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Next-Generation Sequencing vs. Clinical-Pathological Assessment in Diagnosis of Multiple Lung Cancers: A Systematic Review and Meta-Analysis

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ABSTRACT

Accurately distinguishing between multiple primary lung cancers (MPLC) and intrapulmonary metastasis (IPM) is crucial for tailoring treatment strategies and improving patient outcomes. While molecular methods offer significant advantages over traditional clinical-pathological evaluations, they lack standardized diagnostic protocols and validated prognostic value. This study systematically compared the diagnostic and prognostic performance of molecular methods versus clinical-pathological evaluations in diagnosing multiple lung cancers (MLCs), specifically focusing on the impact of next-generation sequencing (NGS) parameters on diagnostic accuracy. A review of 41 studies encompassing 1266 patients revealed that two molecular methods, Mole1 (manually counting shared mutations) and Mole2 (bioinformatics-assisted clonal probability calculation), both demonstrated superior diagnostic accuracy and prognostic discrimination capabilities. Molecular assessment, particularly Mole1, effectively stratified prognosis for MPLC and IPM, leading to significantly improved disease-free survival (DFS: HR = 0.24, 95% CI: 0.15–0.39) and overall survival (OS: HR = 0.33, 95% CI: 0.18–0.58). Further analysis suggests that a minimal panel of 30–50 genes may be sufficient to effectively differentiate prognoses. Compared to Mole1, Mole2 demonstrated greater specificity and stability across various panels, achieving AUC values from 0.962 to 0.979. Clinical-pathological evaluations proved unreliable, not only failing to distinguish prognosis effectively but also exhibiting a potential misdiagnosis rate of 35.5% and 33.6% compared to the reference diagnosis. To improve both cost-effectiveness and diagnostic accuracy, bioinformatics-assisted molecular diagnostics should be integrated into multidisciplinary assessments, especially for high-risk cases where diagnostic errors are common.

1 | Introduction

Currently, lung cancer ranks as the most common malignancy and remains the leading cause of cancer-related mortality worldwide [1]. With the widespread use of thin-slice chest computed tomography (CT), multiple lung cancers (MLCs) have become a common clinical challenge [2, 3]. MLCs comprise multiple primary lung cancers (MPLC) and intrapulmonary

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metastasis (IPM). MPLC generally has a better prognosis, often treated with curative resection, whereas IPM typically requires additional therapies with poorer outcomes [4]. Misclassification of MLC subtypes can result in suboptimal treatment strategies and potentially severe therapeutic errors, underscoring the critical importance of accurate diagnosis.

The diagnosis of MLCs traditionally relies on clinical and pathological information, such as the Martini-Melamed criteria [5], the American College of Chest Physicians (ACCP) guideline [6], and the International Association for the Study of Lung Cancer (IASLC) proposal [7], along with histopathological evaluations (HPE) like comprehensive histology assessment (CHA) [8]. However, the advent of next-generation sequencing (NGS) has revolutionized this, offering a potentially superior approach to the differential diagnosis of MLCs [9]. Despite this, a consensus on the optimal method or established protocol for assessing clonal relationships in MLCs remains elusive. Currently, most studies rely on manual interpretation of NGS results (Mole1), often employing subjective evaluation frameworks and manual mutation counting. The studies on this approach lack efficiency and standardization. While some studies have explored bioinformatics tools for clonal probability analysis (Mole2), these are not yet widely adopted [9]. Beyond the need for comparison between two representative molecular assessment methods, two more key challenges remain to be addressed: optimizing sequencing parameters, particularly determining the ideal sequencing coverage, and developing a cost-effective diagnostic that integrates molecular and clinical data. This study aims to address these challenges through a systematic and comprehensive evaluation of both molecular and clinical-pathological diagnostic approaches for MLCs, with the goal of paving the way for a more accurate, efficient, and standardized diagnostic process.

2 | Methods

Our systematic review followed a preregistered protocol on PROSPERO (CRD42024612366) and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) guidelines to comprehensively evaluate the diagnostic and prognostic performance of molecular and clinical-pathological methods for MLCs.

2.1 | Search Strategy and Eligibility Criteria

The final search was conducted on March 1, 2024. A systematic search of PubMed, Web of Science, Embase, and Scopus was conducted, covering literature from database inception to March 2024. Search terms combined keywords such as "diagnosis" "lung neoplasms" and "high-throughput sequencing" Additional manual screening of reference lists supplemented the search. We included studies that assessed the diagnostic and prognostic performance of molecular and clinical-pathological methods for MLCs, while simultaneously providing extractable genomic profiling data. Studies with fewer than 8 patients, review articles, and case reports were excluded. Full search strategies and eligibility criteria are provided in the supplementary file.

2.2 | Data Extraction and Quality Assessment

Two reviewers (Z.Y.W and X.Q.Y.) independently screened titles and abstracts, with eligible citations undergoing full-text review. Data extraction included study design, sample size, patient demographics, diagnostic methods (e.g., panel size and criteria), and primary outcomes. Discrepancies were resolved through discussion with a third reviewer (Y.T.N). The selection process was summarized in a PRISMA flow diagram (Figure 1). Risk of bias was assessed independently by two authors (Z.Y.W and X.Q.Y.) using the QUADAS-2 tool. Studies with high risk were included, with their limitations acknowledged.

2.3 | Data Analysis

Diagnostic accuracy, assessed using sensitivity, specificity, integrated discrimination improvement (IDI), and area under the receiver operating characteristic curve (AUC), was determined via a Bayesian latent class model (LCM) (Tables S1 and S2). Prognostic stratification analysis employed hazard ratios (HRs) and 95% confidence intervals (CI) pooled using a random-effects model. All statistical analyzes were conducted in R (version 4.3.1). Detailed descriptions of statistical modeling and mutation subsampling procedures are provided in the supplementary file.

3 | Result

3.1 | Study Selection and Quality Assessment

A comprehensive search across four major medical literature databases (PubMed, Web of Science, Embase, and Scopus) yielded 3485 potentially relevant studies (1618, 1210, 433, and 224, respectively). Following deduplication and careful screening, 41 articles met the inclusion criteria and were included in the quantitative analysis, encompassing 1266 patients (Figure 1) [10–50]. Literature quality was assessed using the QUADAS-2 tool (Figure S1), revealing a high risk of bias in 22 of the 41 included articles. Our analysis identified three primary sources of this bias. Firstly, 54.5% (12/22) of high-bias studies relied on non-randomized case selection, including only MPLC cases confirmed through clinical-pathological assessments, potentially introducing selection bias. Secondly, 45.5% (10/22) of high-bias studies prioritized genomic characteristic analysis over evaluating the diagnostic performance of different molecular assessments. Lastly, 36.4% (8/22) of the studies exhibited verification bias due to outdated diagnostic reference standards. To mitigate the impact of high-risk literature, we adopted a selective data extraction strategy, focusing on genomic data from these studies for subsequent molecular assessment and comparison of different molecular evaluation methods.

3.2 | Characteristics of Included Studies

The characteristics of the patients and included studies are shown in Tables 1 and S3. Analysis of the extracted data
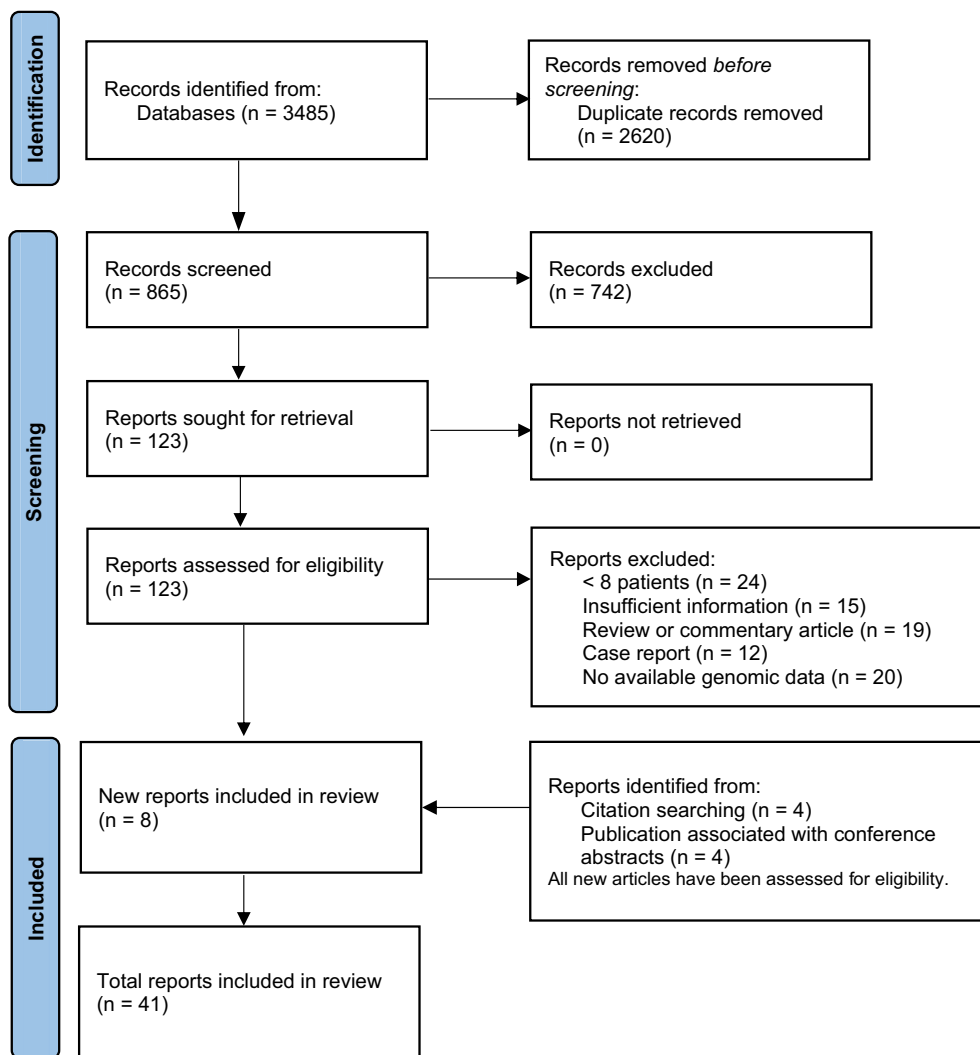
FIGURE 1 | Flow diagram for study selection.

revealed that 82.3% (1042/1266) were cases with double pulmonary nodules, and 36.8% (194/526) had a history of smoking. While multifocal ground-glass opacities (GGO), often indicative of indolent MPLC, are not the primary focus of the included studies, only 86 cases were documented. The majority of cases were clinically and pathologically assessed as "early-stage" nodules with no lymph node metastasis and a diameter of no more than 3 cm (staged as T1N0) if diagnosed as MPLC (Table 1). A total of 5182 tumor-associated single nucleotide variants (SNVs) of tumor mutations were extracted and successfully underwent molecular assessment.

3.3 | Prognostic Stratification Analysis

Although definitive reference diagnoses are unavailable for the included cases, the ability to differentiate prognoses between MPLC and IPM has been proposed as a crucial benchmark for evaluating the efficacy of diagnostic methods in identifying clonal relationships [10, 12–16, 18, 20, 30–32, 38, 40, 46, 48]. As shown in Figure 2, clinical assessment and HPE showed limited effectiveness in distinguishing the prognosis

of different MLCs subtypes, irrespective of whether overall survival (OS) or disease-free survival (DFS) was considered. In contrast, molecular assessments, with the exception of Mole2's suboptimal performance in stratifying OS, exhibited superior performance to clinical-histopathological assessments. Notably, Mole1 identified MPLC with a significantly lower risk of disease progression, yielding a HR of 0.24 (95% CI, 0.15–0.39), which was more favorable compared to the HR for OS, at 0.33 (95% CI, 0.18–0.58). Mole2 was slightly inferior to Mole1, with an HR for distinguishing DFS at 0.51 (95% CI, 0.27–0.98). No significant differences were observed between the results of different studies.

Further analysis revealed that Mole1 exhibited sufficient discriminatory power in studies employing sequencing panels comprising more than 50 genes, as evidenced by its ability to distinguish both DFS and OS (Figure S2). Even when gene panels were limited to 30–50 genes, Mole1 consistently demonstrated a significant capacity to differentiate MLC subtypes, yielding hazard ratios of 0.27 (95% CI, 0.12–0.60) for OS and 0.33 (95% CI, 0.11–0.96) for DFS (Figure 2C). However, this discriminatory power diminished with smaller panels employed.

TABLE 1 | Characteristics of included studies.

Author[a]	Study period	N (patients/tumors)	Synchronous (%)	Reference standard	No. (%) inconclusive cases	Diagnostic molecular methods[c]	No. (%) inconclusive Cases	Suvival analysis	Panel
Arai	1970–2010	12/24	100	HPE	0 (0)	Mole1	8 (67)	NA	1
Asmar	2005–2014	69/154	75.36	HPE	0 (0)	Mole1	29 (42)	NA	4
Belardinilli	2015–2019	10/24	60	CHA + IHC	0 (0)	Mole1	1 (10)	NA	22
Bruehl	2008–2019	32/64	100	HPE	0 (0)	Mole1	8 (25)	Yes	35 (17limited)
Chang	NA	60/128	75	CHA	23 (30)	Mole2	0 (0)	Yes	341–468
Chen	2017–2018	17/35	100	HPE	0 (0)	Mole1	1 (6)	Yes	168
Donfrancesco	2010–2018	24/50	100	CHA + IHC	0 (0)	Mole1	3 (13)	Yes	22
Duan	2016–2018	16/36	100	HPE	0 (0)	Mole1	0 (0)	NA	520
Ezer	2016–2020	61/131	NA	CHA	8 (13)	Mole1	8 (13)	Yes	15/52
Girard	2003–2008	7/14	42.86	MM/ACCP	0 (0)	Mole1	0 (0)	NA	2
Goodwin	2000–2019	40/80	52.5	MM/HPE	0 (0)	Mole1	0 (0)	Yes	50
Goto	2014–2016	24/48	100	Mole1	0 (0)	MoleX	0 (0)	NA	53
Higuchi	2015–2019	37/76	70.27	HPE	0 (0)	Mole1	NA	NA	53
Hu	2018–2020	112/255	92.9	MM	0 (0)	NA	NA	NA	1021
Izumi	2007–2019	17/38	NA	MM	0 (0)	NA	NA	NA	409
Lee	2017–2020	101/208	91.1	ACCP	0 (0)	Mole1	54 (54)	Yes	1
Li	2019–2021	41/94	100	MM	0 (0)	Mole1	5 (12)	NA	500
Liu	2013–2014	38/76	100	HPE	0 (0)	Mole1	4 (11)	NA	1
Liu (A)	NA	6/15	100	MM	0 (0)	Mole1	0 (0)	NA	WES
Liu (B)	NA	15/35	NA	HPE	1 (7)	Mole1	0 (0)	NA	464
Mansuet-Lupo	2010–2012	120/240 (Included 30/60)	72	HPE	0 (0)	Mole1	7 (23)	Yes	22
Pagan	NA	47/108	100	ACCP	0 (0)	Mole1	12 (26)	NA	47
Patel	2014–2015	11/31	45.45	CHA	3 (27)	Mole1	0 (0)	Yes	50
Pei	2017–2019	30/67	83.3	MM	0 (0)	Mole1	0 (0)	NA	808

(Continues)

TABLE 1 | (Continued)

Author[a]	Study period	N (patients/tumors)	Synchronous (%)	Reference standard	No. (%) inconclusive cases	Diagnostic molecular methods[c]	No. (%) inconclusive Cases	Suvival analysis	Panel
Qiu (A)	2019–2019	30/66	100	HPE	0 (0)	Mole1	1 (3)	NA	568
Qiu (B)	2008–2016	44/88	100	HPE	0 (0)	Mole1	26 (59)	NA	22
Qu	2016–2019	8/17	100	MM	0 (0)	Mole1	0 (0)	NA	1
Rodriguez	NA	30/60	50	Mole1	10 (33)	NA	NA	NA	8
Roepman	2003–2014	50/111	72	CHA + IHC	0 (0)	Mole1	14 (24)	Yes	50
Saab	2015–2015	18/52	33.33	HPE	6 (35)	CHA + NGS	1 (8)/5 (28)	NA	50
Takahashi	2002–2013	37/82	48.65	MM/HPE	MM:0 (0) HPE:17 (46)	MoleX	0 (0)	Yes	20
Takamochi	1996–2008	30/68	83.33	MM	0 (0)	Mole1	5 (17)	Yes	2
Vincenten[b]	2007-2015	6/13	83.3	ACCP	0 (0)	Mole1	3 (50)	NA	2
Wang	NA	Training cohort:35/80 Validation cohort:16/36	100	HPE	0 (0)	Mole1	0 (0)	NA	605
Xiao	2004–2015	6/14	100	Mole1	0 (0)	MoleX	NA	NA	50
Xu	2014–2017	50/101	100	ACCP	0 (0)	Mole1	7 (14)	Yes	10
Yang	2007–2020	24/48	45.83	Mole1	10 (42)	MoleX	0 (0)	NA	410–468
Zhang	2018–2021	42/93	100	ACCP	0 (0)	Mole1	3 (7)	NA	10
Zhang (B)	2018–2019	45/101	100	CHA	0 (0)	MoleX	0 (0)	Yes	425
Zheng	2013–2018	18/41	100	HPE	1 (5)	Mole1	3 (17)	NA	4/48
Zhou	2019–2019	19/60	100	MM	0 (0)	Mole1	0 (0)	NA	WES

Abbreviations: ACCP, American College of Chest Physicians; CHA, comprehensive histologic assessment; HPE, histopathological evaluation excluding CHA; HC, Immunohistochemistry; MM, criteria proposed by Martini and Melamed; WES, whole exome sequencing; NA, no report.

[a]Author: Some studies include authors with the same surname, differentiated by suffixes.

[b]Limited cases have extractable mutation data, but the full cohort is sufficiently large for comparison. Only cases with mutation data are shown.

[c]MoleX refers to other rare molecular methods that employ bioinformatics techniques.
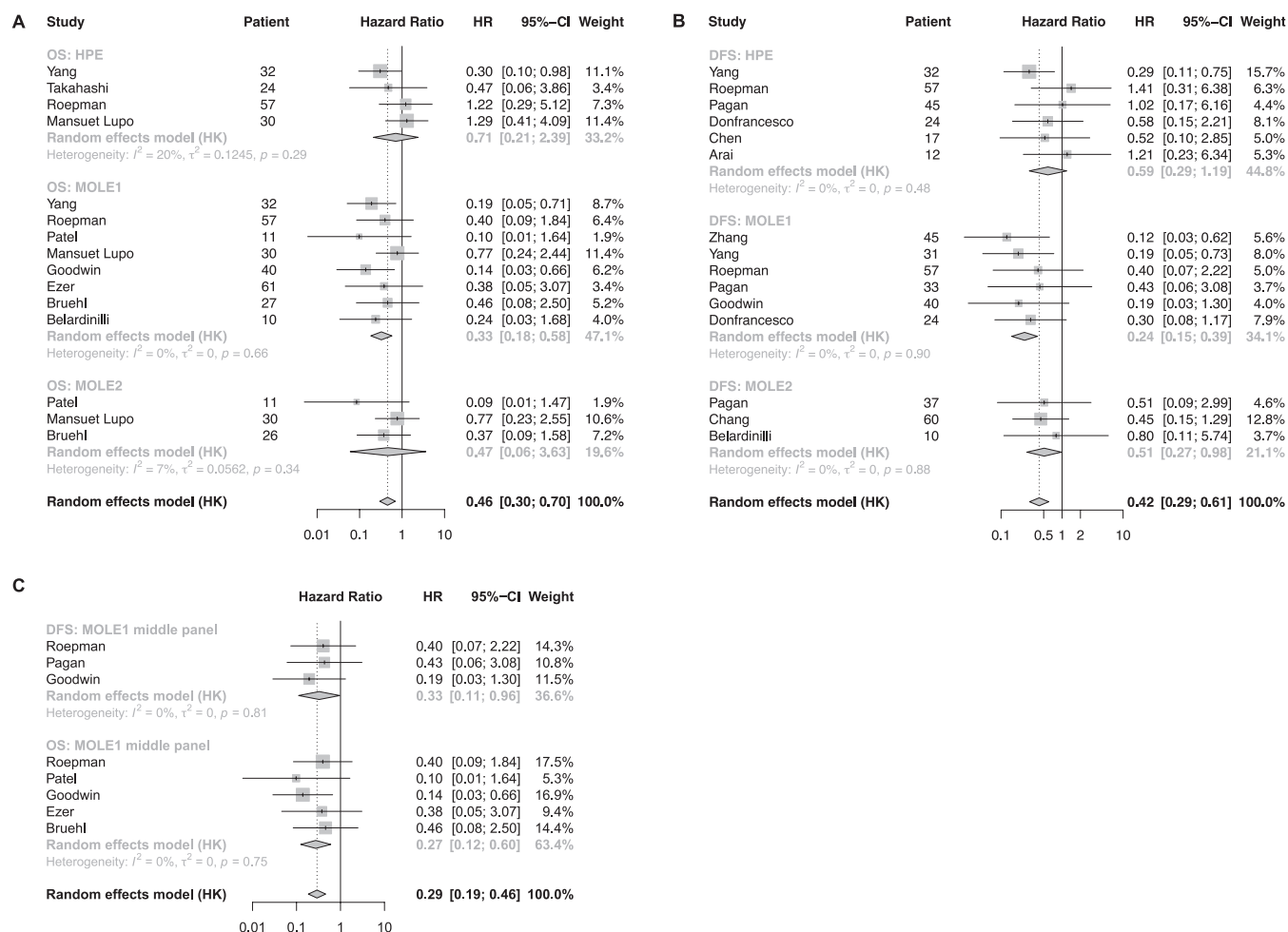
FIGURE 2 | Prognostic analysis of different methods for distinguishing MLCs. (A) Prognostic analysis of OS comparing HPE, Mole1, and Mole2 methods. (B) Prognostic analysis of DFS comparing HPE, Mole1, and Mole2 methods. (C) *OS* and DFS analyzes using Mole1 with middle-size sequencing panels (30–50 genes). OS, Overall survival; DFS, Disease-free survival; HPE, histopathology evaluation.

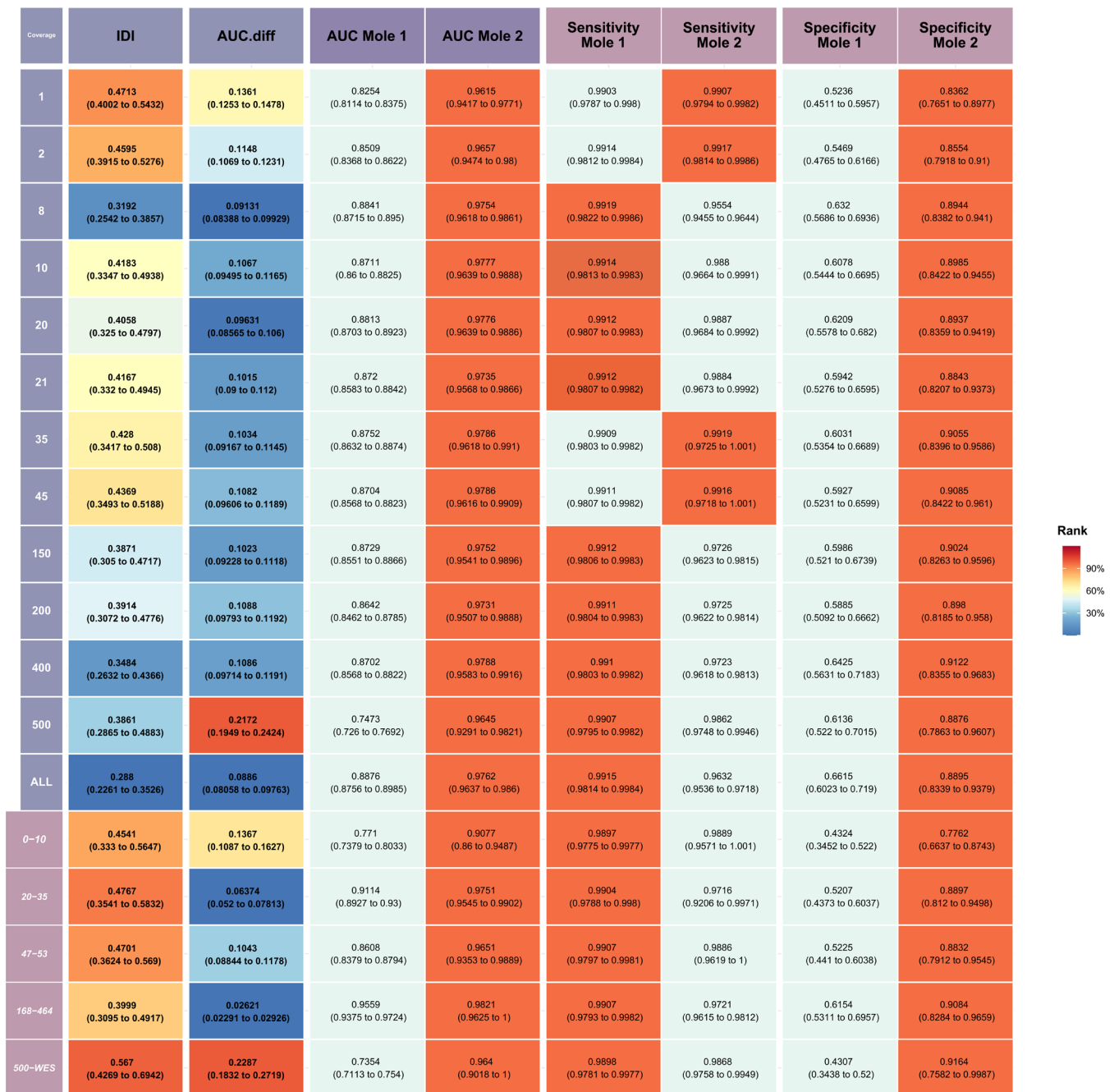## 3.4 | Analysis of Sensitivity and Specificity in Molecular Assessments

By statistically analyzing the diagnostic performance of two molecular methods in simulated MLCs, we determined the necessary prior information. Based on this, we successfully employed a Bayesian latent class model for a comprehensive evaluation of two representative molecular assessments in included cases. We systematically compared the diagnostic performance of the two methods using several metrics, including sensitivity, specificity, IDI, AUC, and the difference in AUC (AUC.diff), based on predicted diagnoses generated by the LCM model.

IDI quantifies the difference in predicted probabilities between the two groups, while AUC.diff assesses the overall discriminatory ability of Mole2 versus Mole1 and evaluates the clinical risk of misdiagnosis by comparing the AUC of the two methods. Both molecular assessment methods demonstrated high accuracy in definitively diagnosed cases. Mole1 achieved an AUC range of 0.747 to 0.888, while Mole2 ranged from 0.962 to 0.979 (Figure 3). Analyzes of IDI and AUC.diff indicate that Mole2 outperformed Mole1 across various sequencing coverages. Notably, the performance gap widened as panel size decreased,

a trend particularly evident in the panel grouping analysis. This trend was less pronounced in the subsampling strategy analysis. Sensitivity and specificity for both methods exhibited an increasing trend with expanding panel size. While both Mole1 and Mole2 demonstrated high and remarkably similar sensitivity values, Mole2 consistently outperformed Mole1 in specificity (Mole1: 0.524–0.662 vs. Mole2: 0.836–0.912), particularly with smaller sequencing panels.

Further analysis of indeterminate (IN) rates reveals the instability of Mole1's performance compared to Mole2. Mole1 produced a significantly higher proportion of IN results (Mole1 43.0% vs. Mole2 32.2%), particularly with panels containing fewer than 50 genes (Figure S3). To achieve comparable diagnostic accuracy to Mole2, Mole1 requires integration with a larger sequencing panel (greater than 100 genes). This finding is further supported by the concordance analysis between the two methods. Even after excluding panels with fewer than 10 genes, agreement remained relatively low across most panel sizes, ranging from 76.6% to 80.6% (Figure S4). Notably, as the sequencing panel expanded, Mole2 demonstrated improved accuracy in identifying inconclusive cases initially diagnosed as such by Mole1 (11.1%–16.9%), while the proportion of Mole1 diagnoses being modified

| Coverage	IDI	AUC.diff	AUC Mole 1	AUC Mole 2	Sensitivity Mole 1	Sensitivity Mole 2	Specificity Mole 1	Specificity Mole 2
1	0.4713 (0.4002 to 0.5432)	0.1361 (0.1253 to 0.1478)	0.8254 (0.8114 to 0.8375)	0.9615 (0.9417 to 0.9771)	0.9903 (0.9787 to 0.998)	0.9907 (0.9794 to 0.9982)	0.5236 (0.4511 to 0.5957)	0.8362 (0.7651 to 0.8977)
2	0.4595 (0.3915 to 0.5276)	0.1148 (0.1069 to 0.1231)	0.8509 (0.8368 to 0.8622)	0.9657 (0.9474 to 0.98)	0.9914 (0.9812 to 0.9984)	0.9917 (0.9814 to 0.9986)	0.5469 (0.4765 to 0.6166)	0.8554 (0.7918 to 0.91)
8	0.3192 (0.2542 to 0.3857)	0.09131 (0.08388 to 0.09929)	0.8841 (0.8715 to 0.895)	0.9754 (0.9618 to 0.9861)	0.9919 (0.9822 to 0.9986)	0.9554 (0.9455 to 0.9644)	0.632 (0.5686 to 0.6936)	0.8944 (0.8382 to 0.941)
10	0.4183 (0.3347 to 0.4938)	0.1067 (0.09495 to 0.1165)	0.8711 (0.86 to 0.8825)	0.9777 (0.9639 to 0.9888)	0.9914 (0.9813 to 0.9983)	0.988 (0.9664 to 0.9991)	0.6078 (0.5444 to 0.6695)	0.8985 (0.8422 to 0.9455)
20	0.4058 (0.325 to 0.4797)	0.09631 (0.08565 to 0.106)	0.8813 (0.8703 to 0.8923)	0.9776 (0.9639 to 0.9886)	0.9912 (0.9807 to 0.9983)	0.9887 (0.9684 to 0.9992)	0.6209 (0.5578 to 0.682)	0.8937 (0.8359 to 0.9419)
21	0.4167 (0.332 to 0.4945)	0.1015 (0.09 to 0.112)	0.872 (0.8583 to 0.8842)	0.9735 (0.9568 to 0.9866)	0.9912 (0.9807 to 0.9982)	0.9884 (0.9673 to 0.9992)	0.5942 (0.5276 to 0.6595)	0.8843 (0.8207 to 0.9373)
35	0.428 (0.3417 to 0.508)	0.1034 (0.09167 to 0.1145)	0.8752 (0.8632 to 0.8874)	0.9786 (0.9618 to 0.991)	0.9909 (0.9803 to 0.9982)	0.9919 (0.9725 to 1.001)	0.6031 (0.5354 to 0.6689)	0.9055 (0.8396 to 0.9586)
45	0.4369 (0.3493 to 0.5188)	0.1082 (0.09606 to 0.1189)	0.8704 (0.8568 to 0.8823)	0.9786 (0.9616 to 0.9909)	0.9911 (0.9807 to 0.9982)	0.9916 (0.9718 to 1.001)	0.5927 (0.5231 to 0.6599)	0.9085 (0.8422 to 0.961)
150	0.3871 (0.305 to 0.4717)	0.1023 (0.09228 to 0.1118)	0.8729 (0.8551 to 0.8866)	0.9752 (0.9541 to 0.9896)	0.9912 (0.9806 to 0.9983)	0.9726 (0.9623 to 0.9815)	0.5986 (0.521 to 0.6739)	0.9024 (0.8263 to 0.9596)
200	0.3914 (0.3072 to 0.4776)	0.1088 (0.09793 to 0.1192)	0.8642 (0.8462 to 0.8785)	0.9731 (0.9507 to 0.9888)	0.9911 (0.9804 to 0.9983)	0.9725 (0.9622 to 0.9814)	0.5885 (0.5092 to 0.6662)	0.898 (0.8185 to 0.958)
400	0.3484 (0.2632 to 0.4366)	0.1086 (0.09714 to 0.1191)	0.8702 (0.8568 to 0.8822)	0.9788 (0.9583 to 0.9916)	0.991 (0.9803 to 0.9982)	0.9723 (0.9618 to 0.9813)	0.6425 (0.5631 to 0.7183)	0.9122 (0.8355 to 0.9683)
500	0.3861 (0.2865 to 0.4883)	0.2172 (0.1949 to 0.2424)	0.7473 (0.726 to 0.7692)	0.9645 (0.9291 to 0.9821)	0.9907 (0.9795 to 0.9982)	0.9862 (0.9748 to 0.9946)	0.6136 (0.522 to 0.7015)	0.8876 (0.7863 to 0.9607)
ALL	0.288 (0.2261 to 0.3526)	0.0886 (0.08058 to 0.09763)	0.8876 (0.8756 to 0.8985)	0.9762 (0.9637 to 0.986)	0.9915 (0.9814 to 0.9984)	0.9632 (0.9536 to 0.9718)	0.6615 (0.6023 to 0.719)	0.8895 (0.8339 to 0.9379)
0–10	0.4541 (0.333 to 0.5647)	0.1367 (0.1087 to 0.1627)	0.771 (0.7379 to 0.8033)	0.9077 (0.86 to 0.9487)	0.9897 (0.9775 to 0.9977)	0.9889 (0.9571 to 1.001)	0.4324 (0.3452 to 0.522)	0.7762 (0.6637 to 0.8743)
20–35	0.4767 (0.3541 to 0.5832)	0.06374 (0.052 to 0.07813)	0.9114 (0.8927 to 0.93)	0.9751 (0.9545 to 0.9902)	0.9904 (0.9788 to 0.998)	0.9716 (0.9206 to 0.9971)	0.5207 (0.4373 to 0.6037)	0.8897 (0.812 to 0.9498)
47–53	0.4701 (0.3624 to 0.569)	0.1043 (0.08844 to 0.1178)	0.8608 (0.8379 to 0.8794)	0.9651 (0.9353 to 0.9889)	0.9907 (0.9797 to 0.9981)	0.9886 (0.9619 to 1)	0.5225 (0.441 to 0.6038)	0.8832 (0.7912 to 0.9545)
168–464	0.3999 (0.3095 to 0.4917)	0.02621 (0.02291 to 0.02926)	0.9559 (0.9375 to 0.9724)	0.9821 (0.9625 to 1)	0.9907 (0.9793 to 0.9982)	0.9721 (0.9615 to 0.9812)	0.6154 (0.5311 to 0.6957)	0.9084 (0.8284 to 0.9659)
500–WES	0.567 (0.4269 to 0.6942)	0.2287 (0.1832 to 0.2719)	0.7354 (0.7113 to 0.754)	0.964 (0.9018 to 1)	0.9898 (0.9781 to 0.9977)	0.9868 (0.9758 to 0.9949)	0.4307 (0.3438 to 0.52)	0.9164 (0.7582 to 0.9987)

Rank
90%
60%
30%

FIGURE 3 | Comparative analysis of diagnostic performance metrics between Mole1 and Mole2. The figure illustrates the performance of Mole1 and Mole2 across various sequencing panels, displaying the IDI, AUC values, AUC.diff, sensitivity, and specificity. Color coding indicates the rank of diagnostic performance for each metric. Specifically, AUC, sensitivity, and specificity are compared for equivalent sequencing panels (within rows), with superior performance highlighted in orange-red. IDI and AUC.diff, conversely, rank performance across different panels (within columns). IDI, Integrated Discrimination Improvement; AUC, Area Under the Curve.

to inconclusive by Mole2 remained below 1.7%. In panel-grouped analysis, the improvement rate reached 7.6%–19.7%, while conversions remained under 3.4%.

These findings suggest that subjective judgment methods like Mole1 may compromise the effectiveness of molecular assessments compared to more objective methods like Mole2, which exhibit greater stability and reliability. Subgroup analyzes further support these findings, despite minor differences (Figure 3).

3.5 | Threshold Effect of Gene Panel Size on Molecular Diagnostic Accuracy

Analysis of molecular assessment results across different panel sizes in all cases revealed a threshold effect on both tumor mutation detection rates and definitive diagnosis rates: beyond a certain sequencing range, the benefits of further panel expansion diminished significantly (Table S4). For example, studies utilizing panels exceeding 100 genes consistently achieved definitive diagnoses
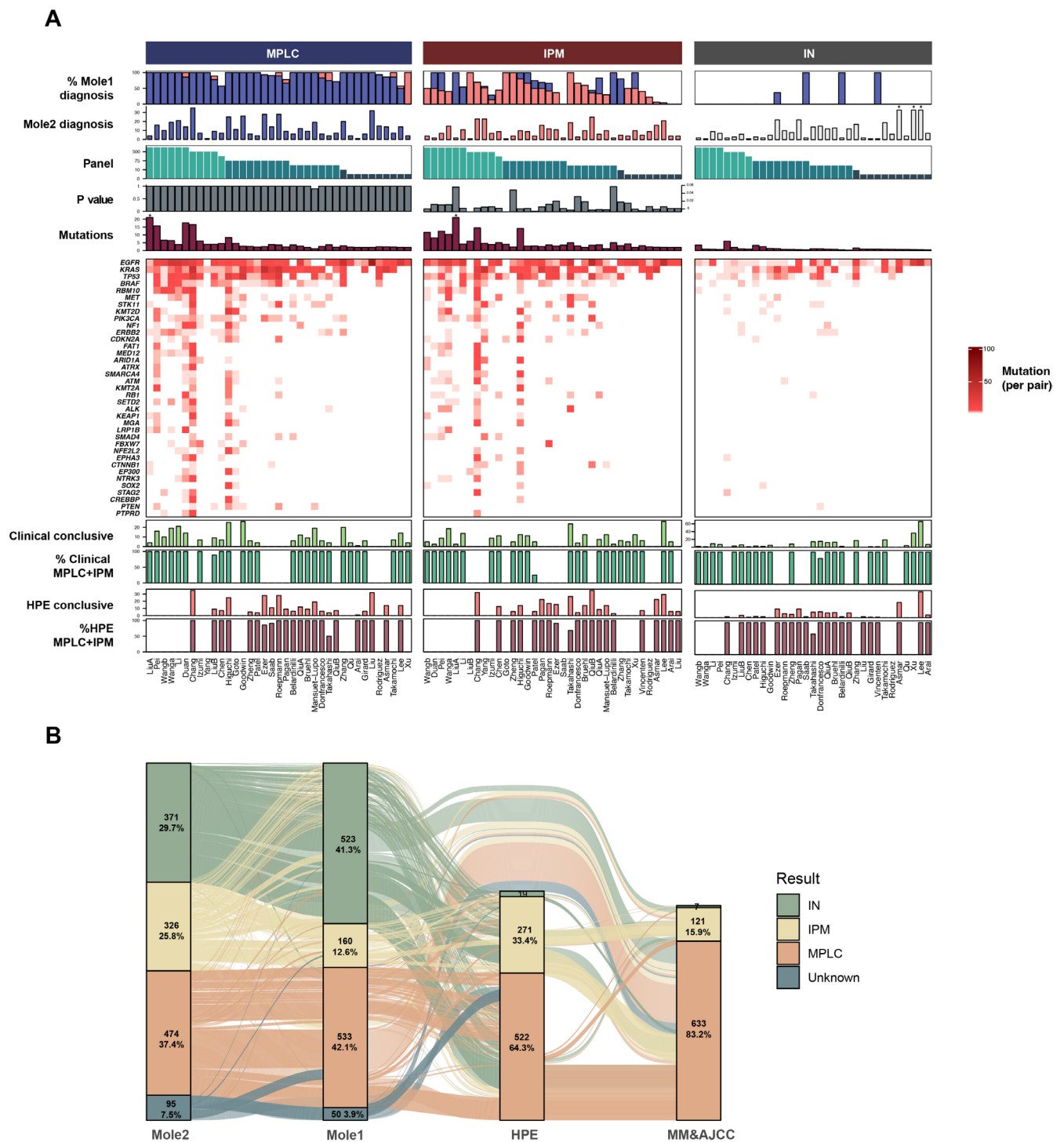
FIGURE 4 | Overview of included studies and diagnostic tracking for included cases. (A) Multidimensional diagnostic landscape was stratified by Mole2 reference classifications, encompassing corresponding Mole1 molecular classification results, tumor mutation heatmaps, gene panel sizes, and diagnostic accuracy rates for clinical or histopathological evaluations. (B) The Sankey diagram visualizes longitudinal shifts in case classifications across clinical, pathological, and molecular evaluations within a cohort of 1266 cases, highlighting diagnostic reclassifications across different approaches.

in 85%–95% of included cases, with minimal difference between studies. Moreover, both the tumor mutation detection rate and improvement rates exhibited rapid initial growth before plateauing (Figures 4A and S3). These findings suggest that in real-world

applications of NGS-based molecular assessment, there is no need to indiscriminately expand molecular testing. Utilizing a panel of an appropriate size is sufficient to meet diagnostic requirements without placing an unnecessary burden on patients.

3.6 | Discrepancies in Diagnostic Accuracies Between Different Diagnostic Assessments

A significant discrepancy exists between clinical-pathological evaluations and molecular assessments. Using Mole2 diagnoses as the reference standard, we found that clinical assessments, including the Martini-Melamed criteria and the non-molecular parts of ACCP criteria and IASLC proposal [10, 13, 15–17, 20, 22–26, 28–30, 32–34, 36, 40, 41, 43, 45, 48–50], demonstrate limited diagnostic accuracy, with potential misjudgments observed in at least one-third of all cases. Subsampling analyzes revealed that the proportion of cases with diagnoses concordant with Mole2 ranged from 16.4% (1 gene) to 66.0% (over 500 genes). Panel-grouped analyzes yielded concordance rates between 16.4%–47.6% (fewer than 10 genes) and a maximum of 61.8% (more than 100 genes). The concordance rate between pathological and molecular assessments is even lower, with over 40% of cases showing diagnostic changes in both subsampling and panel-grouped analyzes (Figure 4B).

The aforementioned findings suggest that rigidly adhering to traditional clinicopathological criteria may be counterproductive in differentiating MLCs. To avoid inconsistencies between diagnostic standards and circular diagnostic processes, we propose a tiered evaluation framework that integrates clinical-pathological assessment criteria with enhanced specificity or sensitivity alongside molecular evaluation, as supported by previous studies and consensus guidelines [14, 30, 51]. To achieve this, we validated different criteria using a cohort of 812 cases with extracted pathological information [10–16, 18, 19, 22, 25, 27, 29–32, 34, 35, 38–40, 42, 48, 49]. Our clinical experience-informed validation yielded several key conclusions [46, 52–54]. Three categories of cases can be confidently diagnosed as MPLC using HPE alone: MLCs with distinct pathological types (e.g., one lesion identified as lung adenocarcinoma (LUAD) and another diagnosed as lung squamous cell carcinoma (LUSC)) are highly likely to be MPLC; MLCs predominantly composed of low-grade components are highly likely to be MPLC; multifocal invasive mucinous adenocarcinomas (IMA) are highly likely to represent IPM. Conversely, two categories strongly warrant molecular evaluation. First, cases with high-grade components require caution, as highlighted by the misclassification of 22 out of 93 similar cases, with 7 reclassified as IPM and 15 as MPLC. Second, cases diagnosed as IPM using HPE, compared to those diagnosed as MPLC, have a higher potential for misdiagnosis (MPLC: 69/284 vs. IPM: 96/207, $p < 0.0001$).

These conclusions offer valuable insights for developing a comprehensive diagnostic framework for suspected MLCs. This stepwise workflow would commence with clinical suspicion of MLCs, followed by pathological screening of surgical specimens. HPE results would then be used to stratify cases into low and high risks of misdiagnosis. Based on this stratification, tailored recommendations for concurrent molecular testing would be provided. This framework aims to guide personalized treatment strategies for patients with suspected MLCs, balancing diagnostic precision with cost-effectiveness.

4 | Discussion

This systematic review and meta-analysis provide the first comprehensive evaluation of molecular and clinical-pathological methods in distinguishing MLCs. We included 41 studies with 1266 patients across diverse clinical settings. Mole1 and Mole2, representing the two most typical and widely used molecular methods, were analyzed alongside the traditional clinical-pathological approaches. Despite variability in panel sizes, Mole2 exhibited superior specificity and comparable sensitivity compared to Mole1. Comparisons between molecular methods and clinical-pathological evaluations showed that molecular approaches exhibited significantly higher accuracy in MLCs diagnosis. While some heterogeneity existed among the included studies, these findings lay the groundwork for refining molecular diagnostic strategies and developing integrated clinical-pathological –molecular diagnostic approaches to improve the management of MLCs.

Mole1 demonstrated robust prognostic stratification capabilities with consistent performance across various panels. However, Mole2 showed less favorable results for OS stratification, contrasting with other analyzes suggesting its superior performance. This discrepancy is likely due to the limited availability of prognostic data. Only three studies provided extractable prognostic data for Mole2, and these studies had relatively short follow-up periods (e.g., Chang et al.'s study reported a median follow-up of only 15 months [14]). This suggests that the observed suboptimal performance of Mole2 may not be an inherent limitation of the methodology, but rather a reflection of the need for further validation through large-scale studies with longer follow-up periods.

Our study reveals that traditional clinical-pathological evaluations, once considered the gold standard for diagnosis, fall short in accuracy. We identified several reasons contributing to their suboptimal performance. First, the clinical diagnostic criteria used in the included studies lacked standardization, resulting in inconsistencies after data integration, even after data harmonization efforts guided by later-published guidelines. Second, only 29.3% (227/774) of cases incorporated analyzes of histological subtypes. The limited adoption of CHA has led to misclassification, particularly in cases with overlapping or ambiguous features.

A combined approach integrating molecular assessments and clinical-pathological criteria represents a pragmatic solution, especially in settings with limited access to molecular diagnostics. HPE can function as an effective initial triage tool for cases with clear diagnostic features, such as those characterized by distinct pathological features identifiable through specific criteria outlined earlier. In these cases, molecular diagnostics can be strategically reserved for more complex or ambiguous cases that necessitate higher diagnostic precision. This integrated strategy optimizes cost-effectiveness, feasibility, and diagnostic yield, ensuring high accuracy while minimizing resource waste.

To address the challenge posed by the lack of a definitive reference diagnosis, we employed a Bayesian modeling approach to conduct diagnostic tests. Given the limited availability of prior information from real MLC cases and the potential biases introduced by simulated data, we performed additional model simulations under mild assumptions. These simulations were designed to avoid extremes, neither assuming a complete absence of prior knowledge nor imposing overly strong priors. The results obtained from this strategy were consistent with our primary findings, as shown in Figures S5 and S6.

This study has several limitations. First, the distribution of sequencing panels across studies was uneven, with a notable scarcity of data available for larger panels, particularly those comprising over 500 genes. While subsampling partially addressed this issue, it could not fully mitigate this limitation. Second, variations in bioinformatics analysis pipelines for NGS, such as variant allele frequency (VAF) detection thresholds and mutation calling protocols (Table S3), introduced potential inconsistencies that may have impacted the study's findings. Third, the prognostic analysis was constrained by the limited number of studies reporting prognostic data and small sample sizes, resulting in considerable uncertainty in effect size estimation. Additionally, genomic analyzes exploring mechanistic differences between MPLC and IPM were constrained by data format inconsistencies, incomplete datasets, and heterogeneity in panel designs and inclusion criteria. Preliminary findings did not reveal substantial differences in driver mutations or pathway-level mutation distributions. Consequently, the main text focuses on results related to diagnostic performance, with mechanistic insights requiring further investigation in future studies.

5 | Conclusions

NGS-based molecular assessments demonstrate superior diagnostic accuracy compared to clinical-pathological evaluations in distinguishing MPLC from IPM. Bioinformatics-assisted molecular methods offer higher specificity and stability across sequencing panels than routine molecular assessments. Integrating molecular assessment with clinical-pathological criteria can streamline the diagnostic process, leading to improved efficiency and cost-effectiveness.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. A. Leiter, R. R. Veluswamy, and J. P. Wisnivesky, "The Global Burden of Lung Cancer: Current Status and Future Trends," *Nature Reviews. Clinical Oncology* 20, no. 9 (2023): 624–639.

2. H. Asamura, K. K. Nishimura, D. J. Giroux, et al., "IASLC Lung Cancer Staging Project: The New Database to Inform Revisions in the Ninth Edition of the TNM Classification of Lung Cancer," *Journal of Thoracic Oncology* 18, no. 5 (2023): 564–575.

3. T. E. Stinchcombe, X. Wang, B. Damman, et al., "Secondary Analysis of the Rate of Second Primary Lung Cancer From Cancer and Leukemia Group B 140503 (Alliance) Trial of Lobar Versus Sublobar Resection for T1aN0 Non-Small-Cell Lung Cancer," *Journal of Clinical Oncology* 42, no. 10 (2024): 1110–1113.

4. L. Jiang, J. He, X. Shi, et al., "Prognosis of Synchronous and Metachronous Multiple Primary Lung Cancers: Systematic Review and Meta-Analysis," *Lung Cancer* 87, no. 3 (2015): 303–310.

5. N. Martini and M. R. Melamed, "Multiple Primary Lung Cancers," *Journal of Thoracic and Cardiovascular Surgery* 70, no. 4 (1975): 606–612.

6. B. D. Kozower, J. M. Larner, F. C. Detterbeck, and D. R. Jones, "Special Treatment Issues in Non-Small Cell Lung Cancer: Diagnosis and Management of Lung Cancer, 3rd Ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines," *Chest* 143, no. Suppl 5 (2013): e369S–e399S.

7. F. C. Detterbeck, V. Bolejack, D. A. Arenberg, et al., "The IASLC Lung Cancer Staging Project: Background Data and Proposals for the Classification of Lung Cancer With Separate Tumor Nodules in the Forthcoming Eighth Edition of the TNM Classification for Lung Cancer," *Journal of Thoracic Oncology* 11, no. 5 (2016): 681–692.

8. N. Girard, C. Deshpande, C. Lau, et al., "Comprehensive Histologic Assessment Helps to Differentiate Multiple Lung Primary Nonsmall Cell Carcinomas From Metastases," *American Journal of Surgical Pathology* 33, no. 12 (2009): 1752–1764.

9. Z. Wang, X. Yuan, G. Jiang, et al., "Towards the Molecular Era of Discriminating Multiple Lung Cancers," *eBioMedicine* 90 (2023): 104508.

10. J. Arai, T. Tsuchiya, M. Oikawa, et al., "Clinical and Molecular Analysis of Synchronous Double Lung Cancers," *Lung Cancer* 77, no. 2 (2012): 281–287.

11. R. Asmar, J. R. Sonett, G. Singh, M. M. Mansukhani, and A. C. Borczuk, "Use of Oncogenic Driver Mutations in Staging of Multiple Primary Lung Carcinomas: A Single-Center Experience," *Journal of Thoracic Oncology* 12, no. 10 (2017): 1524–1535.

12. F. Belardinilli, A. Pernazza, Y. Mahdavian, et al., "A Multidisciplinary Approach for the Differential Diagnosis Between Multiple Primary Lung Adenocarcinomas and Intrapulmonary Metastases," *Pathology Research and Practice* 220 (2021): 153387.

13. F. K. Bruehl, E. E. Doxtader, Y. W. Cheng, D. H. Farkas, C. Farver, and S. Mukhopadhyay, "Does Histological Assessment Accurately Distinguish Separate Primary Lung Adenocarcinomas From Intrapulmonary Metastases? A Study of Paired Resected Lung Nodules in 32 Patients Using a Routine Next-Generation Sequencing Panel for Driver Mutations," *Journal of Clinical Pathology* 75, no. 6 (2022): 390–396.

14. J. C. Chang, D. Alex, M. Bott, et al., "Comprehensive Next-Generation Sequencing Unambiguously Distinguishes Separate Primary Lung Carcinomas From Intrapulmonary Metastases: Comparison With Standard Histopathologic Approach," *Clinical Cancer Research* 25, no. 23 (2019): 7113–7125.

15. X. Chen, J. Lu, Y. Wu, et al., "Genetic Features and Application Value of Next Generation Sequencing in the Diagnosis of Synchronous Multifocal Lung Adenocarcinoma," *Oncology Letters* 20, no. 3 (2020): 2829–2839.

16. E. Donfrancesco, V. Yvorel, F. Casteillo, et al., "Histopathological and Molecular Study for Synchronous Lung Adenocarcinoma Staging," *Virchows Archiv* 476, no. 6 (2020): 835–842.

17. J. Duan, M. Ge, J. Peng, et al., "Application of Large-Scale Targeted Sequencing to Distinguish Multiple Lung Primary Tumors From Intrapulmonary Metastases," *Scientific Reports* 10, no. 1 (2020): 18840.

18. N. Ezer, H. Wang, A. G. Corredor, et al., "Integrating NGS-Derived Mutational Profiling in the Diagnosis of Multiple Lung Adenocarcinomas," *Cancer Treatment and Research Communications* 29 (2021): 100484.

19. N. Girard, C. Deshpande, C. G. Azzoli, et al., "Use of Epidermal Growth Factor Receptor/Kirsten Rat Sarcoma 2 Viral Oncogene Homolog Mutation Testing to Define Clonal Relationships Among Multiple Lung Adenocarcinomas Comparison With Clinical Guidelines," *Chest* 137, no. 1 (2010): 46–52.

20. D. Goodwin, V. Rathi, M. Conron, and G. M. Wright, "Genomic and Clinical Significance of Multiple Primary Lung Cancers as Determined by Next-Generation Sequencing," *Journal of Thoracic Oncology* 16, no. 7 (2021): 1166–1175.

21. T. Goto, Y. Hirotsu, H. Mochizuki, et al., "Mutational Analysis of Multiple Lung Cancers: Discrimination Between Primary and Metastatic Lung Cancers by Genomic Profile," *Oncotarget* 8, no. 19 (2017): 31133–31143.

22. R. Higuchi, T. Nakagomi, T. Goto, et al., "Identification of Clonality Through Genomic Profile Analysis in Multiple Lung Cancers," *Journal of Clinical Medicine* 9, no. 2 (2020): 573, https://doi.org/10.3390/jcm9020573.

23. C. Hu, L. Zhao, W. Liu, et al., "Genomic Profiles and Their Associations With TMB, PD-L1 Expression, and Immune Cell Infiltration Landscapes in Synchronous Multiple Primary Lung Cancers," *Journal for Immunotherapy of Cancer* 9, no. 12 (2021): e003773, https://doi.org/10.1136/jitc-2021-003773.

24. M. Izumi, J. Oyanagi, K. Sawa, et al., "Mutational Landscape of Multiple Primary Lung Cancers and Its Correlation With Non-Intrinsic Risk Factors," *Scientific Reports* 11, no. 1 (2021): 5680.

25. H. Lee, J. H. Park, J. Han, et al., "The High Proportion of Discordant EGFR Mutations Among Multiple Lung Tumors," *Cancers* 14, no. 12 (2022): 3011.

26. P. Li, R. Yang, D. Wang, et al., "Clinical and Genetic Characteristics of Early-Stage Multiple Primary and Independent Primary Lung Adenocarcinoma Patients," *Asia-Pacific Journal of Clinical Oncology* 18, no. 5 (2022): E420–E426.

27. J. Liu, G. Mao, Y. Li, et al., "Targeted Deep Sequencing Helps Distinguish Independent Primary Tumors From Intrapulmonary Metastasis for Lung Cancer Diagnosis," *Journal of Cancer Research and Clinical Oncology* 146, no. 9 (2020): 2359–2367.

28. M. Liu, W. X. He, N. Song, Y. Yang, P. Zhang, and G. N. Jiang, "Discrepancy of Epidermal Growth Factor Receptor Mutation in Lung Adenocarcinoma Presenting as Multiple Ground-Glass Opacities," *European Journal of Cardio-Thoracic Surgery* 50, no. 5 (2016): 909–913.

29. Y. Liu, J. Zhang, L. Li, et al., "Genomic Heterogeneity of Multiple Synchronous Lung Cancer," *Nature Communications* 7 (2016): 13200, https://doi.org/10.1038/ncomms13200.

30. A. Mansuet-Lupo, M. Barritault, M. Alifano, et al., "Proposal for a Combined Histomolecular Algorithm to Distinguish Multiple Primary Adenocarcinomas From Intrapulmonary Metastasis in Patients With Multiple Lung Tumors," *Journal of Thoracic Oncology* 14, no. 5 (2019): 844–856.

31. C. A. Pagan, C. A. Shu, J. P. Crapanzano, et al., "Synchronous Pulmonary Adenocarcinomas," *American Journal of Clinical Pathology* 154, no. 1 (2020): 57–69.

32. S. B. Patel, W. Kadi, A. E. Walts, et al., "Next-Generation Sequencing: A Novel Approach to Distinguish Multifocal Primary Lung Adenocarcinomas From Intrapulmonary Metastases," *Journal of Molecular Diagnostics* 19, no. 6 (2017): 870–880, https://doi.org/10.1016/j.jmoldx.2017.07.006.

33. G. Pei, M. Li, X. Min, et al., "Molecular Identification and Genetic Characterization of Early-Stage Multiple Primary Lung Cancer by Large-Panel Next-Generation Sequencing Analysis," *Frontiers in Oncology* 11 (2021): 653988, https://doi.org/10.3389/fonc.2021.653988.

34. T. Qiu, W. Li, F. Zhang, B. Wang, and J. Ying, "Major Challenges in Accurate Mutation Detection of Multifocal Lung Adenocarcinoma by Next-Generation Sequencing," *Cancer Biology & Therapy* 21, no. 2 (2020): 170–177.

35. Y. Qiu, L. Liu, H. Yang, et al., "Intratumor Heterogeneity of Driver Mutations and TMB Distribution in 30 Early-Stage LUAD Patients With Multiple Lesions," *Frontiers in Oncology* 12 (2022): 952572, https://doi.org/10.3389/fonc.2022.952572.

36. R. Qu, D. Tu, W. Ping, N. Zhang, and X. Fu, "Synchronous Multiple Lung Cancers With Lymph Node Metastasis and Different EGFR Mutations: Intrapulmonary Metastasis or Multiple Primary Lung Cancers?," *Oncotargets and Therapy* 14 (2021): 1093–1099.

37. E. F. Rodriguez, L. H. Tseng, F. De Marchi, et al., "Clonal Origin Evaluated by Trunk and Branching Drivers and Prevalence of Mutations in Multiple Lung Tumor Nodules," *Molecular Diagnosis & Therapy* 24, no. 4 (2020): 461–472.

38. P. Roepman, A. Ten Heuvel, K. C. Scheidel, et al., "Added Value of 50-Gene Panel Sequencing to Distinguish Multiple Primary Lung Cancers From Pulmonary Metastases: A Systematic Investigation," *Journal of Molecular Diagnostics* 20, no. 4 (2018): 436–445.

39. J. Saab, H. Zia, S. Mathew, M. Kluk, N. Narula, and H. Fernandes, "Utility of Genomic Analysis in Differentiating Synchronous and Metachronous Lung Adenocarcinomas From Primary Adenocarcinomas With Intrapulmonary Metastasis," *Translational Oncology* 10, no. 3 (2017): 442–449.

40. Y. Takahashi, K. Shien, S. Tomida, et al., "Comparative Mutational Evaluation of Multiple Lung Cancers by Multiplex Oncogene Mutation Analysis," *Cancer Science* 109, no. 11 (2018): 3634–3642.

41. K. Takamochi, S. Oh, J. Matsuoka, and K. Suzuki, "Clonality Status of Multifocal Lung Adenocarcinomas Based on the Mutation Patterns of EGFR and K-Ras," *Lung Cancer* 75, no. 3 (2012): 313–320.

42. J. P. L. Vincenten, H. F. van Essen, B. I. Lissenberg-Witte, et al., "Clonality Analysis of Pulmonary Tumors by Genome-Wide Copy Number Profiling," *PLoS One* 14, no. 10 (2019): e0223827.

43. X. Wang, Y. Gong, J. Yao, et al., "Establishment of Criteria for Molecular Differential Diagnosis of MPLC and IPM," *Frontiers in Oncology* 10 (2020): 614430, https://doi.org/10.3389/fonc.2020.614430.

44. F. Xiao, D. Liu, Y. Guo, et al., "Survival Rate and Prognostic Factors of Surgically Resected Clinically Synchronous Multiple Primary Non-Small Cell Lung Cancer and Further Differentiation From Intrapulmonary Metastasis," *Journal of Thoracic Disease* 9, no. 4 (2017): 990–1001.

45. L. Xu, J. Chen, Y. Zeng, X. Li, and Z. Zhang, "Differential Diagnosis of Multiple Primary Lung Cancers and Intra-Lung Metastasis of Lung Cancer by Multiple Gene Detection," *Chinese Medical Journal* 135, no. 1 (2022): 86–88.

46. S. R. Yang, J. C. Chang, C. Leduc, et al., "Invasive Mucinous Adenocarcinomas With Spatially Separate Lung Lesions: Analysis of Clonal Relationship by Comparative Molecular Profiling," *Journal of Thoracic Oncology* 16, no. 7 (2021): 1188–1199.

47. Q. Zhang, H. Jia, Z. Wang, et al., "Intertumoural Heterogeneity and Branch Evolution of Synchronous Multiple Primary Lung Adenocarcinomas by Next-Generation Sequencing Analysis," *Frontiers in Oncology* 11 (2021): 760715, https://doi.org/10.3389/fonc.2021.760715.

48. X. Zhang, X. Fan, C. Sun, et al., "A Novel NGS-Based Diagnostic Algorithm for Classifying Multifocal Lung Adenocarcinomas in pN0M0 Patients," *Journal of Pathology. Clinical Research* 9, no. 2 (2023): 108–120.

49. R. Zheng, Q. Shen, S. Mardekian, C. Solomides, Z. X. Wang, and N. R. Evans, 3rd, "Molecular Profiling of Key Driver Genes Improves Staging Accuracy in Multifocal Non-Small Cell Lung Cancer," *Journal of Thoracic and Cardiovascular Surgery* 160, no. 2 (2020): e71–e79.

50. D. Zhou, Q. X. Liu, M. Y. Li, et al., "Utility of Whole Exome Sequencing Analysis in Differentiating Intrapulmonary Metastatic Multiple Ground-Glass Nodules (GGNs) From Multiple Primary GGNs," *International Journal of Clinical Oncology* 27, no. 5 (2022): 871–881.

51. F. Xiao, Z. R. Zhang, X. W. Wang, et al., "Applying Comprehensive Histologic Assessment and Genetic Testing to Synchronous Multifocal Lung Adenocarcinomas and Further Survival Analysis," *Chinese Medical Journal* 132, no. 2 (2019): 227–231.

52. A. G. Nicholson, K. Torkko, P. Viola, et al., "Interobserver Variation Among Pathologists and Refinement of Criteria in Distinguishing Separate Primary Tumors From Intrapulmonary Metastases in Lung," *Journal of Thoracic Oncology* 13, no. 2 (2018): 205–217.

53. T. Y. Chou, S. Dacic, I. Wistuba, et al., "Differentiating Separate Primary Lung Adenocarcinomas From Intrapulmonary Metastases With Emphasis on Pathological and Molecular Considerations: Recommendations From the IASLC Pathology Committee," *Journal of Thoracic Oncology* 20, no. 3 (2025): 311–330.

54. J. C. Chang and N. Rekhtman, "Pathologic Assessment and Staging of Multiple Non-Small Cell Lung Carcinomas: A Paradigm Shift With the Emerging Role of Molecular Methods," *Modern Pathology* 37, no. 5 (2024): 100453.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.