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REVIEW

Multiple Primary Lung Cancers: A New Challenge in the Era of Precision Medicine

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Abstract: With the widespread implementation of lung cancer screening, more and more patients are being diagnosed with multiple primary lung cancers (MPLCs). In the era of precision medicine, many controversies remain in differentiating MPLCs from intrapulmonary metastasis and the optimum treatment choice, especially in patients exhibiting similar histology. In this review, we summarize common diagnostic criteria and novel discrimination methods with a special emphasis on the emerging value of broad panel next-generation sequencing (NGS) for the diagnosis of MPLCs. We then discuss current advances regarding therapeutic approaches for MPLCs. Radical surgery is the main treatment modality, while stereotactic body radiotherapy (SBRT) is safe and feasible for early-stage MPLC patients with inoperable tumors. In addition, immunotherapy and targeted therapy, particularly epidermal growth factor receptor-tyrosine kinase inhibitors, are emerging therapeutic strategies that are still in their infancy. Characteristics of both genomic profiles and tumor microenvironment are currently being evaluated but warrant further exploration to facilitate the application of targeted systematic therapies in MPLC patients.

Keywords: multiple primary lung cancers, MPLCs, diagnosis, surgery, stereotactic body radiation therapy, SBRT, targeted therapy, immunotherapy

Introduction

Worldwide, lung cancer has the highest cancer mortality among multiple types of malignances.[1] With the widespread application of computed tomography (CT) and lung cancer screening, lung cancer patients have a 20–39% reduction in the mortality rate and an improved 5-year survival rate.[1] As lifespans lengthen, up to 15% of patients with lung cancer harbor a second primary lung cancer.[2–6] Furthermore, it has been estimated that the incidence rate of a second primary lung cancer following initial treatment of non-small cell lung cancer (NSCLC) at 3 years, 5 years, 8 years are 5%, 8%, and 16%, respectively.[7] In the case of multifocal lung cancers, discriminating multiple primary lung cancers (MPLCs) from intrapulmonary metastasis (IM) remains a common dilemma in the clinical setting. The main reason for the difficulty in identification is that the histological types are identical in most patients with multifocal lung cancers, with adenocarcinoma being the most frequent (~88%).[2,8,9] Existing diagnostic criteria are mainly based on clinicopathological features,[10–12] and are far from meeting the clinical need. Nonetheless, great efforts have been made worldwide to explore novel and more accurate methods of identifying independent primary tumors from metastasis.

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Most MPLC patients are diagnosed at the early stages of the disease; thus surgical intervention represents one of the most common therapeutic approaches,[13] although it is not applicable to patients with limited pulmonary function or to those with super multiple pulmonary nodules.[14–16] For these patients, novel systematic therapies including targeted therapy and immunotherapy are warranted and should be explored. With the advancement of multiple technologies, particularly next-generation sequencing (NGS), great progress has been made in diagnosing and treating MPLCs. Herein, we performed a literature search of the PubMed and Web of Science (WoS) databases as of May 31, 2020 using the keywords ("multiple primary lung cancer" OR "MPLC" OR "multiple ground-glass opacities" OR "multiple GGOs"), and retrieved the available studies associated with diagnosis and treatment of MPLCs. In this review, we summarize existing diagnostic criteria and therapies for MPLC patients and discuss novel diagnostic methods for discrimination of MPLCs and emerging therapeutic options, with the aim of helping clinicians to better recognize and manage such patients.

Diagnostic Criteria and Stage

Diagnostic Criteria

MPLCs are divided into synchronous MPLCs (sMPLCs) and metachronous MPLCs (mMPLCs). Despite no globally recognized guidelines, three diagnostic criteria for sMPLCs and mMPLCs have been widely used in the clinical setting.[10–12]

Martini et al[10] firstly established the diagnostic criterion for MPLCs in 1975 (Table 1). MPLCs are diagnosed if they present different histological types, while there are subtle differences in the diagnosis of sMPLCs and mMPLCs if tumors share the same histological type. sMPLCs originate from carcinomas in situ, occur in different segments, and have no carcinoma in the common lymphatic drainage sites and extrapulmonary sites. mMPLCs are diagnosed if tumors originate from carcinomas in situ, or are located in different lobes rather than segments, or have a free interval between cancers ≥2 years. The Martini-Melamed criteria based on clinicopathological features are practical and have been put into routine clinical use, but a lack of accuracy exists in distinguishing MPLCs from IM when histological types are identical.

Subsequently, with the development of molecular biology and NGS technology, the Martini-Melamed criteria were revised and improved. The American College of Chest Physicians[11,17,18] added different molecular genetic characteristics as a diagnostic criterion for both sMPLCs and mMPLCs (Table 1). When histological types are

Table 1 Three Common Diagnostic Criteria for Multiple Primary Lung Cancers

Martini Melamed criteria[10]	**ACCP guidelines**[11]	**TNM staging system (8th edition)**[12]
sMPLCs: 1. Tumors physically distinct and separate 2. Histology: A. Different histological types B. Same histological type, but in different segments, lobes, if origin from carcinoma in situ And no carcinoma in lymphatics common to both And no extrapulmonary metastases	**sMPLCs**: 1. Different histology 2. Different molecular genetic characteristics 3. Arising from a separate focus of carcinoma in situ 4. Same histology, if: Tumors in different lobes And no N2, N3 involvement And no systemic metastases	**Clinical Criteria for sMPLCs**: 1. Different histological types on biopsy 2. Arguments favoring sMPLCs: Different radiographic appearance or metabolic uptake Different growth rates Different biomarker pattern Absence of lymphatic or systemic metastases
mMPLCs: 1. Different histological types 2. Same histological type, if: Free interval between cancers ≥2 years Or origin from carcinoma in situ Or second cancer in different lobes and no carcinoma in common lymphatics and extrapulmonary sites	**mMPLCs**: 1. Different histology 2. Different molecular genetic characteristics 3. Arising from a separate focus of carcinoma in situ 4. Same histology, temporarily separated, if: Free interval between cancers ≥4 years And no systemic metastases	**Pathologic criteria for sMPLCs (e.g., after resection)**: 1. Different histological types 2. Clearly different comprehensive histologic assessment 3. Squamous carcinomas arising from carcinoma in situ. 4. Arguments favoring sMPLCs: Different biomarker pattern Free of lymphatic or systemic metastases.

Abbreviations: ACCP, American College of Chest Physicians; sMPLCs, synchronous multiple primary lung cancers; mMPLCs, metachronous multiple primary lung cancers.

identical, different lobes are required for diagnosing sMPLCs in terms of location of tumors and the interval between the development of mMPLCs is extended to four years or above. It has also been suggested that multiple adenocarcinomas should be distinguished based on the proportion of histologic subtypes (eg, lepidic, papillary, micropapillary, acinar), and the consultation of a multidisciplinary team should be taken into consideration in diagnosing MPLCs. However, it is difficult to determine whether a second cancer is primary tumor or a metastatic tumor with an interval of 2 to 4 years between tumors. No specific molecular biomarkers were mentioned.

According to the American Joint Committee on Cancer (AJCC) TNM staging system (8th edition),[12] multiple lung cancers may be considered as clinical sMPLCs if they present different histology on biopsy and as pathological sMPLCs, if the histological types or comprehensive histologic assessment (CHA) are clearly different, or they are squamous carcinomas that arise from carcinoma in situ (Table 1). This was the first time that CHA was added as a diagnostic criterion, but some limitations of this classification should be mentioned. The diagnostic criteria for mMPLCs is beyond the scope of the cancer staging manual and the feature of a clearly different CHA is unclearly defined.

Stage

In 2007, multifocal lung cancers were regarded as IM in the 7th edition of the TNM classification, which categorized multiple same-lobe cancers as T3, ipsilateral different-lobe cancers as T4 and bilateral lung cancers as M1.[19] Subsequently in 2017, the 8th edition of the TNM classification for lung cancer indicated that synchronous or metachronous independent lung cancers should each be classified with a separate TNM stage and be managed individually regardless of the location of the tumors.[12,20,21]

Differential Diagnosis

In clinical practice, it is difficult to differentiate MPLCs from IM solely based on the criteria described above, especially when multifocal lung cancers are of identical histological types. Below, we summarized the role of radiological, clinicopathological and molecular characteristics in distinguishing MPLCs from IM.

Radiological Appearance

CT or positron emission tomography-computed tomography (PET-CT) could aid in the differential diagnosis in the absence of tumor tissues. Multifocal GG/L lung cancers manifest as having ground-glass opacities (GGOs) on CT scans or lepidic cancers on pathology.[12,20,22] Multifocal GG/L lung cancers include adenocarcinoma in situ, minimally invasive adenocarcinoma, and lepidic-predominant adenocarcinoma, and are considered as independent primary tumors.[23–25] A study proposed that multifocal lung cancers with at least one GGO were radiological MPLCs due to a good prognosis.[26] However, early metastasis was detected by two GGOs in the same individual based on many shared mutations, indicating the presence of spread through air spaces (STAS).[14,27] The World Health Organization (WHO) first defined STAS as spread of tumor cells adjacent to the margins of the tumor into air spaces in the surrounding lung parenchyma and suggested that the morphological features of tumor STAS included micropapillary clusters, solid nests, or single tumor cell.[28,29] STAS are connected to tumors by lung alveoli, and the median distance from STAS to the tumor margin is less than 1 cm.[30] There is evidence indicating that STAS is an unfavorable prognostic factor for patients with stage I lung cancers after sublobectomy,[29,31–33] but not for patients after lobectomy.[32] It suggests that STAS may not occur across lobes, so multifocal GG/L lung cancers cannot always be regarded as MPLCs but they may be if they are located on different lobes. Further studies are needed for verification, which will be of great help in classifying patients with multiple lung cancers manifesting as GGOs.

Additionally, PET-CT could discriminate MPLCs from IM based on the difference or ratio of standardized uptake values (SUVs) between tumors in each patient.[34,35] A clinical trial (NCT03679936) is designed to investigate the value of dynamic PET-CT to diagnose MPLCs. However, it's not enough to distinguish MPLCs from IM relying merely on CT or PET-CT. Suh et al[36] established a novel diagnostic algorithm for MPLCs by combining SUVs from PET-CT with radiological features on CT including GGOs, spicule sign, and air-bronchogram. Tumor pairs were diagnosed as MPLCs if one of the following criteria was met: any tumor presents with pure GGOs or GGO-dominant features; both tumors harbor spiculation or air-bronchogram; only one tumor harbors spiculation or air-bronchogram and tumors pairs have more than two grades of SUVs. In contrast to the

histopathological classification, radiological classification achieves a satisfying positive predictive value and provides a convenient and noninvasive way to diagnose MPLCs. But large-scale studies are required to validate its accuracy and utility as a reliable diagnostic criterion.

Recently, artificial intelligence and machine learning approaches have been increasingly applied to biomedical studies including radiology, pathology, and oncology.[37–39] Analyzing features of radiological images by machine learning algorithms could aid in the diagnosis of benign pulmonary nodules, primary, and metastatic lung cancer.[40,41] Therefore, machine learning-based image analysis may represent a novel approach for identifying independent primary tumors in patients with multiple lung lesions. In the future, integrating radiological imaging with pathological and genomic data using artificial intelligence and machine learning may promote more accurate diagnosis for MPLCs.

Histopathological Characteristics

Histopathology is the gold standard for diagnosing lung cancer, but it is difficult to identify MPLCs versus IM when multifocal lung cancers are of the same histological type, particularly adenocarcinoma. According to the 2004 WHO classification, lung adenocarcinoma was defined as morphologically heterogeneous and often had mixed subtypes (acinar, papillary, micropapillary, bronchioloalveolar, and solid).[42] Subsequently, Girard et al[43] developed a histopathological classification to distinguish MPLCs from IM, also known as comprehensive histologic assessment (CHA). Not only percentages of histologic subtypes in 10% increments but also cytologic and stromal characteristics were assessed in this classification. Tumors were considered MPLCs if tumor pairs had different histological types, or the predominant subtypes of adenocarcinomas were different (ie, acinar, papillary), or cytologic and stromal characteristics differed when tumor pairs were squamous cell carcinomas. Tumors were metastatic if they had similar percentages of histologic subtypes or cytologic and stromal characteristics. CHA was confirmed to be highly consistent with the molecular classification.

With the update of the WHO classification to the 2015 version, the subtyping of bronchoalveolar carcinoma was abandoned, and the lepidic component was introduced.[44] Invasive adenocarcinoma was semi-quantitatively assessed in 5% increments according to five subtypes: lepidic, acinar, papillary, micropapillary, and solid. Subsequently, CHA was combined with a low-grade lepidic component to distinguish MPLCs from IM.[45] The low-grade lepidic tumors, resembling type II pneumocytes or Clara cells, had nuclei with slightly irregular shape and size. Compared with CHA, multiple tumors possessing low-grade lepidic component were also considered as MPLCs in this modified CHA.[45] Several studies demonstrated that CHA combined with a low-grade lepidic component could further improve diagnostic accuracy.[45,46]

In summary, CHA alone or combined with a low-grade lepidic component appears to be a less expensive and faster way to distinguish MPLCs but it also has some limitations. First, CHA requires a suitable amount of tumor tissues, so it is not available prior to surgery. Secondly, evaluating a percentage of histological subtype or the stromal characteristics is relatively subjective due to interobserver variations. Finally, CHA may conflict with the molecular classification and currently it is unknown which classification is superior.

Molecular Characteristics

With the development of molecular genetics and NGS, overwhelming evidence has been provided supporting the independent clonal origins of MPLCs, which may contribute to the differential diagnosis of MPLCs from IM. The initial studies sequenced only a few genes such as epidermal growth-factor receptor (*EGFR*) and kirsten rat sarcoma viral oncogene (*KRAS*) to improve discrimination between MPLCs and IM.[2,47,51] However, the clonal origin of multiple tumors is inconclusive when no hotspot driver mutation is identified. Subsequently, NGS screening from 20 to 468 cancer-related genes was applied to identify MPLCs.[52–58]

Seven small studies enrolled a total of 237 patients (range, 11 to 60) and compared the diagnostic efficacy and robustness of the molecular classification with the different histological classifications (Table 2). Overall, NGS provides better discrimination than the clinicopathological classification because it is able to classify more indefinite cases, indicating the importance of NGS. As the numbers of genes sequenced by NGS increase, the ratio of inconclusive cases decreases except in one study. This study sequencing only 20 genes had no equivocal cases, in part because some indefinite cases sharing only one hotspot driver mutation were classified as IM.[55] In other studies,[53,54,56–58] large panel NGS may be more functionally valuable in defining clonal relationship of multiple tumors. Sequencing at least 100 cancer-related genes could confirm the clonal

Table 2 Integration of NGS Data for Discriminating MPLCs from Intrapulmonary Metastasis

Author [year]	MLC, n	Reference standard	Inconclusive[a], n (%)	Diagnostic methods	Genes panel	MPLCs, n	Inconclusive[b], n (%)	NGS: refer[c] (same, n)	Common gene mutations	Convergent evolution
Patel et al [2017][52]	11	AJCC	3 (27)	NGS	50	8	0 (0)[d]	8: 10 (8)	*EGFR/KRAS/TP53/BRAF*	*KRAS*: G12V+G12A/C, *TP53*: Q192X +N210fs*37
Saab et al [2017][53]	18	clinicopathologic, radiologic feature	6 (35)	Histology + NGS	50	17	1 (8)	N/A	*KRAS/EGFR/TP53/BRAF/KIT/ PI3KCA*	*KRAS*: G12V+G12D/ Q61H *EGFR*: 19del+L858R
Roepman et al [2018][54]	50	IHC+AJCC	0 (0)	NGS	50	32	2 (4)	32: 32 (23)	*KRAS/TP53/EGFR/BRAF/ STK11/CDKN2A*	*TP53, EGFR, KRAS*
Takahashi et al [2018][55]	37	MM, histopathology	0 (0) 17 (46)	NGS	20	17	0 (0)[a]	17: 34 (15) 17: 17 (9)	*EGFR/TP53/KRAS/STK11*	*EGFR*: L858R+19del /S768I *HER-2*: F547del+K1152fs
Chang et al [2019][56]	60 (76pairs)	CHA	23 (30) (pairs)	NGS	341 ~468	41 (51 pairs)	1 (1) (pairs)	51: 56 (45) (pairs)	*EGFR/KRAS/ALK/ROS1/MET*	*KRAS*: G12C/D/V+G12C/ D/A/Q61L *EGFR*: L858R+L858R/ 19del /S768I
Donfrancesco et al [2020][57]	24	Pathological features[e]	N/A	NGS	22	15	3 (13)	15: 14 (9) (subtype)	*KRAS/TP53/STK11/HER-2/ EGFR/MET/BRAF*	*KRAS*: G12V+G12C/D *KRAS*: G12D+G12A/C
Higuchi et al [2020][58]	37	histopathology, AJCC	N/A	NGS	53	29	N/A	29: 31 (27) 29: 33 (26)	*TP53/KRAS/EGFR/BRAF/ CDKN2A/STK11*	*EGFR*: L858R+exon 21ins *KRAS*: G12C+G12C

Notes: [a,b]Inconclusive numbers based on reference diagnostic criteria and NGS, respectively; [c]Numbers of MPLC patients diagnosed using NGS or other reference standards, the parentheses indicate numbers of patients diagnosed with MPLCs by both standards; [d]None was inconclusive when both tumors sharing at least one mutation were regarded as metastatic; Pathological features[e] defined as predominant subtypes, lepidic component, nucleoli size, and TTF-1 expression.

Abbreviations: NGS, next-generation sequencing; MPLCs, multiple primary lung cancers; MLC, multifocal lung cancers; IHC, immunohistochemistry; AJCC, American joint Committee on cancer; MM, Martini Melamed criterion; CHA, comprehensive histologic assessment.

relationship of 95% of lung adenocarcinomas although the most appropriate detection number of genes remains unknown. Additionally, as shown in Table 2, the molecular classification by NGS conflicts with the histological classification in many cases, and to some extent compensates for the shortcomings of a histological classification. Due to the inconsistencies of multiple reference diagnostic criteria (Table 2) and the absence of a diagnostic gold standard, additional studies are needed to evaluate the utility of NGS and an appropriate number of genes to be sequenced.

In terms of the diagnostic approach using NGS, some studies sequencing about 50 genes proposed that multiple tumors were independent if they harbored different driver mutations, and were metastatic if they shared gene mutations for even just one common driver mutation.[52,53,55,57] The former could be interpreted by "trunk and branch" theory.[59,60] Early somatic events triggering oncogenesis are the "trunk" of the tumor, and are present at all tumor sites. Late somatic events as tumors progress are the "branches", and are only present in specific tumor subclones. For the latter, it is inappropriate to diagnose IM based on one common driver mutation. *EGFR* and *KRAS* have been reported to be the most frequent driver mutations,[54–56,58] leading to the fair probability of coincidentally sharing one hotspot driver mutation in independent primary tumors. Under these circumstances, combination with histopathology or more comprehensive genomic profiles is essential. Mansuet-Lupo et al[61] already proposed an integrated histomolecular algorithm for MPLCs in which the histological algorithm was decisive when multiple tumors shared one frequent driver mutation *(EGFR* exon 19 deletions or *EGFR* p.L858R or *KRAS* p. G12X). Regrettably, there was no significant difference in the 5-year survival rate between MPLCs and IM, indicating that the histomolecular algorithm still needs to be further improved. Broad panel NGS including 341~468 genes has been shown to allow more robust discrimination of the clonality relationship among multifocal lung cancers.[56] Whole-genome sequencing (WGS) or whole-exome sequencing (WES) provides more comprehensive information for clonality assessment but is unpractical in the clinical setting due to its high cost and requirements for tumor samples.[62,63]

Given that tumor samples are difficult to obtain for gene sequencing for some patients, circulating tumor DNA (ctDNA) released by tumor cells, which is conveniently and noninvasively obtained, may be useful in the detection of gene alterations using NGS.[64] Two prospective clinical trials (NCT02833467; NCT04326751) have been designed to evaluate the feasibility and application value of identifying genetic mutations in ctDNA for MPLC patients, and the initial results are expected.

Apart from point mutations, chromosomal rearrangements may also be applied to identify MPLCs. Murphy et al[65,66] detected somatic breakpoint junctions with mate-pair sequencing technology to determine the lineage of tumors, and found that independent tumors shared few somatic junctions, while metastatic tumors shared many junctions. No indeterminate case for tumor lineage resulted with mate-pair sequencing technology. Additionally, other methods including microsatellite polymorphism, loss of heterozygosity and comparative genomic hybridization have occasionally been applied to discriminate MPLCs and IM.[45,67–69] These methods, especially mate-pair sequencing may be promising in patients who are nondefinitive after integrated with clinicopathological and genetic evaluation. However, they are difficult to conduct in a routine clinical setting due to high cost and the inaccessibility of experimental techniques.

DNA methylation, one of the most intensively studied areas of epigenetics, exerts an important role in cellular biology and transcriptional regulation.[70] Each cell type has a unique DNA methylation profile, which could serve as a tool to trace cell sources of any tumors.[71] Sano et al[72] reported a system of analyzing ten gene promoter methylation status and found that primary lung cancer and metastatic tumors shared the same methylation profile while MPLCs differed. It provides a new tool to identify the clonal relationship of multiple lung cancers. Additionally, characteristics of the tumor microenvironment identified by transcriptome sequencing provides new perspectives for identifying MPLCs.[73] At the protein level, four cancer-related proteins (p53, p16, p27, and c-erbB2) have been reported to be differentially expressed in MPLCs.[74,75] If the sum value of the differences in the expression ratios of four proteins between tumors is more than 90, MPLCs will be diagnosed. It may be convenient but there is no reliable control to evaluate the accuracy in the study. Future proteomic studies may excavate more accurate protein biomarkers for distinguishing MPLCs from IM.

Overall, with regard to accuracy and accessibility, large panel NGS may provide an unprecedented opportunity to identify MPLCs accurately in the clinical setting. Moreover, integration of radiological, histopathological, and comprehensive genomic characteristics by a multidisciplinary team may promote more accurate diagnosis for MPLCs.

Alternative Treatment Options

Surgery: Unknown Optimal Procedure

Given that MPLC patients are mostly diagnosed at an early stage, surgery is the preferred treatment for MPLC patients.[13] However, there is no consensus on the optimum surgical procedure for MPLCs. We systematically reviewed studies about surgical treatments for MPLCs.[4,8,9,76–93] As shown in Table 3, MPLC patients, especially those presenting with GGOs, could achieve a good 5-year overall survival rate (OS; ~95.8%) after surgery and there is a trend toward decreased resection extent and increased survival rate over time. Bilobectomy and pneumonectomy were more common decades ago, while lobectomy and sublobectomy including segmentectomy and wedge resection have become more popular in recent years (Table 3). Currently, controversies remain in choosing either lobectomy or sublobectomy. Shimada et al[84] recommended to resect main cancers (larger size or radiologically invasive) with lobectomy and sub-cancers (any other lung cancers) with sublobectomy. Although Zuin et al[4] revealed lobectomy was superior to sublobectomy for second primary lung cancer with better 5-year survival (57.5% vs 36%; p=0.016), others[8,94] proposed that sublobar resection for the second cancer was safe with acceptable mortality and a 5-year survival rate, especially for patients with limited pulmonary function.

Despite a meta-analysis[13] and the above studies, some difficulties exist in exploring the optimum type of surgery. As shown in Table 3, the diagnostic criteria employed in the studies are diverse and inaccurate. Next, the starting point for calculating OS is non-uniform, with some studies considering the first surgery and others the second surgery. In the future, standardized and larger studies are warranted to investigate the best operation extent under the premise of accurate diagnosis.

Stereotactic Body Radiation Therapy (SBRT): An Alternative to Surgery

For MPLC patients who are medically inoperable due to the presence of severe comorbidities and limited cardiopulmonary reserve, there are still some non-surgical treatments including predominantly SBRT[95] and photodynamic therapy available.[96] SBRT has been confirmed to be more effective and well tolerated for medically inoperable patients with stage I NSCLC compared with standard radiotherapy.[97–99] Some studies have also been conducted to explore the value of SBRT in early-stage MPLC patients.[16,100–108]

As shown in Table 4, ten retrospective studies enrolled a total of 510 (range, 10 to 170) MPLC patients treated with SBRT alone or in combination with other therapies including surgery or conventional radiotherapy. Most of patients could not tolerate an operation due to medical comorbidities, while a few refused surgeries. Median OS fluctuated between 15.5 and 46 months. The 2-year progression-free survival (PFS) and 2-year OS rate were 41.7–95.4%, 30–95.4%, respectively. The 2-year local control rate ranged from 75% to 98.2% with few grade-3 or higher toxicities (0~11%). For MPLC patients whose initial cancer was resected, metachronous SBRT for other tumors could achieve a good survival (3-year OS rate: 95.4%; 3-year PFS rate: 95.4%).[16] Moreover, two studies proposed that mMPLCs patients receiving SBRT had a better 2-year PFS and OS rate in comparison with sMPLCs patients.[101,103] In general, SBRT can achieve a long-term survival and good tumor-control, and is a safe and feasible treatment option for patients with MPLCs, particularly those with limited respiratory function.

Targeted Therapy: Is It Feasible for MPLCs?

Targeted therapy significantly improved the survival of advanced NSCLC patients with driver gene mutations.[109] Molecular alterations like *EGFR* mutation occur frequently in MPLCs, especially those manifesting as GGOs.[110–112] This allows for consideration of targeted therapy, but no large retrospective studies or clinical trials can guide the application of targeted therapy in MPLC patients. Despite well-established paradigms of targeted therapy for NSCLC patients, the clinical scenarios for MPLC patients are unique in that one lesion harboring a targetable mutation is not representative of all lesions. In fact, the discrepancy rate of driver mutations in MPLCs was reported to be as high as 92%,[110,111] which has led to different responses to targeted therapy in MPLC patients, and significant challenges to apply targeted drugs to treat MPLCs.[113–115]

With the development of NGS and genome technology, a theory of "convergent evolution" was proposed in MPLC cases.[63] Heterogeneous driver mutations among tumor foci from the same patient may converge on the same signaling pathway such as *EGFR* and mitogen-activated protein

Table 3 Surgical Treatments and Survival Rates of Patients with MPLCs

Authors [year]	Patients (n)	Diagnosis	Types of surgery					Survival			
			L+SL	L	SL(s)	BiL	P	Median (m)	3-year (%)	5-year (%)	Time to Start for Survival
Rea et al [2001][76]	sMPLCs (19) mMPLCs (61)	MM	11[a] 0	0 49[b]/29[c]	3[a] 10[b]/26[c]	3[a] 0	2[a] 2[b]/6[c]	N/A	N/A	72[b] 51[c]	First surgery Second surgery
Aziz et al [2002][77]	sMPLCs (10) mMPLCs (41)	MM	9	1	N/A	22	15	40	N/A	38	Second surgery
Chang et al [2007][9]	sMPLCs (92)	N/A	14	54	10	8	6	N/A	N/A	35	First surgery
Trousse et al [2007][78]	sMPLCs (125)	MM	15	39	21	9	41	35	61.6[d]	34	First surgery
Leyn et al [2008][79]	sMPLCs (36)	Histology	21	0	3	10	2	49.4	N/A	38	First surgery
Rostad et al [2008][80]	sMPLCs (94)	Histology	11	30	4	8	41	N/A	N/A	27.6	N/A
Finley et al [2010][81]	sMPLCs (175)	Girard	41	59	48	22	5	67.4	64	51	First surgery
Yu et al [2013][82]	sMPLCs (97)	MM, Girard	36	39	14	8	0	38.3	83.1	69.6	First surgery
Zuin et al [2013][4]	sMPLCs (23) mMPLCs (98)	MM	0	106[b]/44[c]	10[b]/60[c]	0	5[b]/ 17[c]	N/A	N/A	76[b] 42[c]	First surgery Second surgery
Ishikawa et al [2014][83]	sMPLCs (93)	MM + CHA	27	28	27	10	1	N/A	93.6	87	First surgery
Shimada et al [2015][84]	sMPLCs (67)	MM, ACCP	19	32	11	4	0	N/A	N/A	95.8(GG) 68(GS)	N/A
Dai et al [2016][85]	sMPLCs (27) mMPLCs (4)	MM	22	0	0	7	2	N/A	75.8	75.8	First surgery Second surgery
Yang et al [2016][8]	sMPLCs (71) mMPLCs (30)	MM	49	0	13	39	0	N/A	84.5	75	Second surgery
Zhang et al [2016][86]	sMPLCs (285)	MM	139	44	59	37	6	N/A	N/A	77.6	First surgery
Cheng et al [2017][87]	sMPLCs (51)	Girard	35	0	9	7	0	N/A	86	67	N/A
Peng et al [2017][88]	sMPLCs (43)	MM, ACCP	21	15	6	0	1	N/A	76.7	N/A	First surgery
Xiao et al [2017][89]	sMPLCs (52)	MM	20	5	18	4	5	52	N/A	40.6	N/A
Zhao et al [2017][90]	sMPLCs (115)	MM	0	94[b]/57[c]	21[b]/58[c]	0	0	N/A	N/A	86.5[b] 69.5[c]	First surgery Second surgery
Chen et al [2019][91]	sMPLCs (95)	MM	58	0	17	20	0	N/A	N/A	87.4	First surgery
Kang et al [2019][92]	sMPLCs (106) mMPLCs (36)	N/A	72	N/A	18	42	0	N/A	N/A	83	Second surgery
Fourdrain et al [2020][93]	mMPLCs (55)	N/A	0	9[b]/35[c]	45[b]/20[c]	1[b]/ 0[c]	0	N/A	77	N/A	N/A

Notes: Inclusion criteria: studies after 2000 of at least 20 patients with MPLCs reporting survival data; [a]Operational types of synchronous MPLCs; [b]The first operation; [c]The second operation; [d]2-year survival rate; GG indicates main cancer is ground-glass-opacity dominant and GS indicates main cancer is solid dominant.

Abbreviations: MPLCs, multiple primary lung cancers; L, lobectomy; (m) SL, sublobectomy or multiple sublobectomies; BiL, bilobectomy; P, pneumonectomy; sMPLCs, synchronous MPLCs; mMPLCs, metachronous MPLCs; MM, Martini and Melamed criterion; N/A, not available; CHA, comprehensive histologic assessment; ACCP, American College of Chest Physicians.

kinase (*MAPK*) signaling pathways. As shown in Table 2, multiple lung cancers in an individual can harbor mutations in identical or different sites of *EGFR* or *KRAS*. *KRAS* mutations occur in approximately 30% of cancers, and therapeutic strategies towards *KRAS*-mutant cancers are currently in preclinical and clinical trial stages.[116] The incidence of *EGFR* mutations in lung adenocarcinoma has been reported to range from 47~64%,[117] while mutations

Table 4 Stereotactic Body Radiation Therapy for Patients with MPLCs

Authors [year]	Patients (n)	SBRT for >1 lesion, n	SBRT for 1 lesion[a], n	Median OS, m	2-year OS, %	2-year PFS, %	2-year LCR, %	Grades of Toxicities, %	Time to Start for Survival
Sinha et al [2006][100]	sMPLCs (8) mMPLCs (3)	9	1	18.5	30	80	80	≥3 (0)	N/A
Creach et al [2012][101]	sMPLCs (15) mMPLCs (48)	s: 11 m: 2	s: 4 m: 46	20	58.5	41.7	90	≥3 (0)	First SBRT
Matthiesen et al [2012][102]	sMPLCs (8) mMPLCs (2)	10	0	15.5	N/A	N/A	95.2	≥3 (0)	N/A
Chang et al [2013][103]	sMPLCs (39) mMPLCs (62)	29	72	46	73.2	67	97.4	> 3 (1)	First therapy Second SBRT
Griffioen et al [2013][104]	sMPLCs (62)	56	6	31	56	62	84	≥3 (0)	N/A
Rahn et al [2013][105]	sMPLCs (6) mMPLCs (12)	27	9	N/A	62	N/A	81.5	≥ 2 (17)	First SBRT
Nishiyama et al [2015][106]	mMPLCs (31)	0	31	46	62*	N/A	N/A	> 3 (3)	N/A
Shintani et al [2015][107]	sMPLCs (18)	15	3	45.6	69.1*	43.2*	77.9*	≥3 (11)	First therapy
Nikita et al [2019][16]	sMPLCs (14) mMPLCs (156)	62	108	45.6	sSBRT: 46.4[b] mSBRT: 79.7[b] Surgery +SBRT: 95.4[b]	sSBRT: 57.5[b] mSBRT: 85.8[b] Surgery +SBRT: 95.4[b]	sSBRT: 75.0[b] mSBRT: 96.0[b] Surgery +SBRT: 98.2[b]	≥3 (3.5)	First therapy
Miyazaki et al [2020][108]	sMPLCs (26)	0	26	N/A	86.3	N/A	N/A	≥3 (3.8)	First SBRT

Notes: Inclusion criteria, studies enrolling MPLCs patients receiving SBRT with reporting survival data; [a]SBRT performed following other therapies including surgery or standard radiation; [b]3-year PFS/OS/LCR.

Abbreviations: MPLCs, multiple primary lung cancers; SBRT, stereotactic body radiotherapy; OS, overall survival; PFS, progression-free survival; LCR, local control rate; sMPLCs, synchronous MPLCs; mMPLCs, metachronous MPLCs; sSBRT, synchronous SBRT; mSBRT, metachronous SBRT (>3 months).

at the same or different sites of *EGFR* gene may occur in up to approximately 45% of *EGFR*-positive MPLC patients manifesting as GGOs.[110,111] Thus, a therapeutic option is available to administer *EGFR*-tyrosine kinase inhibitors for *EGFR*-positive MPLC patients with inoperable lesions. Moreover, Ye et al[113] proposed a novel strategy for MPLCs with diverse molecular profiles. The strategy the authors propose achieves a good efficacy, and involves administering gefitinib for the sensitive lesion initially, followed by resection of the gefitinib-insensitive lesion.

In general, despite the possibility of different responses caused by heterogenous molecular events, targeted therapy, particularly *EGFR*-tyrosine kinase inhibitors, presents a potential alternative therapeutic strategy for MPLC patients, especially for those patients who are medically inoperable. Further investigation is needed to identify common genetic characteristics of all lesions in an individual, which may advance the application of targeted therapy in MPLC patients.

Immune Checkpoint Inhibitors: Should They Be Explored?

Immune checkpoint inhibitors (ICIs) have been approved as first-line therapy for advanced lung cancer, and have dramatically changed its therapeutic scenario.[118] Nonetheless, it is unknown if ICIs are effective for MPLC patients, particularly those whose lesions are dispersed and inoperable. There are two active clinical trials in which ICIs are being tested as first-line therapy or neoadjuvant therapy for MPLCs manifesting as GGOs (phase II: NCT04026841; pilot study: NCT04047186). The uncertainties and emerging questions in the burgeoning field of ICIs are worthy of discussion for MPLC patients.

Firstly, programmed-death ligand 1 (PD-L1) is regarded as the most important biomarker for predicting the efficacy of ICIs in lung cancers;[119] however, little is known about the degree of PD-L1 expression between independent primary tumors in MPLC patients.[120–122] A retrospective study that enrolled 43 MPLC patients with 112 lesions, reported only

13 (30.2%) patients harbored positive PD-L1 expression and 12 of 13 had variable levels of PD-L1 expression.[121] Another study revealed inconsistent expression of PD-L1 in 11 of 23 patients with MPLCs, and none of the patients was PD-L1-positive for all independent lesions.[122] These studies reveal that the expression level of PD-L1 is not high and is heterogenous as a whole, which presents a significant challenge for treating MPLC patients with ICIs. Secondly, tumor infiltrating lymphocytes have been associated with the response to ICIs.[119,123] However, characteristics of the tumor microenvironment in patients with MPLCs are unclear. A reported case with synchronous lung adenocarcinoma and squamous cell carcinoma was confirmed to have different proportions of tumor infiltrating lymphocytes in two lesions by performing transcriptomic sequencing.[73] A clinical trial (NCT04026841) is undergoing to evaluate the characteristics of tumor microenvironment among MPLC patients by performing RNA and T cell receptor sequencing, but the results will require a substantial length of time to be obtained. Finally, genetic testing is not performed in patients with MPLCs manifesting as GGOs who are enrolled in these clinical trials receiving ICIs treatment. GGOs are often adenocarcinomas that have a relatively higher incidence of driver gene mutations such as *EGFR* mutation.[124] There is evidence available indicating that NSCLC patients harboring oncogenic driver alterations, particularly *EGFR* mutations and *ALK* fusions, have a lower objective response rate to ICIs.[125] Similarly, the efficacy of ICIs in MPLC patients may be affected by driver gene mutation status. Therefore, future studies should consider additional factors including the tumor microenvironment and driver gene mutation status before applying ICIs to treat MPLCs.

Conclusion

Despite great difficulties in discriminating MPLCs from IM, important advancements have been made over the past decades. The current technological advancements allow us to differentially diagnose MPLCs from IM based on a combination of radiological features on CT or PET-CT, histopathological analysis, and molecular characteristics (eg, gene mutations, chromosomal variations, DNA methylation). Broad panel NGS plays a key role in identifying MPLCs, and helps to establish an accurate diagnostic criterion. Importantly, it is recommended that a multidisciplinary team integrate the above resources and be involved in the diagnosis and management of MPLCs.

To date, radical surgery represents the main therapy for MPLCs, but the optimal extent of surgical intervention remains to be standardized. For medically inoperable patients with MPLCs, SBRT is an alternative that presents good efficacy and safety. Additionally, immunotherapy and targeted therapy particularly with *EGFR*-tyrosine kinase inhibitors are emerging therapeutic options. These novel systematic therapies warrant further investigation of characteristics of both genomic profiles and tumor microenvironment to facilitate their application in MPLCs.

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