately detected, so it was concluded that whether preoperative ctDNA can be detected or not does not affect the postoperative MRD monitoring results (Figure 5). At the same time, the study found that the important reason affecting MRD monitoring is the anatomical factors of simple brain metastasis.

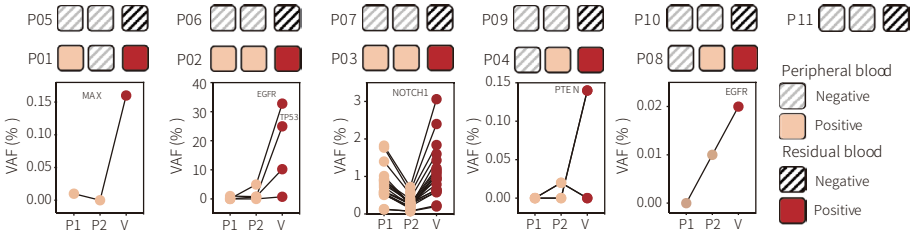

Figure 4. Comparison between 2 peripheral blood and venous residual blood ctDNA test results in 11 additional patients with stage I NSCLC

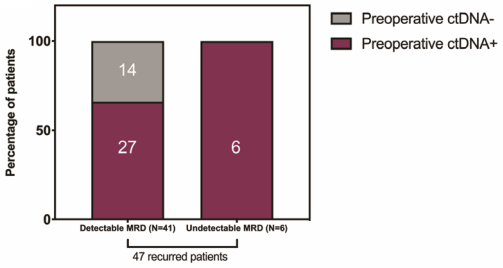

Figure 5. Relationship between postoperative MRD monitoring and preoperative ctDNA detection in recurrent patients

Summary

This prospective research facilitated by MGI's DNBSEQ sequencing platform demonstrates a significant correlation between ctDNA-MRD detection and clinical outcomes in lung cancer patients.

MGI's DNBSEQ sequencing platform continues to support cutting-edge research in the field of cancer research. The study defines potentially cured populations as those with persistently negative MRD detection and explores the predictive value of MRD for adjuvant therapy. It indicates that adjuvant therapy is of no value for patients with consistently negative MRD detection, and clarifies that the presence of Nonshedding tumors before surgery does not affect postoperative MRD monitoring.

DNBSEQ-T7 Genetic Sequencer

References

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Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G400RS	900-000170-00
	Genetic Sequencer DNBSEQ-T7RS	900-000236-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	MegaBOLT Bioinformatics analysis accelerator	900-000555-00
	ZTRON Pro Appliance（T500）	900-000444-00
Library Prep	MGIEasy Universal Library Conversion kit (App-A)	1000004155
Sequencing Reagents	DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE100)	1000016950
	DNBSEQ-T7RS High-throughput Sequencing Set (FCL PE100) V2.0	1000028455

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