Review Article on Oligometastatic NSCLC: Definition and Treatment Opportunities

A narrative review on tumor microenvironment in oligometastatic and oligoprogressive non-small cell lung cancer: a lot remains to be done

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Objective: In this review, we aim to collect and discuss available data about the role and composition of tumor microenvironment (TME) in oligometastatic (OMD) and oligoprogressive (OPD) non-small cell lung cancer (NSCLC). Furthermore, we aim to summarize the ongoing clinical trials evaluating as exploratory objective the TME composition, through tissue and/or blood samples, in order to clarify whether TME and its components could explain, at least partially, the oligometastatic/oligoprogressive process and could unravel the existence of predictive and/or prognostic factors for local ablative therapy (LAT).

Background: OMD/OPD NSCLC represent a heterogeneous group of diseases. Several data have shown that TME plays an important role in tumor progression and therefore in treatment response. The crucial role of several types of cells and molecules such as immune cells, cytokines, integrins, protease and adhesion molecules, tumor-associated macrophages (TAMs) and mesenchymal stem cells (MSCs) has been widely established. Due to the peculiar activation of specific pathways and expression of adhesion molecules, metastatic cells seem to show a tropism for specific anatomic sites (the so-called "seed and soil" hypothesis). Based on this theory, metastases appear as a biologically driven process rather than a random release of cancer cells. Although the role and the function of TME at the time of progression in patients with NSCLC treated with tyrosine-kinase inhibitors and immune checkpoint inhibitors (ICIs) have been investigated, limited data about the role and the biological meaning of TME are available in the specific OMD/OPD setting.

Methods: Through a comprehensive PubMed and ClinicalTrials.gov search, we identified available and ongoing studies exploring the role of TME in oligometastatic/oligoprogressive NSCLC.

Conclusions: Deepening the knowledge on TME composition and function in OMD/OPD may provide innovative implications in terms of both prognosis and prediction of outcome in particular from local treatments, paving the way for future investigations of personalized approaches in both advanced and early

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disease settings.

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Clinical approach to oligometastatic and oligoprogressive NSCLC

The definition of oligometastatic disease (OMD) in non-small cell lung cancer (NSCLC) is still rather heterogeneous (1): indeed, most of the studies limit such definition to the presence of no more than five metastatic sites, involving maximum two organs, while other involve patients with three or less metastatic sites. Nevertheless, it is important to notice that the majority of patients enrolled in dedicated clinical trials have a single metastasis (2,3). From the analysis of a case-based survey involving 10 real-life patient cases discussed in a multidisciplinary context by a panel of NSCLC experts, a conservative OMD definition emerged, which include number of organs involved, mediastinal nodes status and prospects for radical treatment, as crucial components for daily practice decision-making (4). A consensus statement about the definition and staging of OMD NSCLC was then formulated by a pan-European multidisciplinary group: a maximum of five individual metastases (excluding the primary site), involving a maximum of three organs, was proposed; fluorodeoxyglucose F18-labeled positron emission tomography and computed tomography (FDG PET-CT) and brain assessment were recommended as mandatory, while biopsy and/or additional dedicated imaging were proposed in selected cases, usually after multidisciplinary team discussion (5). Recently, a consensus involving experts from the European Organisation for Research and the Treatment of Cancer (EORTC) and European Society for Radiotherapy and Oncology (ESTRO) has developed an oligometastatic disease classification system which should be applied to all cancer patients treated with local ablative therapy (LAT). Furthermore, the authors proposed a dynamic oligometastatic state model, underlying the dynamic transitions between oligorecurrent, oligoprogressive, and oligopersistent disease, depending on the response to local and systemic therapy (6).

The timing of metastases appearance seems to have a crucial impact in terms of prognosis. Patients affected by metachronous OMD without nodal involvement are considered at "low-risk" and experience a better overall survival (OS; 5-year OS 47.8%), while synchronous metastases with one or more positive lymph nodes (N1/N2) define the patient at "high-risk" of developing further metastases with a worse prognosis (5-year OS 13.8%), as defined by an individual patient data metanalysis (7). Nevertheless, both groups appear to benefit from local therapies.

Another subgroup of NSCLC that may have a prognostic benefit from an ablative treatment (usually surgery or radiotherapy) for one or more metastatic sites includes patients who develop disease progression in one or a limited number of metastatic sites, after achieving disease stability or objective response to systemic treatment. Such entity is widely recognized as oligoprogressive disease (OPD) and its biological behavior is likely to be related to tumor heterogeneity, which, under the selective pressure of the applied systemic treatment, promotes the development of one or more clones harboring intrinsic resistance mechanisms, and/or to the crosstalk between cancer cells and the surrounding tumor microenvironment (TME), whose behavior can also be modulated in a pro- or anti-tumor fashion by the ongoing treatment (8).

Although physicians should be aware that patients affected by OMD may benefit from a more aggressive approach (local therapies) than the "classical" stage IV NSCLC patients, at this time the only recognized tool to define oligometastatic disease is through imaging as a surrogate and still controversial biomarker for low metastatic potential. Initial studies aimed at assessing the potential role of ablative therapies on outcome of OMD NSCLC, have focused on patients with isolated brain or adrenal metastasis, showing better OS with local treatment (9). Most of these studies combined systemic treatment and local consolidative therapy, stereotactic body radiation therapy (SBRT) in particular, demonstrating a significant improvement in both

progression free survival (PFS) and OS, as compared with systemic treatment/observation alone (10,11). Local ablative treatment of residual or synchronous oligometastatic sites in patients affected by NSCLC harboring epidermal growth factor receptor (EGFR) mutations treated with tyrosine kinase inhibitors (TKIs) appears to be safe and feasible with promising survival outcome (12,13). Similar encouraging results were observed in oncogene-addicted lung cancer experiencing OPD during TKI treatment (14,15). Studies encompassing surgical resection of oligometastatic sites, which may give deeper insights into the biology and underlying mechanisms of OMD/OPD NSCLC, are currently ongoing.

Most of the available evidence exploring the potential role of TME interactions in NSCLC was obtained regardless of the specific pattern and timing of disease progression; indeed, to the best of our knowledge, this review represents the first attempt at systematically analyze available data about the role of NSCLC/TME interactions in the specific setting of OMD/OPD.

We present the following article in accordance with the Narrative Review reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-1134).

Materials and methods

A comprehensive PubMed and ClinicalTrials.gov search was performed on July/August 2020, to identify the published and ongoing studies exploring the role of TME in oligometastatic/oligoprogressive NSCLC. The following keywords were used: tumor microenvironment, TME, NSCLC, oligoprogressive, oligometastatic, oncogene addicted, immunotherapy, LAT. In order to acquire a complete and in-depth perspective on this emerging topic, all original articles and reviews investigating the role of TME in (oligo)progression and (oligo)metastasis were considered. Abstracts not published in extenso, case reports and non-English full texts were excluded. All inclusion criteria were evaluated in title, abstract, and full text of original papers, by two independent reviewers.

Role and composition of TME in NSCLC genesis and progression

In recent years TME has emerged as a crucial determinant of cancer onset, biological behavior, and progression (16).

NSCLC microenvironment is characterized by: (I) rich angiogenesis, providing an efficient pro-tumoral oxygen supply, and (II) florid immune environment composed by cytokines and immune cells and usually related to the chronic exposure of lung tissue to inhalant toxic agents, mainly cigarette smoking (17). Immune response occurring in the respiratory tract includes prompt phagocytosis of inhalant pathogens and particles, recognized by alveolar macrophages, the most represented leukocytes in lower airways, through pattern recognition receptors (PRRs) (18,19). In addition, epithelial cells of the respiratory tract contribute to immune response not only by exerting a mechanical function, but also modulating secondary cytokines and chemokines (IL-1β, IL-6, -7, -8, TNF-α), reactive oxygen species, adhesion molecules, and other lung-specific factors such as surfactant proteins and β-defensins, in response to specific cytokines, such as interferon-γ (INF-γ) and IL1-β (20). Of note, lung tissue exposed to cigarette smoking is usually enriched in myeloid-derived suppressor cells (MDSC)-like cells, lacking the immunosuppressive functions that they acquire after tumor development (21). Finally, CD8+ and CD4+ T cells (with a predominance of the T-helper 1 subtype expressing INF-γ) and dendritic cells, whose recruitment and maturation is stimulated by granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by CD4+ T-cells, are also present in lung tissue (22,23).

TME in lung cancer progression and metastatization

The role of TME in cancer progression is widely established. Metastatic spread represents a multi-step process, based on a series of intricate interactions between cancer cells and various TME components. The metastatic potential of tumor cells depends on both the chemotactic factors secreted by the target organ and the intrinsic predisposition of neoplastic cells to home, proliferate and survive in that specific site (17,24).

The first step of the metastatic process consists in the growth of the primary mass, invading local tissues (*Figure 1*). Degradation of the basement membrane, due to the coordinated action of integrins, proteases and adhesion molecules (L1CAM, L1 cell adhesion molecule, and CRMP1, collapsin response mediator protein 1) represents a crucial event at this stage (25,26). At the same time, cancer cells gain a migratory phenotype through shape modification (becoming more spindle-shaped), loss of polarity, and adhesion molecules' switch from E-cadherin to N-cadherin and vimentin (27). This process is universally recognized as "epithelial-to-mesenchymal transition"

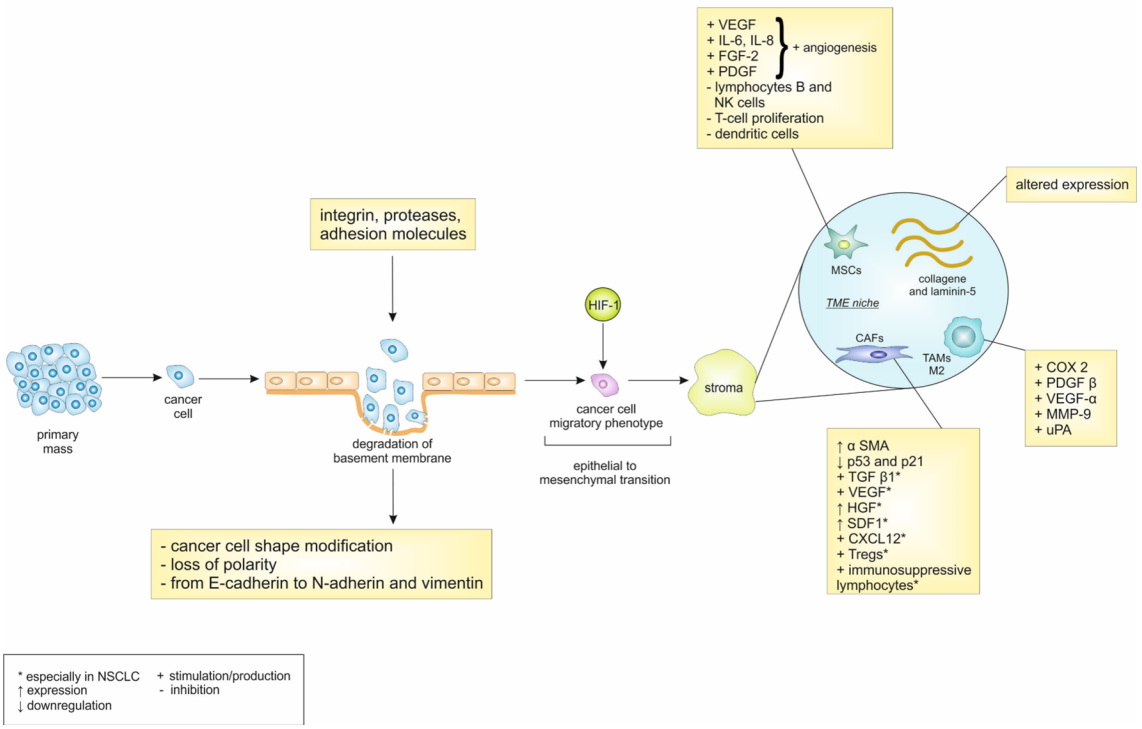

Figure 1 Local invasion of the stroma and composition of tumor microenvironment. HIF-1, hypoxia-inducible factor 1; TME, tumor microenvironment; MSCs, mesenchymal stem cells; VEGF, vascular endothelial growth factor; IL-6 and IL-8, interleukin-6 and interleukin-8; FGF-2, fibroblast growth factor; PDGF, platelet-derived growth factor; TAMs M2, tumor-associated macrophages M2; COX-2, cyclooxygenase-2; PDGF-β, platelet-derived growth factor beta; VEGF-α, vascular endothelial growth factor alpha; MMP-9, matrix metalloproteinase-9; uPA, urokinase-type plasminogen activator; αSMA, alpha smooth muscle actin; TGF β-1, transforming growth factor beta-1; HGF, hepatocyte growth factors; SDF1, stromal cell-derived factor 1; CXCL12, C-X-C motif chemokine 12; Tregs, regulatory T cells.

(EMT) and is strongly induced, among other stimuli, by the hypoxic microenvironment created by the rapid growth of the primary tumor mass not balanced by efficient angiogenesis; hypoxia leads to the accumulation of hypoxia-inducible factor 1 (HIF-1), which in turns promotes the transcription of EMT genes (28).

Once they have invaded the stroma, tumor cells interact with extracellular matrix (ECM) and stromal cells, thereby creating a microenvironment that is similar to the one of the wound healing (17,29), with cancer associated fibroblasts (CAFs) as the predominant cell type able to promote tumor growth, modulate drug response and mediate protease-mediated ECM degradation (30). CAFs express high levels of alpha smooth muscle actin (αSMA) and downregulate p53 and p21 (stress-response genes) (31,32). CAFs and tumor cells produce transforming growth factor beta-1 (TGF-β1), which has an ambivalent biological role: in early stages of tumor growth it exerts growth inhibitory functions, while in advanced stages it promotes tumor growth and progression (33,34), through increased expression of anti-apoptotic factors (as Bcl-2) and modulation of the expression of adhesion molecules and integrins (35). In lung adenocarcinoma, CAFs have been confirmed to produce TGF-β, vascular endothelial growth factor (VEGF), and other pro-metastatic factors like hepatocyte growth factors (HGF) and stromal cell-derived factor 1 (SDF1), also known as C-X-C motif chemokine 12 (CXCL12) (36,37), as well as to induce regulatory T cells (Tregs) and immunosuppressive lymphocytes (38).

Another cell population playing a crucial role in NSCLC invasion and metastasis is represented by the mesenchymal stem cells (MSCs) (39), which sustain an immunosuppressive status by reducing dendritic cells maturation (40), T-cell proliferation, B-lymphocytes

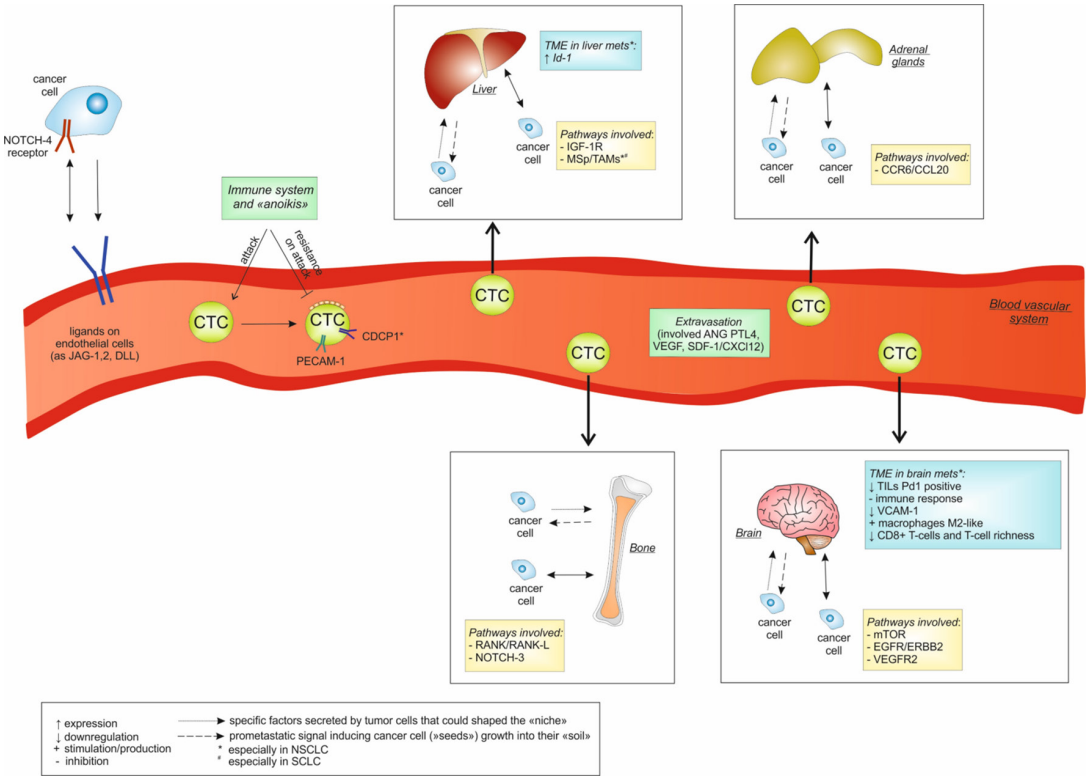

Figure 2 Intravasation/extravasation of cancer cells and "seed and soil" theory. JAG-1,2, Jagged ligands 1,2; DLL, delta-like ligand; CTC, circulating tumor cell; RANK/RANK-L, receptor activator of nuclear factor kappa-B/receptor activator of nuclear factor kappa-B ligand; PECAM-1, the platelet and endothelial cell adhesion molecule-1; CDCP1, CUB domain containing protein 1; TME, tumor microenvironment; mets, metastases; ANGPTL4, angiopoietin-like factor 4; VEGF, vascular endothelial growth factor; SDF1, stromal cell-derived factor 1; CXCL12, C-X-C motif chemokine 12; IGF-1R, insulin-like growth factor receptor 1; MSp/TAMS, macrophage-stimulating protein/tumor-associated macrophages; mTOR, mammalian target of rapamycin; EGFR/ERBB2, epidermal growth factor receptor/receptor tyrosine-protein kinase erbB-2; VEGFR-2, vascular endothelial growth factor receptor 2; TILs PD-1 positive, PD-1-positive tumor infiltrating lymphocytes; VCAM-1, vascular cell adhesion molecule-1; CCR6/CCL20, chemokines.

and NK cells activation (41). Moreover, MSCs stimulate angiogenesis, through the local production of factors such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF-2 and -6), IL-6, IL-8, VEGF, and angiopoietin-1 (17).

Finally, NSCLC stroma is particularly enriched in tumor-associated macrophages (TAMs), which can be divided into two main subtypes, M1 and M2, characterized by anti- and pro-tumorigenic properties, respectively. NSCLC cells recruit TAMs by secreting IL-17 and promote a prostaglandin E2 (PGE2)-mediated M2 switch (42). Pro-tumorigenic M2 TAMs enhance tumor invasiveness by secreting cyclooxygenase-2 (COX2), PDGF-b, VEGF-a, HGF, cathepsin k, matrix metalloproteinase-9 (MMP-9) and urokinase-type plasminogen activator (uPA). The interaction between MMP-9 and uPA has a specific role in NSCLC invasion (43).

With regard to the interaction with ECM proteins, the expression of collagens and laminine-5 is altered in NSCLC, with the latter correlating with an upregulation in EGFR-AKT signaling (17). Lysyl-oxidase (LOX) is upregulated in response to hypoxia, enhancing tumor invasiveness by modulating focal adhesion kinase (FAK) signaling and creating cross-links among collagen fibers (44). Moreover, tumor cells directly modulate ECM structure and function, by increasing the production of specific MMPs (1, 2 and 9), whose polymorphisms have been associated with NSCLC risk and survival, suggesting the existence of a complex and bidirectional interaction between cancer cells and ECM (45,46).

The second step of the metastatic process consists in the

"intravasation" of cancer cells into the blood and lymphatic vascular systems to reach distant target organs (*Figure 2*). The specific mechanisms involved at this stage are not yet completely clarified, but two models have been proposed: a passive dissemination or an active mechanism following a cytokine gradient (17). Among the ligand-receptor pairs potentially involved, there are SDF1-CXCL12 and CXCR4-CXCR7. The binding of CXCL12 to CXCR7, instead of CXCR4, reduces the expression of MMP12 and increases VEGFα, stimulating the angiogenic pathway (47,48). Furthermore, NSCLC cells express NOTCH1-4 receptors, which binding to their ligands JAG-1 and -2, DLL (delta-like) -1, -3 and -4 on stromal endothelial cells can induce intravasation and trans-endothelial migration (49).

Once passed through the blood vessel walls, circulating tumor cells (CTCs) are exposed to multiple threats, including immune recognition and immune-mediated elimination. To avoid immune attack, After entering the bloodstream CTCs are rapidly covered by an activated platelets layer (50), which hides antigen presenting domains with L- and P-selectins and tissue factor (51,52). Another mechanism involved in CTCs removal is a phenomenon called "anoikis", an apoptotic response to the loss of interaction between ECM and integrins (53). Metastatic NSCLC express markers of anoikis-resistance, such as the platelet and endothelial cell adhesion molecule-1 (PECAM-1) and CUB domain containing protein 1 (CDCP1) (54). Of note, metastatic cancer cells exist in the bloodstream also as disseminated tumor cells (DTC) and circulating tumor microemboli (CTM), whose biological significance and function are not yet well characterized (17).

Once at their destination, CTCs need to extravasate through the well-developed and almost impermeable host vessel walls (17). Cancer cells extravasation is an active process, comparable to the diapedesis of leucocytes, and involves several players, such as angiopoietin-like factor 4 (ANGPTL4) (55), VEGF and SDF-1/CXCL12 (56), as well as adhesion molecules (57). The destination of CTCs is not only influenced by "mechanical" factors, such as the direction of the blood flow: validated models propose that the microenvironment of target tissues produce pro-metastatic signals inducing cancer cells (the "seeds") to survive and growth into their new "soil" (58). One underlying mechanism assumes the existence of a pre-metastatic niche, produced in response to specific factors primarily secreted by the tumor (59). Such niche has a fibrotic nature and contains a high number of activated fibroblasts and VEGFR-positive immature myeloid cells, able to create and maintain a suitable environment for adhesion and invasion. Recent evidence suggest that such "niche" could be shaped by both the primary tumor and MSCs through the induction of an immunosuppressive microenvironment, favored, for example, by the expression of programmed death ligand-1 (PD-L1), which binds to PD-1 on macrophages, T and B cells thus inhibiting their activation, and the recruitment of suppressive cells as TAMs, tumor associated neutrophils (TANs) and Tregs (60).

In terms of target organs, it is likely that metastatic cells show a selective tropism, dependent on the activation of specific pathways and the expression of adhesion molecules. In NSCLC, the most frequent sites of metastatization are represented by liver (33–40%), brain (15–43%), bone (19–33%) and adrenal glands (18–38%) (61).

Brain metastases seem to follow a CXCL12 gradient and show the activation of CXCR4/CXCL12-mediated anti-apoptotic pathways (62,63). Signaling pathways strongly involved in brain metastases include mTOR, EGFR/ERBB2, and VEGFR2 (64). Moreover, available studies support the existence of a more immunosuppressive microenvironment in brain metastases, as compared to the primary tumor, potentially related to several factors: (I) limited quantity of PD-1-positive tumor infiltrating lymphocytes (TILs) (65); (II) inhibition of pathways involved in leukocyte extravasation, dendritic cell maturation, and Th1 immune responses; (III) reduced expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1); (IV) presence of pro-tumorigenic M2-like macrophages; (V) reduced CD8+ T cell and TILs infiltration and (VI) limited T-cell richness and density (66).

Bone metastases from NSCLC are typically osteolytic, induced by the interaction between osteoblasts and osteoclasts with the crucial mediation of receptor activator of nuclear factor kappa-B ligand (RANKL), which promotes bone degradation and thus the release of ECM factors, further attracting metastatic cells. A preclinical study suggested that increased expression of RANK, RANKL and osteoprotegerin (OPG) is correlated with a higher metastatic potential to the bone; these proteins are frequently detected in bone metastases from NSCLC. In this light, a direct production of RANKL from metastatic sites may further facilitate the migration of RANK-positive cancer cells (67). NF-κb, a crucial mediator of the RANKL pathway, can also be activated by EGFR and KRAS signaling, frequently boosted in NSCLC, through the phosphorylation of IκB, an inhibitory protein that segregates NF-κb into the cytosol. Therefore, NF-κb can dimerize, translocate into the nucleus and act as a transcription factor for several genes involved

in the inflammation process, innate and adaptive immune response, and carcinogenesis (68). In addition, an increased expression of Notch-3 contributes to TGF-β-driven EMT and to development of bone metastasis in NSCLC (69).

Increased expression of insulin-like growth factor receptor 1 (IGF-1R) represents one of the main potential "triggers" of metastatic spread to the liver. IGF-1R signaling is involved in DNA synthesis, anchorage-independent cell growth, apoptosis, cell migration and tissue invasion (70), as well as in the production of altered collagen IV, III and XVIII (71). Integrin alpha2 binding to collagen IV has been associated to anoikis resistance and hepatic metastatization (72). The chemotactic factor macrophage-stimulating protein (MSP) promotes liver metastases when overexpressed in small-cell lung cancer (SCLC) and induces liver recruitment of TAMs producing pro-angiogenic growth factors (73). Finally, NSCLC-related liver metastases usually express high levels of inhibitor of differentiation (Id-1), whose suppression inhibits TGFβ expression and EMT (74).

Biological mechanisms underlying adrenal gland metastatization are still unclear. Ligand-receptor complex CCR6/CCL20 is likely to be involved, as CCR6 has been found overexpressed in adrenal metastases from lung cancer (75).

Role of TME at progression during TKIs or immunotherapy

TME and progression to anti-EGFR TKIs

Given the clinical implications of progressive disease in EGFR-mutant NSCLC, TME status at the time of progression to anti-EGFR TKIs has been investigated. Tumor cells produce molecules able to promote cell growth regardless of EGFR activation, such as IL-6, growth-arrest specific protein-6 (GAS6), HGF and EGF, in an autocrine manner (76). Alterations in cell-to-cell adhesion are also involved, with increased levels of N-cadherin and integrin β1 and suppression of Serpin B2, thus resulting in a more invasive behavior due to ECM degradation (77,78). CAFs and MSCs express several molecules (as CXCR4, IL6-R, HGF, AXL, TGFβ-R) involved in TKIs resistance through EMT promotion, enhanced cell survival by MAPK and JAK-STAT signaling, and apoptosis inhibition through increased Bcl-2 activity (76). The interaction between HGF and mesenchymal-epithelial transition factor (MET) plays a crucial role in mediating EGFR-TKIs resistance. Available data reported that the CAFs of TKI-resistant tumors express higher levels of HGF and that the addition of an anti-HGF antibody may restore response to TKI *in vitro* (79). In clinical practice, MET targeting represents a crucial strategy in trying to overcome MET-driven resistance to EGFR-TKIs (80).

EMT is another crucial mechanism of resistance, mediated by the autocrine and paracrine action of TGF-β (81), leading cancer cells to progressively lose the epithelial markers, while increasing expression of the mesenchymal ones (as vimentin) (82). As discussed above, a hypoxic environment promotes HIF-1α expression by cancer cells, inducing TKI resistance through activation of the TGFα-IGFR1 pathway and enhanced angiogenesis mediated by VEGF secretion (83,84).

Immune cells involved in TKIs resistance are mainly TAMs, producing VEGF and promoting activation of MAPK, NF-κB, TGF-β and RAS pathways (85). A retrospective study including 70 patients treated with anti-EGFR TKIs reported relevant prognostic differences according to TME composition with a worse outcome for the combined presence of high PD-L1 tumor expression and high levels of CD8+ TILs [median PFS 2.4 months, response rate (RR) 14.3%] (86).

TME and progression to anti-ALK TKIs

Regarding resistance to anaplastic lymphoma kinase (ALK) inhibitors, available data suggest that the activation of EGFR and MET pathways can mediate cell growth, bypassing the oncogenic ALK signaling (87). Hypoxia as well may induce EMT and resistance to ALK inhibitors in ALK-rearranged NSCLC (88).

TME and progression to immunotherapy

Immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1, both in monotherapy and in combination with chemotherapy, have demonstrated to remarkably improve overall survival in locally advanced and metastatic lung cancer (89). Nevertheless, response to ICIs is hardly predictable, with PD-L1 expression being the only validated predictive biomarker so far. In order to clarify the individual response to ICIs, tumors have been stratified according to the presence of immune cells in their TME. This has led to a first splitting into "hot" and "cold" tumors. "Hot" or inflamed tumors are characterized by strong T-cell infiltration (particularly composed of CD8+ lymphocytes), high genomic instability (resulting in a high amount of neoantigens), and a pre-existing immune response with high levels of chemokines (such as CCL5, CXCL9, CXCL10)

recruiting T-cells and a strong expression of IFN-γ to support their anti-tumor function. On the other hand, "cold" tumors show poor T-CD8+ infiltration, with high amounts of macrophages and fibroblasts and a low tumor mutational burden (90,91). Moreover, TGF-β prevents response to anti PD-L1 and leukemia inhibitory factor (LIF) cytokine inhibits the production of T-cell attracting chemokines (90). A third phenotype has been proposed, defined as "altered", further divided into "excluded" tumors, where immune cells are confined at the edge of the tumor with no infiltration signs, and "immune-suppressed" tumors, with a low degree of immune cells reflecting an immunosuppressive microenvironment (91). The overexpression of tryptophan metabolites, particularly kynurenine, produced by the indoleamine 2,3-dioxygenases (IDO-1), has been associated with an immune-suppressive TME (92). A preliminary study conducted on 26 patients treated with ICIs reported an enhanced activity of this enzyme in the subgroup experiencing early disease progression, while a lower kynurenine/tryptophan ratio correlated with better outcome (92,93).

A rich TME infiltration by TILs (CD8+ and CD4+) has been associated with a favorable prognosis in different tumors, including NSCLC (94). Currently, a growing body of data suggests that specific phenotypes of immune cells and the diversity of immune repertoire might correlate with ICIs efficacy (95). In detail, the TCF7+/TCF7– CD8+ ratio is predictive of response and survival in melanoma patients treated with ICIs (96). In another study, differential expression of CD39 between two populations of tumor-infiltrating CD8+ was associated to better recognition of tumor-associated antigens (97). The emerging role of TILs in lung cancer microenvironment, alongside with a high clonal mutation burden due to carcinogen exposure, are leading to the development of adoptive T-cell therapy approaches, using autologous TILs enriched with T cells specifically recognizing clonal neoantigenic epitopes by selective expansion; such approach may offer a further chance to patients who become resistant to anti PD1/PD-L1 therapy (98).

Peripheral blood immune cell populations, reflecting tumor immune microenvironment, are currently under investigation as putative predictive and prognostic factors, potentially suitable for non-invasive immune monitoring. MDSCs are an immature population of myeloid cells with a strong immunosuppressive activity, inhibiting in particular T- and NK-cell functions (99). In this light, high numbers of circulating or infiltrating MDSCs may portend worse prognosis and reduced response to ICIs in NSCLC and other cancer types. The interaction between TME and ICIs is reciprocal, however, as MDSCs levels and function are, in turn, influenced by ICIs. Bertelli *et al.* reported a case of strong reduction in MDSC1 (a CD14+ and CD124+ subset of MDSC) number and suppressive activity in a NSCLC patient treated with durvalumab after chemo-radiotherapy, clinically and radiologically corresponding to long-lasting disease remission (100). Probably the most deeply investigated blood biomarker is the neutrophil/lymphocyte ratio (NLR), or the dNLR [neutrophils-(leucocytes-neutrophils)], whose value at baseline appears to correlate with response to ICIs. A recent metanalysis has confirmed that NSCLC patients treated with ICIs have poorer OS and PFS when their baseline NLR or dNLR is high (101). Together with baseline LDH, dNLR constitutes the Lung Immune Prognostic Index (LIPI): a poor LIPI score (dNLR ≥3 and LDH > ULN) is associated with poor PFS, OS and disease control rate, as compared to an intermediate (dNLR >3 OR LDH > ULN) or good (dNLR <3 and LDH < ULN) score, in a retrospective analysis of 466 patients treated with ICIs; conversely, LIPI stratification was not predictive of response to chemotherapy (102).

The role of gut microbioma, recently defined as a key component of TME (103), in ICIs response is yet to be clarified. The presence and health of gut microbioma are of primary importance in the development of both a local (gut lymphoid tissue, lamina propria CD4+ cells, IgA production) and systemic immunity, as many bacterial-derived molecules [as lipopolysaccharide (LPS), peptidoglycan, flagellin] enhance the activity and correct maturation of innate immune cells such as dendritic cells, macrophage and neutrophils. In mice, adaptive immunity has been demonstrated to depend on gut bacteria as well, as germ-free mice poorly developed B follicles and T-cell zones within spleen and lymph nodes (104). An analysis performed on 89 melanoma patients treated with anti-PD1 showed a more efficient antigen presentation and effector T function in the subgroup with a higher heterogeneity in gut microbioma composition, while patients with a lower diversity had limited intratumoral lymphoid and myeloid infiltration and weakened antigen presentation capacity, conditioning a worse response to ICIs (95). Microbioma heterogeneity seems to be affected by antibiotic therapy. Derosa *et al.* retrospectively reported a significant decrease in PFS and OS in NSCLC patients who received antibiotics, particularly within the first 30 days from ICIs start (105). Microbioma composition was evaluated in a study involving NSCLC and renal cell cancer receiving

anti-PD-1 treatment. A prevalence of *Akkermansia Muciniphila* was associated with a better clinical outcome, as it was detectable in 69% of patients who responded to ICIs, along with a well-represented number of commensals such as *Ruminococcus spp.*, *Alistipes spp.*, and *Eubacterium spp.*; conversely, *A. Muciniphila* could only be detected in 34% of patients who progressed or died. When mice with antibiotic gut sterilization received fecal microbiota transplant with stool of responder patients or an oral gavage with *A. Muciniphila*, response to anti-PD1 was restored, demonstrating a crucial role of gut microbioma composition on the outcome to anti-PD1 therapy (106).

TME in oligometastatic and oligoprogressive cancers, including NSCLC

Despite the fact that local microenvironmental interactions are arguably crucial in determining metastatic development and therapy resistance in specific locations, limited data are available about the role of TME in oligometastatic and oligoprogressive solid tumours. The first attempt at characterizing factors potentially able to influence the rate of success or failure of oligo- and widespread metastases was reported by Reyes and Pienta (24,107): the "quality" of the primary microenvironment is dependent from multiple factors, including pH, oxygenation, amount of nutrients, interaction with supporting host cells, and quality/quantity of immune response. Epithelial cancer cells growing into a highly vascularized environment, enriched in nutrients, are less likely to evolve towards a more aggressive behaviour through EMT, becoming more motile and leaving a relatively hostile original site; as the quality of the primary TME decreases, the generation of lethal, mesenchymal-like clones increases (24,107).

Few studies explored the biology of oligometastases and oligoprogression in oncogene-addicted NSCLC, particularly in NSCLC cases harboring *EGFR* mutations (108). Metastatization patterns in *EGFR*-mutant NSCLC are often characterized by progression in a limited number of anatomic sites, such as the brain. These patterns of progression/metastatization are likely to represent a manifestation of intratumor and intertumor genomic heterogeneity (109-112). In 2008, Taniguchi *et al.* described the intratumor heterogeneity of *EGFR* mutations, reporting the presence of both *EGFR*-mutant and *EGFR*-wild type clones in 6 out of 21 patients treated with gefitinib (109). Moreover, discordant *EGFR/BRAF/KRAS* mutational patterns were detected between primary tumors and lymph node metastases (110,111). Chen *et al.* described, in paired samples of primary lung adenocarcinoma and regional/distant metastases, an overall discordance rate in *EGFR* mutation status of 13.9%. Furthermore, the authors showed that a significant proportion of patients (29%) who developed resistance to *EGFR* TKIs, had a significant heterogeneity between the primary tumor and metastatic sites or between individual metastatic sites (112). Whether and which local microenvironmental interactions may influence metastatic fitness and/or therapy resistance of specific tumour clones (e.g., *EGFR*-mutant versus *EGFR*-wild type) in different metastatic locations, however, remains to be established.

Preliminary data trying to unravel the biological bases of OMD/OPD in lung cancer are enriched by pivotal studies available in other solid tumors. An analysis performed on 335 matched metastases from 575 renal cell carcinoma samples suggested rapid progression and reduced OS in metastatic specimens harboring *9p* loss, low intratumor heterogeneity, and a higher proportion of genomic somatic copy-number alterations. In this study, the few patients experiencing "attenuated progression" (defined as the development of a single metastatic site within 6 months from surgery or multisite progression more than 6 months after surgery) were affected by tumors showing high intratumoral heterogeneity, low rate of somatic copy number alterations, *PBRM1* and *SETD2* driver mutations (113). Another study performed in patients undergoing partial hepatectomy for colorectal cancer metastases identified three main molecular subtypes ("classic", "immune" and "stromal") according to their peculiar molecular profile. Among them, the "stromal" cohort, associated with prevalent KRAS, EMT, and angiogenesis signaling pathways, was associated with a higher metastatic potential with an increased risk of developing extrahepatic metastatic disease. Interestingly, the "immune" molecular subtype, mainly characterized by high immune infiltration, interferon alpha and gamma signatures, activation of p53 pathways, overexpression of innate and adaptive immune genes, identified a relatively indolent subgroup which developed clinically evident metastases limited in number (oligometastatic state) (114).

An interesting finding is that microRNAs may have a role in mediating the metastatic potential. Khodarev *et al.* profiled microRNAs derived from cancer patients with oligo- or poly- lung metastases from different primary sites treated with SBRT or surgery, in order to examine their potential role in driving the oligometastatic phenotype. These microRNAs were defined as "oligomiRs". Among

them, one common locus at 14q32 associated with carcinogenesis and stem cell functioning, encoded for a significant number of oligomiRs (115).

Notwithstanding the limited amount of available data about TME in oligometastatic disease, borrowing the available evidence from the metastatic setting may provide additional insights for clinical/translational studies in this setting. In particular, in the context of clinical trials, when a bioptic procedure is feasible, comparing the study of TME (i.e., exploring the composition in terms of immune cells such as fibroblasts, TAMs, immature myeloid cells and Tregs, and the expression of VEGF, PD-L1 and adhesion molecules) between primary tumor and oligometastatic site and between different oligometastatic sites may offer a chance to further deepen the knowledge about the mechanisms supporting the OMD process.

Ongoing studies including LAT for oligometastatic and oligoprogressive NSCLC

The real impact of TME in oligometastatic and oligoprogressive lung cancer still represents an untapped field, presumably because patients with only one or a few metastatic sites (at diagnosis or at progression) are often not considered candidate to surgery and routine re-biopsy does not represent the standard of care. As highlighted above, an increasing amount of data supports a potential positive impact of LAT, radiotherapy or surgery, in addition to systemic therapy, for OMD or OPD lung cancer patients. In the majority of ongoing trials, the collection of tissue/cytological samples at baseline is mandatory, but only a few studies also collect surgical specimens deriving from the removal of OMD/OPD sites: in particular, only one study (NCT03827577) requires the surgical removal of both the primary tumor and/or all oligometastases. Studies encompassing the collection of tumor (and/or blood) material offer a concrete chance to deepen the current knowledge about TME in OMD/OPD lung cancer, possibly revealing a dynamic landscape of cell infiltration and genetic alterations able to drive the oligometastatic/oligoprogressive process (116) (*Table 1*).

Conclusions

To date, the real impact and biological meaning of TME in OMD and OPD lung cancer is still far to be clarified.

Table 1 Ongoing clinical trials including TME evaluation in OMD/OPD lung cancer

Phase	ClinicalTrials.gov identifier	Setting	N	Treatment	Type of tumor sample	Primary endpoints	Secondary endpoints	Status
IIb	NCT04216121	OPD EGFR-mutant NSCLC	39	LAT (SBRT or surgery) + first line osimertinib	Surgical specimen[1]	PFS2	Time to next line systemic therapy; patterns of progression to LAT; QoL	Active, recruiting
II	NCT02759835	OPD EGFR-mutant NSCLC	37	LAT (surgery, RT, radiofrequency ablation) before or after the start of osimertinib(any line)	Surgical specimen[1] Liquid biopsy	PFS, PFS2	RR, OS	Active, not recruiting
II	NCT03808662	OPD triple negative breast cancer or NSCLC	160	SBRT to all the OPD sites *vs.* SOC	Tissue biopsy[2]	PFS	OS	Active, recruiting
II	NCT04255836	OMD NSCLC	35	Durvalumab + first line (carboplatin + paclitaxel or cisplatin + pemetrexed) + SBRT	Tissue biopsy[2]	PFS	ORR, OS, Safety	Active, not recruiting
II/III	NCT02759783	Extracranial OMD prostate cancer, breast cancer or NSCLC	245	SOC +/− SBRT	Tissue biopsy[2]	PFS	OS, local lesion control, QoL, FFWMD	Active, not recruiting

Table 1 (*continued*)

Table 1 (*continued*)

Phase	ClinicalTrials.gov identifier	Setting	N	Treatment	Type of tumor sample	Primary endpoints	Secondary endpoints	Status
II	NCT02316002	OMD NSCLC	51	Pembrolizumab after definitive therapy	Tissue biopsy or surgical specimen[2]	PFS		Active, not recruiting
III	NCT03391869	Stage IV NSCLC	270	Nivolumab + ipilimumab +/– LCT	Tissue biopsy or cytological sample[2]	OS; OS in OMD subgroup	PFS, TANM	Active, recruiting
III	NCT02076477	OMD NSCLC	420	CT (cisplatin + docetaxel or pemetrexed) + RT	Tissue biopsy or cytological sample[2]	RR	PFS	Active, recruiting
II	NCT03965468	OMD NSCLC	47	Durvalumab + carboplatin-paclitaxel + SBRT on all the metastatic sites + surgery or radical RT on the primary	Surgical specimen[1]	PFS	OS, OR, DoR, distant PFS	Active, recruiting
II	NCT03410043	Stage IIIB or IV EGFR-mutated NSCLC	143	Osimertinib +/– LAT (surgery and/or RT)	Surgical specimen[1]	PFS	OS; PFS in OMD subgroup; TANM; time to progression of target lesions	Active, recruiting
III	NCT03827577	OMD NSCLC	195	SOC +/– LAT of all metastatic sites and resection of the primary	Surgical specimen[3]	OS		Active, recruiting

[1], surgical specimen available if LAT is performed with surgery of the oligometastatic sites; [2], baseline tissue biopsy, cytological sample or surgical specimen (either on primary tumor or on metastasis); [3], surgical specimen of primary and/or of all oligometastases. N, number; OPD, oligoprogressive disease; NSCLC, non-small cell lung cancer; LAT, local ablative therapy; SBRT, stereotactic body radiotherapy; PFS, progression free survival; PFS2, progression free survival (at second progression); QoL, quality of life; RT, radiotherapy; RR, response rate; OS, overall survival; SOC, standard of care; OMD, oligometastatic disease; ORR, overall response rate; FFWMD, freedom from widespread metastatic disease; LCT, Local Consolidation Therapy; TANM, Time to Appearance of New Metastases; CT, chemotherapy; OR, overall response; DoR, duration of response.

Ongoing trials exploring the efficacy of LAT in this setting, in particular those involving surgery as the main therapeutic approach to treat oligometastases, may help to provide crucial information. Deepening the knowledge on TME composition and function in OMD/OPD may provide innovative implications in terms of both prognosis and prediction of outcome from local treatments. Moreover, new candidate player(s) able to drive and/or support oligometastatization or oligo progression may emerge, paving the way for future investigations of targeted therapeutic approaches in both advanced and early disease settings.

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