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LETTER

Unveiling molecular incomplete response (mIR) *via* ultra-deep NGS: a novel concept contrasting pathologic complete response (pCR) in occult lymph node micrometastases of non-small cell lung cancer

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Accurate assessment of lymph node status is the cornerstone of staging and treatment decision-making in non-small cell lung cancer (NSCLC). Currently, conventional histopathology remains the gold standard. However, clinical reality presents the following challenge: 30%–40% of patients with pathologic node-negative (pN0) disease eventually have a recurrence or distant metastasis[1]. While liquid biopsy [specifically circulating tumor DNA (ctDNA)-based molecular residual disease (MRD) detection] has revolutionized postoperative surveillance[2,3], blood-based MRD reflects the systemic tumor burden and may lack sensitivity for detecting occult metastases within regional lymph nodes, which can act as "sanctuaries" for residual tumor cells. This issue is particularly pertinent in the era of neoadjuvant immunotherapy, during which some patients achieve a radiologic or even pathologic complete response (pCR) yet still relapse. These discrepancies raises two critical questions: Does conventional pathology underestimate staging due to limited sensitivity? Is "pCR" equivalent to "molecular complete clearance"? Systematic, deep molecular assessment of dissected lymph nodes may offer a unique perspective.

Lymph node micrometastasis typically refers to tumor clusters between 0.2 and 2.0 mm in diameter[4]. Although widely studied, traditional molecular techniques (e.g., polymerase chain reaction and immunochemistry) often have high false-positive rates or suboptimal specificity[5,6]. Next-generation sequencing (NGS), which is widely used for blood MRD, can target tissue-specific mutations with high sensitivity and specificity, and distinguish tumor signals from normal background noise[7].

To address this issue, a single-center retrospective exploratory study utilizing ultra-deep NGS was conducted to evaluate lymph nodes from high-risk pN0 NSCLC patients. The analysis led to the introduction of a novel hypothesis-generating concept (molecular incomplete response [mIR]), which we propose as a molecular counterpart to pCR. We observed that prognosis varies significantly among patients achieving a pCR after neoadjuvant therapy. Notably, two patients with stage IIIB squamous cell carcinoma who achieved a pCR after neoadjuvant chemo-immunotherapy (confirmed by conventional pathology) subsequently relapsed and died (23 and 30 months post-surgery). Retrospective ultra-deep NGS of the dissected lymph nodes revealed definitive tumor-specific molecular signals in multiple nodal stations, despite negative histologic findings.

Prompted by these findings, the cohort was expanded to include 10 high-risk NSCLC patients who were treated between 2019 and 2022. Given the small sample size, this study was exploratory and hypothesis-generating in nature. All patients underwent radical resection with systematic lymphadenectomy and confirmed to be pN0 (AJCC 9th edition). High-risk was defined as poorly differentiated adenocarcinoma, non-keratinizing squamous cell carcinoma, or other high-grade histologies such as pulmonary sarcomatoid carcinoma. The cohort included five patients with recurrences, including the two pCR cases and five matched non-recurrence controls. The study was approved by the Institutional Review Board of the Tianjin Cancer Hospital Airport Hospital (Approval No. LWK-2024-0012), and all patients provided informed consent.

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Our methodology utilized a personalized ultra-deep NGS strategy based on whole-exome sequencing (WES). WES was first performed on the primary tumor (mean depth ≥ 500×) to identify somatic mutations. A customized panel targeting up to 50 clonal mutations and core lung cancer genes was then designed for ultra-deep sequencing of dissected lymph nodes (median depth > 10,000×). This strategy maximized sensitivity for detecting trace tumor DNA (**Figure S1**).

Of the 10 conventional pN0 patients, 8 harbored molecular evidence of micrometastases in the lymph nodes (**Figure 1**). This rate is notably higher than the 12%–58% reported using traditional techniques[6,8,9], highlighting the superior sensitivity of NGS and suggesting that lymph node micrometastases may be highly prevalent in high-risk NSCLC.

The clinicopathologic data and the lymph node MRD status of all 10 patients were collected (**Table 1**). The most thought-provoking finding involved the two abovementioned pCR patients (P4 and P5). Despite achieving the gold-standard pCR endpoint (ypT0N0), low-frequency but definitive tumor signals [mean variant allele frequency (VAF): 0.1% and 0.23%] were detected in lymph nodes by retrospective ultra-deep NGS. We define this state as mIR, representing the presence of residual molecular disease in patients otherwise classified as pCR. This concept indicates that neoadjuvant therapy may eradicate macroscopic disease but resistant or dormant tumor clones can persist within the lymph node microenvironment[10]. These cells, which are not visible by light microscopy, retain the potential for recurrence.

The discrepancy between pCR and mIR underscores a critical clinical blind spot. Whereas pCR is currently considered the optimal surrogate for survival in neoadjuvant trials, mIR, which is only detectable *via* ultra-deep NGS, may identify a subset of patients with MRD who remain at high risk for recurrence. In these cases, pCR may offer a "false reassurance," suggesting a need to integrate molecular assessment into the definition of treatment response.

Interestingly, lymph node MRD status did not significantly distinguish between the recurrence (n = 5) and non-recurrence (n = 5) groups in the limited cohort (**Table S1**). We acknowledge that the lack of statistical significance (P = 0.455) was highly likely due to a Type II error resulting from insufficient statistical power in the extremely small cohort, as well as confounding effects from varying pathologic responses among patients.

Furthermore, although mIR represents regional MRD, several mIR-positive patients in the cohort had distant systemic recurrences (e.g., brain and abdominal cavity). This finding suggests that the surgically resected mIR-positive lymph nodes are not the direct source of subsequent recurrence. Rather, the presence of trace MRD in regional lymph nodes serves as a surrogate indicator of the tumor intrinsic resistance to neoadjuvant therapy and the biological propensity for systemic micro-dissemination. If dormant tumor clones can survive the neoadjuvant regimen within the regional lymph node microenvironment, it is highly probable that similar drug-resistant clones exist in occult distant sites.

We acknowledge several major limitations in this study. First, the extremely small cohort size (n = 10) severely limited the statistical power, rendering the results susceptible to the influence of individual cases. Consequently, this work should be strictly interpreted as an exploratory, hypothesis-generating study rather than a definitive clinical evaluation. Second, the core concept of mIR contrasting pCR is heavily based on only two specific cases (P4 and P5). Third, paired plasma ctDNA MRD data were only available for 5 of 10 patients, limiting our ability to fully compare the added value of lymph node mIR over established blood MRD approaches. Fourth, there was a demographic imbalance in our cohort (male-to-female ratio, 9:1), which limit the generalizability of our findings. Finally, considering that pCR is still debated as an adoptable surrogate endpoint by regulatory authorities, routine lymph node NGS testing remains an experimental concept far from clinical application.

In conclusion, the statistical power was insufficient to evaluate mIR as an independent prognostic factor in multivariable models. Future large-scale, prospective studies with matched primary tumor, lymph node, and longitudinal plasma samples are warranted to validate the prognostic value of mIR.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Meng Lu, Ran Zhang, Peng Chen, Jian You.
Collected the data: Meng Lu, Ran Zhang, Haidi Xu, Bingsheng Sun.
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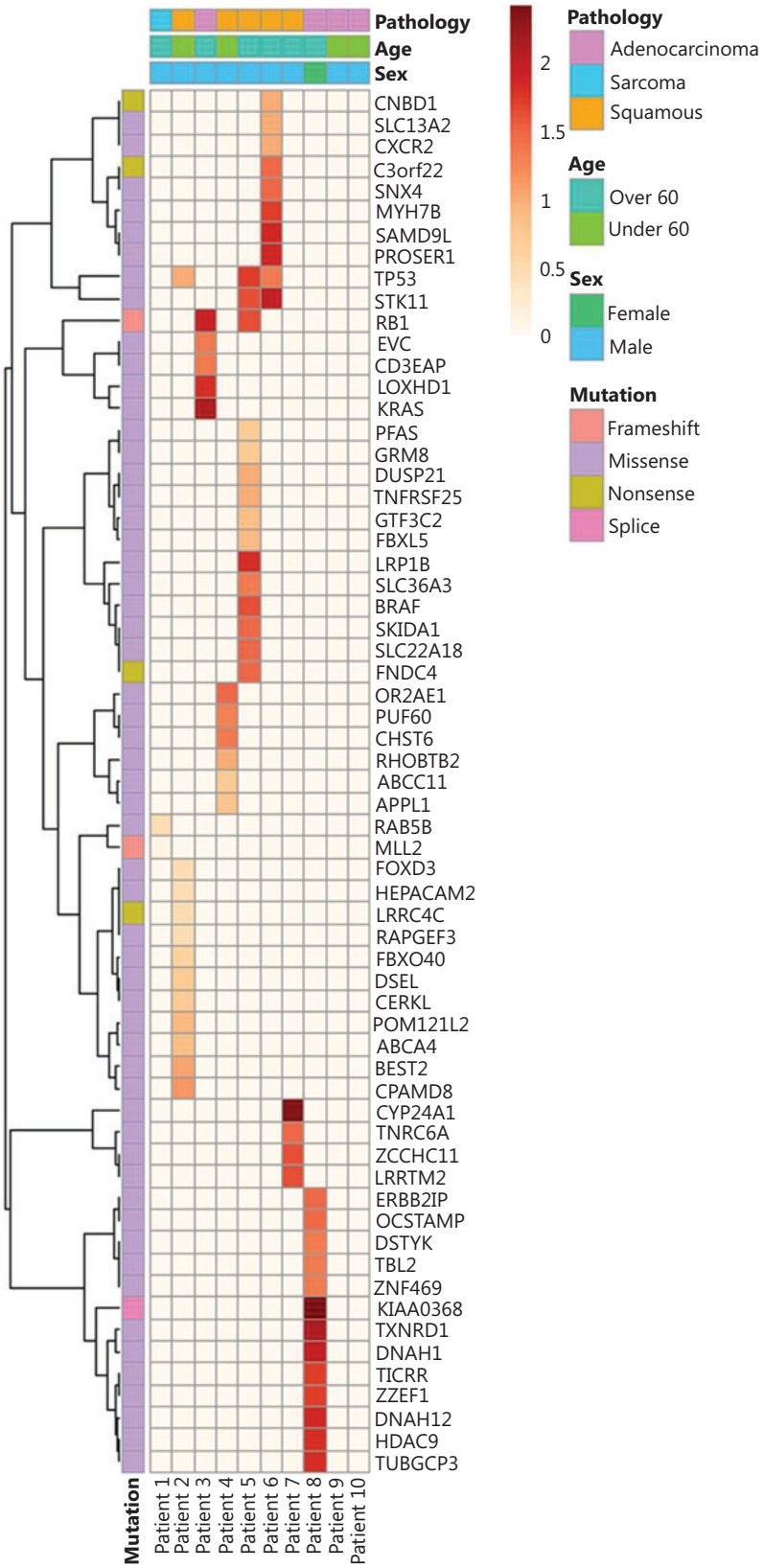


Figure 1 Heatmap of gene mutation sites in lymph nodes of all patients. The vertical axis represents 10 patients and the horizontal axis shows tumor gene mutation types in the lymph nodes with color intensity indicating mutation frequency.

Table 1 The clinicopathologic data and the lymph node MRD status of all 10 patients

Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Gender	Male	Male	Male	Male	Male	Male	Male	Female	Male	Male
Age (years)	70	59	65	58	67	62	66	64	55	54
Smoking history (pack-years)	0	100	1,600	1	1,040	1,600	1,200	0	600	1,200
Tumor location	Peripheral	Central	Peripheral	Peripheral	Central	Peripheral	Peripheral	Peripheral	Peripheral	Peripheral
Maximum diameter (cm)	7.7	6.5	3	7.9	6.0	4.0	2.2	2.8	1.5	0.8
Clinical stage	3A	2B	1B	3B	3B	1B	1A	1A	1A	1A
CYFRA-21-1 (ug/L)	0.97	1.93	3.04	2.02	1.04	2.92	4.42	1.82	1.54	2.17
CA19-9 (U/mL)	4.99	6.36	8.05	18.3	12.62	0.6	8.43	10.84	11	2.7
CEA (ug/L)	0.9	2.17	13.01	2.68	2.76	2.72	3.87	2.47	2.68	3.05
NSE (ug/L)	34.3	17.4	21.17	13.2	19.2	13.26	11.2	25.07	11.6	8.63
SCC (ng/mL)	1.1	12.3	1.1	1.5	1	0.8	1.3	0.5	0.9	1.2
Neoadjuvant therapy	No	No	No	Yes	Yes	No	No	No	No	No
Surgical approach	Thoracoscopic	Open	Thoracoscopic	Open	Open	Thoracoscopic	Thoracoscopic	Thoracoscopic	Thoracoscopic	Thoracoscopic
Extent of excision	RUL	RML + RLL	RLL	RLL	LUL	RUL	RUL	RUL	LUL	Segmentectomy of LLL
Pathologic type	PSC	SCC	IAC	SCC	SCC	SCC	SCC	IAC	IAC	IAC
TP53 mutation	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
KRAS mutation	No	No	Yes	No	No	No	No	No	Yes	Yes
TMB (mut/Mb)	163	283	375	215	654	131	324	34	247	127
Follow-up (months)	12	53	76	40	43	76	70	73	68	55

Table 1 Continued

Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
Recurrence status	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No
DFS (months)	12	20	58	23	30	-	-	-	-	-
Recurrence sites	Pleural, abdominal cavity	Mediastinal lymph nodes	Brain, pulmonary, pleural, hilar lymph nodes	Hilar lymph nodes	Brain, abdominal cavity	-	-	-	-	-
Tumor-related deaths	Yes	No	No	Yes	Yes	No	No	No	No	No
MRD status of lymph nodes	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative
Dissected lymph node stations	1.2.3P.4.7.9.10.11	1.2.3P.4.7.8.10.11	2.4.7.9.11	1.2.4.7.9.10.11	3A.5.6.7.8.9.10.11	2.4.7.9.10.11	2.4.7.11	2.4.9.10.11	5.6.7.9	9.10
Evaluated lymph node stations	7.9.10	4.7.8	2.4	4.7.7.7	5.6.7.10	4.7.10.11	2.4	2.4.10.11	5.6.7	9.10
Lymph node MRD+ stations	10	4.7.8	2.4	4.7.7.7	5.6.7.10	4.7.11	2.4	2.4.10.11	-	-
Lymph node MRD+ rate	1/3	3/3	2/2	4/4	4/4	3/4	2/2	4/4	0	0
Number of gene mutations	2	13	6	9	17	14	4	21	0	0
mVAF (%)	0.02	0.06	0.5	0.1	0.23	0.28	0.8	0.45	0	0
MSAF (%)	0.03	0.11	1.0	0.2	0.6	0.9	2.1	0.9	0	0
Latest MRD status of blood	Not tested	Not tested	Not tested	Not tested	Not tested	Negative	Negative	Negative	Negative	Negative

RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; PSC, pulmonary sarcomatoid carcinoma; SCC, squamous cell carcinoma; IAC, invasive adenocarcinoma; DFS, disease-free survival; mVAF, mean variant allele frequency; MSAF, maximum somatic allele frequency.

Data availability statement

The data generated in this study are available upon request from the corresponding author.

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