Review Article on Oligometastatic NSCLC: Definition and Treatment Opportunities

Diagnostic and prognostic biomarkers in oligometastatic non-small cell lung cancer: a literature review

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Objective: This review aims to summarize the possibilities of recently discovered molecular diagnostic techniques in lung cancer, by evaluating their impact on diagnosis, monitoring, and prognosis in oligometastatic disease.

Background: Oligometastatic non-small cell lung cancer (OM-NSCLC) is currently defined based on morphological rather than biological features. Major advances in the detection of molecular biomarkers in cell-free tumoral DNA and the models of oncogene addiction make as feasible an early diagnosis and guide the therapeutic decision-making progress to improve the prognosis.

Methods: This narrative review EXAMINES current approaches of diagnosis, monitoring, and prognosis of OM-NSCLC and describes the fast-evolving therapeutic scenario of this disease. We provide an overview of the powerful capability of liquid biopsy techniques applied to blood and fluid and we focus on the technological advancement of circulant biomolecular factors in OM NSCLC pathology, starting from apparently simpler models such as oncogene addicted tumors to evaluate themselves in the light of treatment with immune-checkpoint inhibitors.

Conclusions: A better understanding of spatial and temporal evolution of oligometastatic diseases would contribute to a more accurate diagnosis and tailored treatment. Data from prospective clinical trials in the early stage of disease, coupled with knowledge of genetic characteristics of lung tumors, are warranted. These efforts would lead to improving the possibility to eradicate the residual disease in these low burden tumoral settings, thus enhancing the definitive cure perspectives.

Keywords: Circulating biomarkers; oligometastatic; lung cancer

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Introduction

Since the first observations on growth and the metastatic spreading of non-small cell lung cancer (NSCLC), it has been clear that some tumors remained confined and indolent in few organs for a long time (1). The "oligometastatic" condition—hereafter called oligometastatic NSCLC—was initially defined as an intermediate stage between locally advanced and widely disseminated disease (1). However, this definition was inaccurate and did not discriminate between primary oligometastatic disease and oligorecurrence, and different cut-off numbers of metastases or organs involved were used (1).

Recently, the European Organization for Research and Treatment of Cancer Lung Cancer Group (EORTC-LCG) published a consensus about the clinical definition of oligometastatic NSCLC (2) and described different patterns among de-novo status, repeat or induced oligometastatic disease in collaboration with the European Society of Radiotherapy (ESTRO) (3).

All definitions summarize a phenotypic rather than a genotypic condition; however, describing genetic features and microRNA signatures may be pivotal in the diagnostic process of oligometastatic disease (4). In clinical practice, genotyping to identify oligometastatic-NSCLC (OM-NSCLC) is not feasible, and the diagnosis of a clinical oligometastatic status per se is a prognostic factor.

Indeed, among clinical factors, metachronous versus synchronous metastases, N-stage, and adenocarcinoma histology may stratify the risk of progressive disease and death (5).

Lacking biomarkers of OM-NSCLC, it is impossible to differentiate an oligometastatic disease that grows slowly and remains in this clinical status from an oligometastatic disease that continues to proliferate and spreads in multiple organs. Furthermore, it is not possible to distinguish the definition of metastasis in multinodular lung disease from multiple primary lung tumors with the prognostic consequences and the therapeutic strategy of the case only from histopathological evidence (6,7).

Many efforts had been done to discriminate multiple primary lung cancers from intrapulmonary metastasis. The first attempt was based on the expression of four cancer-related proteins—p53, p16, p27, and C-erbB2 (8). Subsequently, the TRACERx program, using the whole-exon sequencing, revealed a more complex genetic scenario (9). Tissue genetic profiling and liquid biopsies resulted as effective techniques to achieve the correct diagnosis of oligometastatic disease and ameliorate the individualized therapeutic strategy.

Tissue-based biomolecular biomarkers can more accurately identify those patients who might benefit from local therapy, describing mRNA expression, microRNA expression, DNA mutations, epigenetic changes. In these procedures, poor quality of tissue and tumor heterogeneity—spatial and temporal—may be limiting (10).

Blood-based biomarkers are attractive, since they do not require invasive biopsies and may explore many tumoral components such as proteins, microRNAs, circulating tumor cells, ctDNA, and exosomes. These techniques can be repeated, if necessary, and better reflect tumor molecular heterogeneity, either temporal or spatial, than a single biopsy (11,12).

ctDNA is quantitively related to tumor burden and is a more objective measure of total body disease burden than imaging. Based on these observations, ctDNA profile can be useful to determine the minimal residual disease (MRD), defined as a small volume of tumor cells remaining after treatment in patients who do not have clinical evidence of disease, in early-stage or oligometastatic solid tumors and may allow predicting the outcomes in oligometastatic patients (13). In OM-NSCLC, ctDNA profile can be applied both to identify those patients who are eligible for local ablative treatment and to follow them after ablative treatments that completely eradicate metastatic deposits and lead to a definitive cure.

This review summarizes current approaches of diagnosis, monitoring, and prognosis of OM-NSCLC and describes the fast-evolving therapeutic scenario of this disease. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/tlcr-20-1067).

Methods

We performed an updated literature search for papers published up to October 2020 about the role of diagnostic and prognostic biomarkers for OM-NSCLC throughout different medical research databases like PubMed, Scopus, and Web of Science, as well as an evaluation of abstracts reported on principal international cancer congresses (ASCO, ESMO, IASLC) websites.

We employed for the search the following terms: "oligometastatic non-small cell lung cancer", "biomolecular markers", "predictive biomarkers", "prognostic biomarkers", "liquid biopsy", and "oligometastatic non-small cell lung cancer". We retrieved and analyzed also data of completed

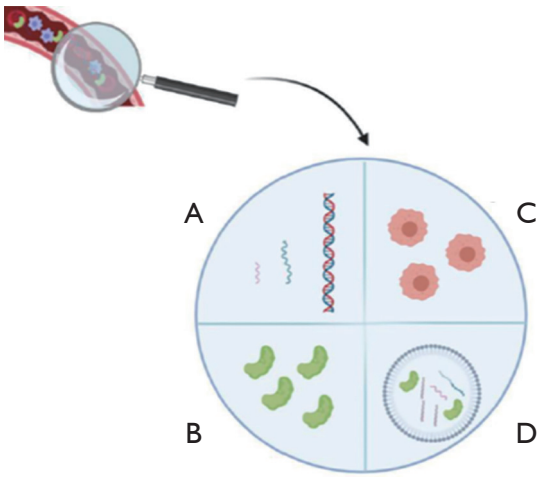

Figure 1 "Liquid biopsy" traditionally represents a peripheral blood sample withdrawal. Into the blood stream different analytes of clinical interest may be recovered: (A) circulating tumor nucleic acids (DNA and RNA), (B) Protein, (C) circulating tumor cells and (D) extracellular vesicles.

or ongoing clinical trials about this specific topic.

Given the hugeness of the data concerning the predictive and prognostic factors of response to treatments, we have focused on the technological advancement of circulant biomolecular factors in OM NSCLC pathology, starting from apparently simpler models such as oncogene addicted tumors to evaluate themselves in the light of treatment with immune-checkpoint inhibitors.

Biological techniques

Interventional pathologists play a pivotal role in the molecular characterization of OM-NSCLC, triaging the appropriate technique for the mini-invasive evaluation of the dynamic changes related to the genomic landscape of tumors. Cytological specimens may be the first ideal source of DNA for next-generation sequencing (NGS); fine needle aspirations (FNAs) are safe and cost-effective procedures that allow an optimal sampling of deep lesions via radiological guides (ultrasound/computed tomography). Traditional or liquid-based preparations are suitable for the application of exome sequencing or customized panels and, especially, May Grunwald Giemsa (MMG)-stained slides can be easily microdissected to obtain enriched samples. Supernatants may provide adequate material for the detection of driver mutations in oncogene-addicted cancers, helping to avoid the sacrifice of diagnostic stained smears (14). Especially during the natural history of long-lasting cancers, the possible contribution of core-needles as repeat biopsies is debated. Traditionally, processed formalin-fixed paraffin-embedded (FFPE) samples have been compared to paired PAXgene® tissue fixed paraffin-embedded (PFPE) tissues, which might be superior for DNA and RNA integrity, particularly in low-yield samples (15). Cytology and micro-histological approaches might work in a complementary fashion (16), however, when neoplastic sites are not accessible or clinicians have not the possibility to apply an interventional procedure with the requested advantage, the recourse to liquid biopsy is completely justified. The best way to guarantee multitasking management of precious oncological samples is the careful choice of the most appropriate way to take on the bioptic target and the medium to maximize the extraction of nucleic acids from small specimens.

The term "liquid biopsy" defines not only cell-free DNA (cfDNA), shed into the bloodstream by tumor and non-tumor cells, but also other relevant biological molecules and macrostructures, such as microRNA, circulating free RNA, extracellular vesicles, and circulating tumor cells (*Figure 1*) (17). CtDNA represents the specific fraction of tumor-derived cfDNA to assess the molecular status of epidermal growth factor receptor (*EGFR*) (18-21) at the resistance from first-line treatment with a first or second-generation EGFR tyrosine kinase inhibitors (TKIs) or the basal setting when tumor tissue is absent or not adequate for predictive analysis (18).

At the moment, the correlation between ctDNA levels and tumor burden (22) or the possibility to adopt liquid biopsy in non-advanced stage settings [such as cancer interception, early detection, and MRD (23,24)], represent research hot-topics.

A correlation among ctDNA levels and disease burden and specific metastatic sistes has been widely demonstrated (22). In patients with advanced-stage solid, the ctDNA concentration in the bloodstream is about 100 times higher concerning early-stage patients (25). In a recent prospective case-control sub-study by Liu *et al*, targeted methylation analysis of cfDNA was performed to detect and localize multiple cancer types across all stages on 2,482 cancer patients (>50 different cancer types) and 4207 non-cancer people (26). The overall specificity of 99.3% in cancer detection was reached. Interestingly, the authors highlighted that the detection rate was higher in advanced stages. As far as tumors are concerned, sensitivity was 18% in stage I, 43% in stage II, 81% in stage III, and 93% in stage IV (26).

Table 1 Tumor ctDNA shareability in NSCLC patients with intra- or extra-thoracic metastasis

First author	Methodology	Sensitivity intra-thoracic metastasis	Sensitivity extra-thoracic metastasis
Oxnard *et al*.	BEAMing PCR	75.0%	86.3%
Wu *et al*.	allele-specific quantitative real-time PCR kit	60.0%	76.9%
Tseng *et al*.	Peptide nucleic acid-zip nucleic acid polymerase chain reaction clamp	23.8%	78.0%
Kasahara *et al*.	dsPCR	50.0%	78.8%
Thress *et al*.	cobas EGFR Mutation Test and BEAMing dPCR	18.2%	77.8%
Jenkins *et al*.	cobas EGFR Mutation Test and NGS	50.6%	72.1%

BEAMing PCR, beads, emulsion, amplification, magnetic polymerase chain reaction; dsPCR; digital solid polymerase chain reaction; *EGFR*, epidermal growth factor receptor; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction.

In this scenario, another key factor is represented by the tumor dimension. An overall tumor volume of 10 cm^3 seems to be the cut-off value to reach an adequate sensitivity level when considering ctDNA analysis (27). More recently, as discussed below, the integration of different biological levels (genetics, transcriptomics, and proteomics) may be a fascinating tool to overcome the limitations of ctDNA analysis, to implement liquid biopsy even for early-stage patients and cancer interception (28).

Data from proteomic analyses indicated that proteins of extracellular vesicles and particles of 426 human samples could serve as reliable biomarkers for cancer detection (29).

Another crucial issue related to the ctDNA analysis in solid tumors is associated with the specific metastatic site. The ctDNA "shareability" into the bloodstream is strictly dependent, not only on the tumor type (*Table 1*) (30-35) but also on the metastatic site. As an example, in a really interesting pooled analysis by Passiglia *et al*, considering a set of ten studies, for a total of 1425 NSCLC patients, the sensitivity of ctDNA based *EGFR* mutation testing was significantly higher in patients with M1b *vs*. M1a disease stage (odds ratio, OR: 5.09; 95% CI: 2.93–8.84) regardless the use of digital (OR: 5.85, 95% CI: 3.56–9.60) or non-digital polymerase chain reaction technologies (OR: 2.96, 95% CI: 2.24–3.91) (36). These well-structured analyses showed that the specific metastatic site significantly affected the accuracy of ctDNA based *EGFR* mutations analysis in NSCLC patients (36).

In most cases, advanced-stage cancer patients show a central nervous system, pleural, or peritoneal metastasis. In a recent prospective study by Villatoro *et al*, 42 cerebrospinal fluid (CSF), pleural effusion, and/or ascites were used as a source of ctDNA in advanced NSCLC and melanoma patients and compared with results obtained from paired blood samples in 22 cases. The results obtained underline that fluids close to metastatic sites are superior to blood for the detection of relevant mutations (37). These important results can be useful in the oligometastatic patients, for the ctDNA molecular characterization to define the treatment strategy.

Also in this scenario, as previously discussed for tumor stage and size, the integration of different "omics" levels, may represent a key "weapon" to overcome the limitations related to the metastatic site location, not only for NSCLC patients but also for other types of solid tumors.

Multidimensional liquid biopsy analysis

The integration between the epigenetic fingerprints and the mutational profile of ctDNA is exemplified by the GRAIL Inc. program (26). Based on the evidence of Liu *et al.* (26), this innovative approach is focused on ctDNA sequence methylation assessment, through the analysis of 100,000 methylation regions (covering ~1 million CpG sites), by using a highly efficient targeted bisulfite NGS and machine learning, able to identify the abnormal methylated ctDNA region.

Another example of different omics level integration is represented by CancerSEEK (Thrive Earlier Detection Corp.) that combines the detection of ctDNA mutation profile and protein expression associated with eight types of cancers, including ovarian, liver, stomach, pancreatic, and esophageal cancers, with a median sensitivity of 70%.

Despite the exciting results, both GRAIL and CancerSEEK (and other similar approaches) are not ready for clinical implementation and larger studies are currently ongoing for the clinical validation of these novel tools.

Liquid biopsy real-time monitoring for OM-NSCLC

One of the most fascinating opportunities offered by liquid biopsies is the possibility to track clonal evolution during anticancer treatments, pinpointing the emergence of resistant clones before radiographic progression and monitoring MRD. The term MRD refers to the evidence of a small number of cancer cells that remain in the body during or after treatment and are associated with prognostic and therapeutic relevance. Liquid biopsy is particularly suitable for this scope due to its minimally invasive nature that allows multiple evaluations over time without significant risks for the patient.

Several recent studies have evaluated the use of cfDNA analysis for real-time monitoring during EGFR TKIs in EGFR-mutated NSCLCs using different methodologies, including digital droplet PCR (ddPCR) (38-41), next-generation sequencing (NGS) (37,40), and Peptide nucleic acid–zip nucleic acid clamp PCR (32). Collectively, these studies have shown a promising role for longitudinal monitoring of *EGFR* mutations in cfDNA through liquid biopsy, allowing the identification of acquired resistance mechanisms, such as the secondary *EGFR* mutation *T790M* after the 1st/2nd generation EGFR TKIs, even before radiographic progression. The use of NGS platforms offers the opportunity to simultaneously evaluate different mechanisms of resistance with high sensitivity and should be preferred over PCR-based methods (42). Whether the identification of mechanisms of acquired resistance in cfDNA before radiographic progression should lead to a therapeutic switch from a line of therapy to another (for instance, different EGFR TKIs) is a matter of debate and is currently under evaluation in the EORTC phase II study APPLE trial (NCT02856893).

In the context of OM-NSCLC and oligoprogressive disease, the use of cfDNA analysis could be of high clinical relevance (*Figure 2*), since multiple clinical studies have recently reported that cfDNA dynamics are a strong prognostic factor during anticancer therapies in advanced NSCLC, including EGFR TKIs (43-45) and immune checkpoint blockage with PD-(L)1 inhibitors (46-48). The dynamics of ctDNA have been shown to correlate with the outcome of advanced NSCLC patients treated with PD-(L)1 inhibitors and could better discriminate equivocal radiographic patterns of response, such as pseudoprogression and/or oligoprogression. In addition, ctDNA changes seem to correlate with pathologic response in early NSCLC after neoadjuvant PD-(L)1 blockage (46). Using an ultrasensitive liquid biopsy test (Cancer Personalized Profiling By Deep Sequencing, CAPP-Seq), Zhang *et al.* showed that ctDNA analysis can noninvasively identify MRD in NSCLC patients with long-term benefit to PD-(L)1 blockade (≥12 months), with 93% of patients with undetectable ctDNA levels progression-free versus none of the patients with detectable ctDNA (P<0.0001) (49). Albeit limited by small sample size (n=31), the study is hypothesis-generating and the use of liquid biopsy might allow personalized strategies for a treatment duration of immune checkpoint blockade and enable early intervention in patients at high risk for progression (49).

Data on OM-NSCLC are scant. Recently, an exploratory analysis of a randomized phase II study evaluating local consolidative therapy versus maintenance therapy or observation was reported (50). Plasma NGS was performed on 21 patients using a 1,021 cancer gene panel. At early follow-up timepoints, patients treated with local consolidative therapy were associated with a lower detected mutation burden. Furthermore, in a small subset of patients (n=6) with available serial samples for ctDNA analysis, the first increase of ctDNA mutation burden preceded radiographic progression by a median of 6.7 months (range, 2.9–17.9 months) (49). These results are in line with previous findings in early-stage NSCLC (stage I–III), where ctDNA detection after a curative intent treatment precedes radiographic progression in 72% of the patients by a median of 5.2 months (13). Furthermore, recently Chabon *et al.* showed that pretreatment ctDNA levels in stage I-IIIA NSCLC were associated with prognosis in terms of both disease-free and distant metastasis-free survival (51), suggesting that persistence of ctDNA detection after curative-intent treatments might reflect the presence of micrometastases (52). Collectively, these results indicate that ctDNA levels are associated with tumor burden and might be used as a minimally invasive monitoring tool after curative intent therapies in early-stage NSCLC and/or local consolidative therapies in OM-NSCLC, allowing personalized therapeutic strategies in positive cases.

Whether ctDNA changes during treatment of OM-NSCLC could represent a novel reliable biomarker will be further investigated in the ongoing phase II EXTEND basket trial (NCT03599765) (53), assessing the efficacy

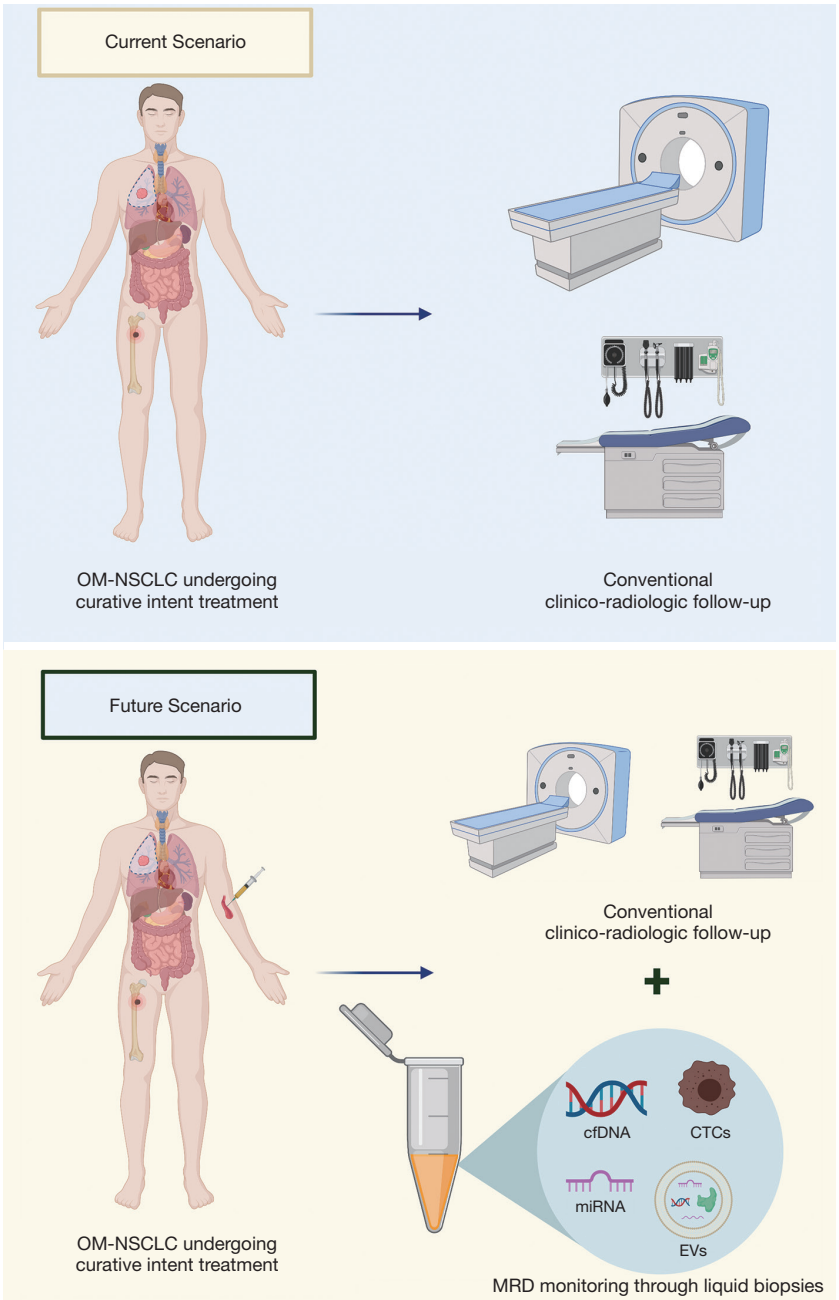

Figure 2 MRD monitoring through liquid biopsies in the context of OM-NSCLC. OM-NSCLC, oligometastatic non-small cell lung cancer; cfDNA, cell free DNA; CTCs, circulating tumor cells; miRNA, micro RNA; EVs, extracellular vesicles; MRD, minimal residual disease; Credit: Created with BioRender.

and safety of upfront local consolidative therapy in OM solid tumors, and the phase III LONESTAR trial (NCT03391869) (54), evaluating the impact of local consolidative therapy after nivolumab-ipilimumab in stage IV NSCLC.

Prognostic and predictive biomarkers for the OM-NSCLC

Two clinical factors have been associated with the OS and prognostic stratification of patients with OM-NSLC, i.e., the type of metastatic presentation (synchronous versus

metachronous) and N status (5), and several candidate biomarkers are under investigation to predict the outcome of patients with OM-NSCLC and their response to treatments.

Besides tumor-sample-based biomarkers, such as the PD-L1 expression, the high-microsatellite instability (MSI-H), the tumor mutational burden (TMB), the presence of tumor-infiltrating lymphocytes (TILs), and gene expression profiling (GEP) (55,56) liquid and other non-tumor sample-based biomarkers may offer the advantage to overcome the lack of tissue, the tumor heterogeneity, and the different adaptive mechanisms of tumors cells to treatments (57) and will be briefly described (*Table 2*).

Relevant vectors from liquid biopsies are cfDNA/ctDNA (and the associated level of blood TMB), peripheral blood mononuclear cells (PBMSCs), soluble mediators (i.e., proteins), circulating tumor cells (CTCs), exosomes, and microRNA (58).

As previously discussed in this review, in EGFR mutated NSCLCs, the presence of ctDNA and its quantity can be related to tumor volume, stage, and possible discrimination between specific metastatic sites (i.e., M1b *vs.* M1a) (22,25,35). Their sensitivity could be variable depending on tumor shedding. The presence and the levels of cfDNA/ctDNA could be explored in the OM-NSCLC for prognostic stratification and treatment monitoring.

Based on cfDNA and ctDNA, their levels, blood TMB (bTMB), and allelic variant frequencies have been explored in advanced NSCLC as biomarkers predictive of PD-1/PD-L1 tumor expression and outcomes under immune-checkpoint inhibitors (ICIs). A high bTMB has been correlated with the favorable clinical outcome under ICIs independently by PD-L1 expression on tumor tissue (59) and could be correlated to tissue TMB (60). Changes in levels of ctDNA (61) and the allelic frequency of distinct mutations measured in ctDNA (47,61,62) showed concordance with disease response to ICIs and could assist radiographic assessments (58). Therefore, these biomarkers could be helpful to better define genomic profiling between the OM-NSCLCs and towards the non-OM-NSCLC and to monitor treatment response to treatment.

Other types of circulating biomarkers

As previously discussed, the emerging concept of liquid biopsy includes also other biological relevant molecules and macrostructures, such as miRNA, cell-free RNA, extracellular vesicles and circulating tumor cells (16). The simultaneous analysis of these different analytes, in combination with data obtained from the ctDNA mutational analysis may represent a novel advice to overcome the limitations related to the low sensitivity of liquid biopsy in some settings, such as cancer interception, early stage disease analysis and MRD (16).

The role of other liquid biopsy components, such as circulating tumor cells (CTCs) and extracellular vehicles (EVs) is far less defined in the context of OM-NSCLC. The presence of ≥5 CTCs before chemotherapy was significantly associated with worse prognosis in advanced NSCLC (52). Their role in OM-NSCLC is still unclear, albeit some indirect evidence suggests a promising role in this peculiar clinical scenario. For instance, in early-stage NSCLC (stage I) treated with stereotactic body radiotherapy (SBRT), pretreatment levels of CTCs (using a cut-off of ≥5 CTCs/mL) and posttreatment persistence are significantly associated with increased risk of recurrence outside the targeted treatment site (63). Furthermore, longitudinal CTC monitoring in patients with locally advanced NSCLC treated with chemo-radiotherapy predicted the outcome, with most of the patients having undetectable CTCs on initial post-radiotherapy draw and CTCs re-emergence predicted disease progression before radiographic evidence of recurrence (64). These results suggest a potential application of CTCs in the context of OM disease and deserve further confirmation in prospective studies.

The presence and the amount of blood CTCs could represent another potential biomarker to differentiate OM-NSCLCs and compare to non-OM-NSCLCs, to predict prognosis and inform treatment strategies. For instance, fewer CTCs were detected among NSCLC patients with OM than in those with non-OM brain metastases, while the presence of CTCs and their thresholds of ≥2 and ≥5 CTCs/7.5 mL of blood were independent factors for overall survival in the OM disease (65). This could suggest more intensive treatments in patients with OM-NSCLC with brain metastases and high levels of CTCs. Furthermore, PD-L1+ CTCs were associated with poor OS and treatment failure (58).

PD-L1 mRNA and protein levels in circulating extracellular vesicles (often referred to as exosomes) may also have the potential to predict tumor response to anti-PD1/PD-L1 antibodies. Cancer cells release exosomes from the endocytic compartment into the plasma and they play a role in influencing processes involved in tumor progression, including immunoediting and drug resistance, by transporting nucleic acids, proteins and lipids to nearby

Table 2 Candidate non tumour-sample-based biomarkers for the OM-NSCLC

Biomarker	Parameter	Correlation findings	Potential applications for the OM-NSCLC
Liquid			
cfDNA/ctDNA	Levels	Tumour volume	Prognostic stratification
		Tumour stage	Treatment monitoring
		Metastatic site	
		Response to ICIs	
bTMB from cfDNA/ctDNA	High/low	Tumour PD-L1 or TMB	Profiling
		Response to ICIs	Treatment monitoring
Allelic variants from ctDNA	Frequency	Response to ICIs	Profiling
	Changes		Treatment monitoring
CTCs	Levels	Prognosis	Prognostic stratification
		Response to ablative RT	Treatment orientation and monitoring
PD-L1 mRNA and protein levels from exosomes	Levels	Response to ICIs	Prognostic stratification
	Changes		Adaptive response to ICIs
Soluble PD-L1 and PD-1	Levels	Prognosis	Profiling
		Metastatic site	Prognostic stratification
		Response to ICIs	Treatment monitoring
		Response to TKIs	
PBMCs: PD-1/PD-L1+ T cell subtypes and CTCs, NK	Frequency	Prognosis	Prognostic stratification
	Levels	Response to ICIs	Adaptive response to ICIs
NLR ± LDH	Levels	Prognosis	Profiling
		Response to ICIs	Prognostic stratification
Non-liquid			
Radiomics	Features	Histological and molecular features	Profiling
		Prognosis	Prognostic stratification
		Response to ICIs	Response to ICIs
MTV and TLG from FDG-PET scan	Levels	Prognosis following ablative RT	Prognostic stratification
			Response to ablative RT
Gut microbiota	Species	Response to ICIs	Profiling
		irAEs	Response to ICIs
			Toxicity

OM-NSCLC, Oligometastatic non-small cell lung cancer; bTMB, blood tumour mutational burden; cfDNA, circulating cell-free DNA; CTC, circulating tumours cells; ctDNA, circulating tumour DNA; FDG-PET, fluorodeoxyglucose (FDG)-positron emission tomography (PET); ICI, immune checkpoint inhibitors; irAEs, immune-related adverse events; LDH, lactate dehydrogenase; MTV, metabolic tumor volume; NK, natural killers; NLR, neutrophil-to-lymphocyte ratio; PBMCs, peripheral blood mononuclear cells; PD(L)-1, programmed cell death (ligand)-1; RT, radiotherapy; TKI, tyrosine kinase inhibitor; TLG, total lesion glycolysis.

or distant cells (66). PD-L1 protein levels in circulating exosomes and changes in plasma levels of PD-L1 positive exosomes, by the analysis of PD-L1 mRNA, were related with treatment outcome to PD-1/PD-L1 inhibitors in melanoma and NSCLC patients (67,68). The presence of PD-L1 on tumor-derived exosomes may allow them to target PD-1+ CD8 T-cells as a mechanism of the adaptive response of the tumor cells to T cell reinvigoration, thus allowing them to evade immune response at the effector stage (67).

The soluble form of PD-L1 (sPD-L1) levels have not been related to the tumor PD-L1 (69-71). However, high baseline sPD-L1 levels were associated with poor prognosis and abdominal metastases (69,72), whilst low baseline sPD-L1 levels were linked to treatment efficacy (68) and increasing levels to treatment failure (70). High sPD-1 levels predicted a favorable outcome to the TKIs (73).

PBMCs include T-cells (≈70%), B-cells (≈15%), monocytes (≈5%) and natural killer cells (≈10%) whose proportion may considerably differ by showing the activity of the immune system and play an important role as immunological biomarkers (74). Furthermore, PD-1 expression can be measured on PBMCs and is elevated among CD4+ T-cells from advanced NSCLC patients compared with healthy donors, while no correlation between PD-L1 expression on tumor cells and PD-1 expression on CD4+ and CD8+ T cells was found (75). High PD-L1 CD4+ T cells were associated with unfavorable outcome in OS and PFS and failure to ICIs (75), whilst an early (within 4 weeks) increase in PD-1+ CD8+ T cells predicted better response to anti-PD1 agents (76). A high baseline number of NK-cells and their increase during ICIs was associated with treatment response (77).

The neutrophil-to-lymphocyte ratio (NLR) is a surrogate for tumor-associated inflammation and likely represents the frequency and activity of myeloid-derived suppressor cells, that hinder T-cell proliferation and expansion (78). A high NLR and its combination with high lactate dehydrogenase or tumor PD-L1 expression level were related with worse outcomes to ICIs, but not to chemotherapy (79-81).

PD-L1 protein levels in exosomes, sPD-L1/PD-1 levels, PD-1/PD-L1 expression on T cells and CTCs and NLR could, therefore, be prognostic and useful to explore possible differences in the adaptive response to PD-1/PD-L1 inhibitors in the OM-NSCLC.

However, although promising, the use in clinical practice of these soluble biomarkers is currently limited by data heterogeneity, lack of direct comparison of assays, vectors, and validations (58). No liquid biomarker has been currently tested in randomized phase III trials, although the first trial is ongoing (82).

Recently, deep learning approaches applied to the quantitative analysis of radiological images (namely the radiomics approach) has been explored as a diagnostic and risk-layering tool to characterize specific tumors features (such as the PD-L1 expression or the TMB) and predict patients' prognosis and response to treatments by offering interpretable artificial intelligence (AI) models able to merge a high amount of information from different vectors (83). Of course, the utility of radiomics in the OM-NSCLC could vary from assisting the histological and molecular diagnosis of the tumor to profiling better this disease subgroup and predict prognosis and response to treatments.

Another imaging biomarker that could have a role for the prediction of outcome following ablative radiotherapy of the OM-NSCLC could be the metabolic burden of the disease as defined by the metabolic tumor volume and total lesion glycolysis of all lesions by the fluorodeoxyglucose positron emission tomography as both resulted as independent prognostic factors for the OS (84).

Finally, the study of gut microbiota, that could be obtained by stool cultures or molecular techniques (i.e., sequencing, metagenomics), has associated various species to the disease response and immune-related adverse events (irAEs) from ICIs and could be useful to profile the OM-NSCLC and predict disease response and toxicity from the ICIs.

Diagnostic and prognostic biomarker in oligometastatic NSCLC: are we ready from prime time?

Liquid biopsy and ctDNA profiling are usually employed in clinical practice to detect the EGFR T790M mutation and the subsequent sensitivity to Osimertinib (85). These techniques are extremely sensitive and specific and are currently recommended for T790M testing when tissue biopsy is not feasible (86). In some oligometastatic diseases, the difficulties to obtain a fresh biopsy make the liquid biopsy the unique way to employ a treatment driven by an actionable target, and different sources of ctDNA, such as liquor, have been explored. Even in these cases, Osimertinib was active in presence of EGFR T790M (87).

Dynamic changes of EGFR mutations in plasma or cerebrospinal fluid during the EGFR tyrosine kinase inhibitor treatment are directly linked to clinical activity and monitoring these fluctuations may be useful to decide

the strategy in the management of intra and extracranial disease (88).

The open-label, single-arm, prospective APOLLO study enrolled patients with EGFR T790M positive NSCLC with brain metastatic spread, treated with Osimertinib (89). In twelve patients with paired cerebrospinal fluid and plasma sample, a low concordance between cerebrospinal fluid/plasma EGFR T790M status positivity (8.3%) was detected, underlying the differences of the microenvironment of intra and extracranial sites. However, a higher concordance was seen in EGFR mutation-sensitive del19 and L858R between plasma and cerebrospinal fluid (100% and 75%, respectively).

The clearance of T790M in cerebrospinal fluid after 6 weeks of treatment displays a higher intracranial response rate and a trend in better median progression-free survival.

This trial is the last example of a series of trials (90) that demonstrated that early clearance of EGFR mutation in plasma may correlate with positive activity and efficacy outcomes. The presence of EGFR T790M variant allelic frequency (VAF) rather than its clearance is predictive of Osimertinib activity (85).

The cut-off points of plasma VAF at the beginning of therapy and the convenient time to detect VAF clearance during treatment (i.e., 3–6 weeks) are still a matter of debate and the detection of resistance mutation is not enough to change accordingly the therapeutic strategy.

Even if some experiences demonstrated that the occurrence of plasma mutation resistance could anticipate the clinical progression of a median of 3 months, no prospective trials with complete accrual indicated if it is opportune to change the therapeutic behavior (91).

The ongoing APPLE trial is a unique example of a prospective clinical trial in which the therapeutic strategy is adapted after the detection of T790M mutation at fixed timepoints in ctDNA in EGFR mutant TKI treatment-naïve patients. The trial compared continuing gefitinib until disease progression according to RECIST or switching to Osimertinib when plasma T790M arose and clarified the predictive power of liquid biopsy in this setting (82).

In other oncogene-addicted lung cancers, such as ALK-driven tumors, ctDNA by calculating maximum allelic frequency correlates with tumor burden and predicts the response to ALK inhibitors. In these experiences, maximum plasma allelic frequency and ALK alteration VAF are independent surrogates of ctDNA and high disease burden.

ALK fusion/ mutations have been found in more than 2/3 of plasma analyzed patients; therefore, a personalized therapeutic strategy that chooses active ALK inhibitors according to ALK-driven resistance mutation detected would become reasonable. However, also for ALK fusion or mutated NSCLCs, no prospective trials are still published about therapeutic change driven by dynamically testing of plasma ALK VAF.

Like ALK-harboring tumors, liver, adrenal glands, and bone are metastatic sites that correlate with a higher burden of ctDNA shedding (92); for instance, the plasma assay is more sensitive when the central nervous system is the prevalent site of metastasis (93,94).

In oncogene-addicted OM-NSCLC, ctDNA profiling is an attractive tool in both diagnostic settings when the tissue biopsy is hard to collect and monitoring of therapeutic activity. However, no conclusion about the prognosis could be done without a link to an elevated ctDNA burden at the diagnosis (*Figure 3*) (95).

The need for coupling prognostic information and predictive activity of therapeutic strategy is more evident in the field of non-oncogene addicted oligometastatic NSCLC, eventually eligible for immunotherapy. Recently, a comprehensive analysis of ctDNA VAF from solid tumors with a large representation of lung tumors treated with durvalumab combined or not with tremelimumab demonstrated that a higher basal VAF was associated with poor prognosis while the reduction of ctDNA during treatment had a predictive meaning (49). Baseline mean or maximum VAF was associated with median OS and not with the overall response rate (ORR), tumor burden or other prognostic information, as showed in multivariate analysis (95). Therefore, ctDNA can be considered as a prognostic factor rather than a predictor of response to immune-checkpoint inhibitors.

The ctDNA dynamics correlated to the clinical benefit of immunotherapy, as the reduction of ctDNA mean VAF measured at 6 weeks was associated with ORR and OS (62).

Data that correlate mean VAF and prognosis associated with immunotherapy have been already reported, but the cut-offs of mean ctDNA VAF to distinguish poor from good prognosis have not been established yet (96).

The detection of stable or increased levels in circulating DNA VAF is an interesting phenomenon to evaluate the activity of disease when instrumental imaging scans restage a stable disease, as per RECIST criteria. Again, the lack of results from well-conducted prospective trials does not allow to draw a firm indication about the impact on therapeutic strategy.

Thus, the liquid biopsy approach may represent a

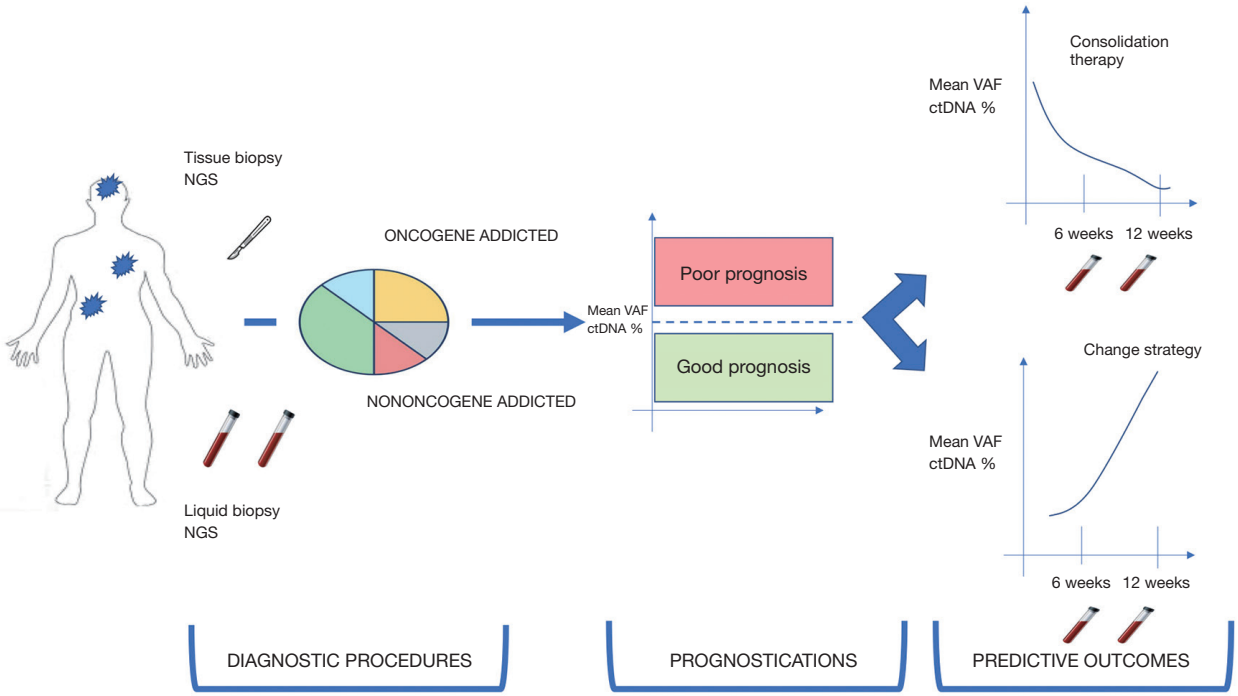

Figure 3 Flow chart proposal of diagnostic, predictive and prognostic tools to manage OM-NSCLC in the future. NGS, next generation sequencing; VAF, variant allelic frequency; ctDNA, cell tumoral DNA.

valuable option in OM-NSCLC to overcome issues related to invasive tissue sampling procedures. In addition, the selection of a "liquid biopsy" more close to the metastatic site (e.g. CSF in brain metastasis) may overcome the limitations of a low shedding of tumor-related analytes into the bloodstream, leading to false-negative molecular results.

Conclusions

Clinical data available to date are not focused on OM-NSCLC setting and the scenario that is more likely to be employed arises from oncogene-addicted tumors and NSCLC during a deep response to the drug.

In the setting of NSCLC, liquid biopsy developed in early-stage disease or when a MRD is expected during active treatment is feasible; however, since a low ctDNA shedding is expected due to a low tumor burden, ultra-sensitive assays are necessary. In addition, the liquid biopsy approach can overcome the limitation of tissue-based approaches related to the spatial and temporal molecular heterogeneity that may significantly limit the adoption of the best treatment option in OM-NSCLC patients.

In OM-NSCLC, liquid biopsy can be a diagnostic tool, seeking oncogene mutation typically expressed in lung cancers; this technique may overcome the difficulties due to small tissue biopsy in low metastatic spreading disease or localized in difficult to reach the site (i.e., brain localization).

From a prognostic point of view, the mean ctDNA VAF seems to be the only biological factor that emerges from literature, even if it has not been specifically studied in the OM-NSCLC setting.

The main point is that the oligometastatic disease is currently described using clinical and morphological definitions rather than its biological features; data from prospective clinical trials in an early stage of disease, coupled with knowledge of genetic characteristic of lung tumors are warranted since they may further clarify diagnostic and prognostic features of this sub-group of disease (97). These efforts would lead to improving the possibility to eradicate the residual disease in these low burden tumoral settings, enhancing the definitive cure perspectives.

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Footnote

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