Clinical Impact of the Epithelial-Mesenchymal Transition in Lung Cancer as a Biomarker Assisting in Therapeutic Decisions

Julien Ancel, Maxime Dewolf, Gaëtan Deslée, Béatrice Nawrocky-Raby, Véronique Dalstein, Christine Gilles, Myriam Polette

To cite this version:

Julien Ancel, Maxime Dewolf, Gaëtan Deslée, Béatrice Nawrocky-Raby, Véronique Dalstein, et al.. Clinical Impact of the Epithelial-Mesenchymal Transition in Lung Cancer as a Biomarker Assisting in Therapeutic Decisions. Cells Tissues Organs, 2022, 211, pp.91-109. 10.1159/000510103. hal-03051871

HAL Id: hal-03051871
https://hal.science/hal-03051871

Submitted on 15 Feb 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Clinical impact of EMT in lung cancer as a biomarker for assisting therapeutic decisions

Julien Ancel\textsuperscript{1,2}, Maxime Dewolf\textsuperscript{2}, Gaëtan Deslée\textsuperscript{1,2}, Béatrice Nawrocki-Raby\textsuperscript{1}, Véronique Dalstein\textsuperscript{1,3}, Christine Gilles\textsuperscript{4,*,δ} and Myriam Polette\textsuperscript{1,3,δ}.

\textsuperscript{1} Inserm, Université de Reims Champagne Ardenne, P3Cell UMR-S1250, SFR CAP-SANTE, 51092 Reims, France
\textsuperscript{2} Service de pneumologie, Hôpital Maison Blanche, CHU de Reims, 51092 Reims, France
\textsuperscript{3} Laboratoire de pathologie, Hôpital Maison Blanche, CHU de Reims, 51092 Reims, France
\textsuperscript{4} Laboratory of Tumor and Development Biology, GIGA-Cancer, University of Liège, 4000 Liège, Belgium

Short Title: EMT in lung cancer management

δ Equal contribution

*Corresponding Author

Christine Gilles, Laboratory of Tumor and Development Biology, GIGA-Cancer, University of Liège, 4000, Belgium.

E-mail: cgilles@uliege.be
Keywords: non-small-cell lung cancer; epithelial–mesenchymal transition; therapy; resistance.
Abstract

Lung cancer is one of the most common solid cancers and represents the leading cause of cancer death worldwide. Over the last decade, examining epithelial-mesenchymal transition (EMT) in lung cancer has gained major interest. Here, we review clinical and histological features of non-small cell lung cancer (NSCLC) associated with EMT. We then aim at establishing EMT potential clinical implications in current therapeutic options including surgery, radiation, targeted therapy against oncogenic drivers and immunotherapy.
Introduction

Lung cancer is responsible for the highest number of cancer-related deaths worldwide. About 85% of lung cancers are classified as non-small cell lung cancers (NSCLC), subsequently histologically subdivided as adenocarcinoma (AC) (about half of NSCLC cases) and squamous cell carcinoma (SCC) (about 30% of NSCLC cases). Regarding staging, more than a half of cases are diagnosed at a metastatic or advanced stage without curative options. Over the past years, innovative therapies such as molecularly targeted drugs and immunotherapies have emerged and have led to overall improvements in cancer treatment. However, global 5-year survival rate for NSCLC patients remains under 20% (Bray et al., 2018; Hirsch et al., 2017). Even in localized stages with a surgically resectable tumor, 5-year survival rates drastically drop from 60% for stage IIA disease to 36% for stage IIIA disease, according to the 8th edition staging project of the International Association for the Study of Lung Cancer (IASLC) (Goldstraw et al., 2016). Promising and innovative strategies are ongoing, especially in neo-adjuvant (Uprety et al., 2020) and adjuvant (Broderick, 2020; NCT02595944; NCT02486718; NCT02504372) contexts, that could improve the dramatic landscape of NSCLCs (Kris et al., 2020; Otaibi et al., 2019). Nevertheless, such a bad record also highlights the need to further refine characterization of tumor specimens in order to improve clinical patient management.

Examining epithelial-mesenchymal transition (EMT) in lung cancer has gained major interest in the last decade. EMT is a term widely used now to describe the loss of epithelial features and the acquisition of mesenchymal traits by epithelial cells in various physiological and pathological conditions. In cancer, EMT is considered as a dynamic and reversible process, thereby generating various phenotypes. Some hybrids may have enhanced plasticity allowing them to adapt to various tumor micro-environments encountered during cancer progression and metastatic dissemination (Bhatia et al., 2020; Dongre and Weinberg, 2019; Yang et al.,...
2020). As such, EMT has been shown to endow tumor cells with many properties that may provide them a selective advantage to overcome different steps of cancer progression including enhanced invasive potential, enhanced survival, stimulation of angiogenesis, immune escape, resistance to apoptotic signals and niching properties (Francart et al., 2018; Pastushenko and Blanpain, 2019). EMT is molecularly complex, diversified and context-dependent. This complexity certainly contributes to slow down the implementation of EMT consideration in clinical routine. EMT programs may indeed be triggered by several factors such as transforming growth factor-β (TGF-β) (Miyazono et al., 2018), fibroblast growth factor (FGF) (Katoh and Nakagama, 2014), epidermal growth factor (EGF) (Shaurova et al., 2020), Notch or Wnt signaling pathways (Patel et al., 2019; Yuan et al., 2014). Activated signaling pathways converge onto a set of transcription factors (EMT-TFs) such as Snail, ZEB and Twist family (Zeisberg and Neilson, 2009). A dynamic balance finally represses expression of epithelial related-genes such as E-cadherin and induces expression of genes coding for mesenchymal markers such as vimentin. Importantly, EMT rather supports early steps of the metastatic dissemination, i.e. tumor invasion, survival in the blood stream and early niching. After an eventual period of dormancy, a reversal towards more epithelial phenotypes, so called mesenchymal-epithelial transition (MET), is considered to occur at secondary sites to support metastatic outgrowth. Although EMT features have been described for many years in numerous histological types of cancer, discussion remains regarding EMT characterization in tumors. Pan-cancer studies are based on most consensual and proposed canonical markers such as E-cadherin (CDH1), vimentin (VIM), N-cadherin (CDH2) and fibronectin (FN1). Using NSCLC cell lines and tumor samples, some studies established EMT-signatures. From these NSCLC reports, some markers such as DSP, TJP1, CLDN4, ERBB3, GALNT3 and CDS1 appear commonly associated with CDH1 epithelial related-genes while MMP-2, AXL, ZEB1/2, NRP-1 and TWIST clustered with VIM and FN1 mesenchymal
related-genes (Antony and Huang, 2017; Goossens et al., 2017; Karacosta et al., 2019; Shao et al., 2019; Wong et al., 2018; Wushou et al., 2014; Zheng and Kang, 2014).

We discuss here the potential utility of examining and considering EMT in clinical practice. Although some reports highlight a contribution of EMT in small cell lung cancer (Ito et al., 2017) and malignant pleural mesothelioma (Schramm et al., 2010) with specific implications respective to their cell type origin, we will focus this review on NSCLC, the most frequent lung cancer in which EMT has now been extensively analyzed. We aim at pointing to clinically relevant features related to EMT process that could help clinicians to identify disease contexts. We further propose to examine the beneficial contribution of EMT in clinical management from early/advanced stages to metastatic conditions in order to meet current clinical challenges. Considering the poor outcome of resectable lung cancer, a crucial challenge is indeed to identify independent and powerful predictors of patient global outcomes that are currently lacking. On another hand, advanced and metastatic conditions are associated with ineluctable therapy resistances to current available strategies including chemotherapies, radiotherapy, targeted therapies and immunotherapies. In these clinical conditions, considering EMT as a biomarker could allow a refinement of patient management.

**NSCLC biomarkers related to EMT**

Aiming at defining the clinical relevance of EMT as a biomarker, we will first confront EMT status with pathological features of lung tumor cells, and then unravel relationships bridging EMT to two cornerstones of both lung carcinogenesis and clinical management that are oncogenic drivers and immune profiles.

*a. EMT and histopathological features*

Literature emphasizes that EMT features are quite commonly observed in lung cancer both in AC and in SCC (Kidd et al., 2014; Mittal, 2018). For example, Dauphin et al. described until 50% of tumors cells with a mesenchymal phenotype (Dauphin et al., 2013). Mesenchymal
traits were shown to be even more frequent in other lung histological types such as large cell neuroendocrine carcinomas (Galván et al., 2014), sarcomatoid carcinomas (Thomas et al., 2012) or pleomorphic carcinomas which are typical of mesenchymal switch (Kondo et al., 2018; Miyahara et al., 2015). Mesenchymal features have also been associated to a low degree of differentiation in NSCLC (Bian et al., 2019; Dauphin et al., 2013; Matsubara et al., 2014; Z. Wang et al., 2019). Otherwise, the spatial distribution of EMT features within a tumor is very heterogenous. EMT attributes have thus frequently been reported in so-called invasive fronts, at the interface with extracellular matrix, correlating with invasiveness and metastatic potential (Maeng et al., 2014). EMT has also been associated with hypoxic zones and inflammation-rich areas (Dominguez et al., 2017), emphasizing a major contribution of specific tumor microenvironments (TME) in EMT induction/regulation (Foster et al., 2014; Hung et al., 2009; Yang and Wu, 2008). Accordingly, experimental data strongly support that EMT may be induced/regulated by ECM components, inflammatory mediators and other soluble factors secreted by stromal cells, and hypoxia (Lou et al., 2016; Mittal et al., 2016). Inversely, EMT+ tumor cells have also been reported to secrete higher levels of soluble factors crucially impacting the tumor microenvironment (e.g.: VEGF stimulating angiogenesis, chemokines impacting immune cell recruitment). At last, among classically examined clinicopathological parameters in lung cancer is also immune infiltration. Thus, EMT also modulate immune cell-infiltration (De Matteis et al., 2019; Dominguez et al., 2017) through well identified molecular regulatory networks (Markopoulos et al., 2019). For instance, Chae et al. reported reduced CD4 T-cell and CD4/CD8 T-cell infiltration respectively in lung AC and SCC with tumor cells displaying mesenchymal attributes (EMT+) (Chae et al., 2018). Inversely, increased activated B-cells and regulatory T-cells were reported, although some differential infiltration patterns need to be further defined between AC and SCC. Additionally, EMT+ tumors were also found to over-express multiple immunosuppressive cytokines such as IL-10, TGF-β, IL-6 or IL-11 (Y. N. Jiang et al., 2019; Q. Zhang et al., 2017;
Zhao et al., 2018; Zheng et al., 2019). A narrow crosstalk and regulatory loops between stromal cells and EMT-derived cells are thus established, contributing to the formation of particular areas favoring tumor invasion and dissemination. Main EMT-associated markers and activation pathways are illustrated and summarized in Figure 1.

In addition to EMT determination in lung primary tumors, examining EMT in Circulating Tumor Cells (CTCs) has also gain major interest. CTCs indeed today appear as promising biomarkers in lung cancer (Y. Li et al., 2018; Maly et al., 2019; Milano et al., 2018). Unlike tumor biopsies, CTCs allow a live assessment of disease progression and could thus help to predict metastasis and monitor therapeutic response. Numerous studies today report the presence of EMT-shifted hybrid CTCs and CTC clusters in NSCLC patients (G. Li et al., 2018; Lindsay et al., 2017; Sawabata et al., 2020). Interestingly, Manjuntha et al. observed that the intensity of EMT markers staining (vimentin and fibronectin) was higher in EMT+ CTCs than in patient-matched NSCLC tumor tissues (Manjunath et al., 2019). Validating the importance of EMT characterization and supporting its utility for clinicians, Xu et al. classified CTCs into three subpopulations from epithelial, intermediate and mesenchymal phenotypes and reported that mesenchymal CTCs were more commonly found in patients in the metastatic stages of different types of cancers (Wu et al., 2015). Additionally, several reports associated a mesenchymal shift in CTCs as predictor of poor outcomes in NSCLC (Li et al., 2017, p. 4; Liu et al., 2018). Miguel-Perez et al. thus identified mesenchymal CTCs as an independent prognostic factor for relapse-free survival, with an impact on overall survival in resected lung adenocarcinomas (de Miguel-Pérez et al., 2019). Moreover, similar data were obtained in a prospective and controlled cohort (Manjunath et al., 2019). Among metastatic stages, EMT sub-classification could allow to further refine those with poor evolution profile (Y. Wang et al., 2019). EMT status in CTCs could also be useful as predictor of therapeutic response (Liao et al., 2014; Milano et al., 2018; Togo et al., 2017). Despite these numerous data validating the clinical relevance of examining EMT in CTCs, the variability of
methodologies used to enrich and isolate CTCs combined with the molecular complexity of EMT certainly introduces biases in our comprehension of EMT-related CTC heterogeneity. It seems worth mentioning that many CTC isolation devices are based on the expression of specific epithelial biomarkers (such as EpCAM). Subpopulations of EMT-derived CTCs, supposedly expressing lower levels of many membrane epithelial markers, may thus fail being detected by such methods. To address this limitation, alternative enrichment devices exploiting physical properties of CTCs are developed to isolate label-free CTCs and facilitate the study of EMT heterogeneity in the CTC population (Alix-Panabières et al., 2017; Genna et al., 2020; Nicolazzo et al., 2019).

All in all, EMT is a frequent event in NSCLC, observable both in the primary tumor site and in CTCs. Its association with unfavorable clinicopathological features justifies the proposition to consider EMT as a potential marker to predict patient outcomes. Methodological standardization and identification of the most relevant molecular markers are nevertheless necessary steps before a routine clinical utilization.

b. EMT and oncogenic drivers

Our growing molecular knowledge of cancer somehow shook some dogmas and continuously redirects lung cancer management. Several targeted therapies constitute the current arsenal to combat lung cancer, most of which are specifically directed against recognized lung cancer oncogenic drivers (Figure 2). Among major genetic modifications associated with NSCLC, KRAS, EGFR and ALK addictive mutations or alterations are probably the main ones (Arbour and Riely, 2019).

-KRAS

To date, KRAS mutation is the most common molecular alteration encountered in NSCLC but remains with no effective therapies targeting tumors harboring mutant variant of KRAS, despite many clinical trials (Aran and Omerovic, 2019; Yang et al., 2019). Experimental data
suggest that \textit{KRAS} mutations contribute to support mesenchymal changes, alone but often in
synergy with other EMT-inducing factors (Arner et al., 2019). For instance, mutant \textit{KRAS} and
\textit{TP53} cell lines established from a lung cancer transgenic mouse model were found to display
important EMT/MET plasticity. A shift toward a mesenchymal phenotype was shown to
depend on a well-described miR-200/ZEB regulatory loop and to promote metastasis
(Gibbons et al., 2009). Deciphering further \textit{KRAS} mutant/EMT relationships, Singh \textit{et al.}
showed that, within \textit{KRAS} mutant cell lines, two sub-groups were distinguished based on their
\textit{KRAS} dependency to maintain their viability. The \textit{KRAS}-dependent NSCLC cell line treated
with the classical EMT-inducer (TGF-β1) acquired \textit{KRAS} independency, contrary to untreated
cell line. Thus, mesenchymal switched NSCLC cell lines harbored a \textit{KRAS} independency and
inversely, supporting a close relationship between EMT and loss of oncogene addiction
(Singh \textit{et al.}, 2009). Examining \textit{KRAS} molecular status and EMT phenotype as tandem
biomarkers could thus harbor a particular significance and also refine patient stratification and
therapeutic strategies. Considering until now deceiving effectiveness for drugs targeting
\textit{KRAS} addiction, selected patients with \textit{KRAS}-mutant NSCLC harboring epithelial phenotype
could thus benefit from \textit{KRAS} inhibitors.

- \textit{EGFR}

As the second most common molecular addiction occurring in NSCLC, activating mutations
of the epidermal growth factor receptor (EGFR) gene are especially involved in lung
adenocarcinoma without smoking history. A large majority of these activating mutations (85-90\%)
occurs by exon 19 deletion (about 45\%) or exon 21 L858R mutation (about 45\%)
(Castellanos \textit{et al.}, 2017). In \textit{EGFR}-mutated advanced lung cancer patients, many randomized
phase III trials have revealed that treatment with first-, second- and now third-generation
EGFR tyrosine kinase inhibitors (TKIs) resulted in an improved outcome compared to
standard chemotherapy in first line (Soria \textit{et al.}, 2018). Erlotinib even provided a similar
efficacy than chemotherapy in second line in \textit{EGFR} wild type tumor with a better tolerance
(Ciuleanu et al., 2012). However, cancer progression fatally occurs after a median of 12-month treatment and almost all patients who strongly responded to EGFR-TKI acquire resistance over time. Frequently, the mechanism of resistance is a acquired \textit{EGFR} mutation (Rotow and Bivona, 2017). In half of the cases, this second mutation is T790M point mutation in exon 20 of the \textit{EGFR} gene (Kobayashi et al., 2005) that can be triggered by osimertinib, a dedicated EGFR-TKI (Carlisle and Ramalingam, 2019). Among patients with tumor harboring mutation of EGFR and developing EGFR-TKIs resistance, a significant part does not exhibit mechanisms of genotypic resistance. Such EGFR-independent mechanism of resistance includes EMT, occurring in about 5% of cases (Bronte et al., 2018; Lim et al., 2018). Interestingly, tumors from patients developing resistance to TKIs exhibit mesenchymal traits while maintaining their original EGFR-activating mutation. Moreover, Sequist et al. did not observe EMT features in patient developing a resistance mechanism mediated by T790M \textit{EGFR} mutation (Sequist et al., 2011), supporting that T790M does not drive EMT. In patients harboring T790M mutation and treated with osimertinib, progression also unfortunately occurs, and a C797S tertiary mutation has been identified. A tertiary resistance could be also associated to and/or supported by EMT, even in non-C797S mutation-harboring patients. Although MET gene amplification may more frequently drive the underlined mechanism of resistance in this context (Del Re et al., 2019). Those clinical observations linking EGFR-TKIs resistance to the emergence of EMT phenotypes are supported by \textit{in vitro} and \textit{in vivo} preclinical studies (Tulchinsky et al., 2019; X. Zhu et al., 2019). For example, TGF-β1, Insulin-like growth factor 1 receptor (IGF1R) and Notch-1 pathways, known to be potent EMT-inducers, seem to be crucial actors in resistance mechanism (Cortot et al., 2013; Rho et al., 2009; Soucheray et al., 2015; Suda et al., 2011; Xie et al., 2013; Zhou et al., 2015). This phenotypical switch appears to be reversible and could thus be promising in combined therapies (Witta et al., 2006). By using dasatinib, Sesumi et al. inhibited EMT induction by
TGF-β in EGFR-mutant NSCLC cell lines. For NSCLC already harboring erlotinib resistance with mesenchymal features, dasatinib monotherapy failed to restore both an epithelial phenotype and sensitivity to EGFR-TKIs. However, combining erlotinib and dasatinib prevented EMT-mediated resistance to EGFR-TKI and resulted in T790M mutation of resistance (Sesumi et al., 2017).

- \textit{ALK}

Among ALK molecular alterations, ALK translocations with a fusion partner correspond to the second targetable oncogenic driver to date in NSCLC (Du et al., 2018). Following the ALEX trial, Alectinib became the gold standard of ALK-rearranged related TKI with an increase of overall survival (Hida et al., 2017). As similarly observed in EGFR inhibitor management, some ALK inhibitor resistance inevitably occurs and is mediated most of time by an acquired \textit{ALK} mutation (Katayama, 2018). As for resistance to EGFR-TKI, EMT has been observed and proposed as a non-oncogenic resistance pathway (Peters and Zimmermann, 2018). Gainor et al. explored different mechanisms of ceritinib resistance in 12 re-biopsies and observed that 5 specimens displayed mesenchymal traits. Interestingly, some of them also harbored a concomitant second ALK-mutation of resistance (Gainor et al., 2016). Similarly, Gower et al. described acquired EMT characteristics in tumor harboring ALK-TKI resistance. However, reversible models of EMT did not allow to restore sensitivity to ALK inhibitors, suggesting that EMT process can be associated but not required for ALK-TKI-resistance. Deciphering further this EMT/ALK-mutation status, Fukuda et al. performed microdissection analyses in a tumor resistant to ALK-rearranged related TKI. This tumor concomitantly harbored an acquired \textit{ALK} mutation of resistance (L1196M) in epithelial-like tumor area while no additive mutation was found in mesenchymal-switched tumor area. Taken together, those observations suggest that EMT can both be independent and additive mechanisms underlying ALK-TKI-resistant cancers (Fukuda et al., 2019). To investigate the EMT and ALK-TKI resistance relationship, Kogita et al. established NSCLC cell line with...
EML4-ALK rearrangement and showed that hypoxic condition was associated to ALK-TKI-resistance by an EMT-dependent signaling (Kogita et al., 2014). Beyond molecular mechanisms involving hypoxia induced-factors, epithelial splicing regulatory protein 1 (ESRP1) (Voena et al., 2016), three other EMT-related pathways were involved in ALK mutant cancers as proteoglycans, HIF-1 and FoxO signaling pathways and ECM-receptor interaction as reported by Wei et al. (Wei et al., 2018). All together, these observations suggest that resistance to ALK-TKI can be associated with mesenchymal features even though EMT is not the sole driver of resistance.

- Others

Among other targetable oncogenic drivers, some molecular alterations seemed related to EMT. For example, EMT process has been described as dysregulated in BRAF mutant cancers such as primary cutaneous melanoma or papillary thyroid carcinoma (Mitchell et al., 2016). Possibly due to low prevalence of BRAF mutant in lung cancer (less than 1%), only few works reported BRAF/EMT interplay in NSCLC (Urbanska et al., 2020). With a structural similarity to ALK but lower prevalence, ROS1 alterations are also oncogenic drivers targetable in clinical management (Lin and Shaw, 2017). Gou et al. described a mesenchymal polarization in NSCLC cell line with CD74-ROS1 G2032R mutation, leading to increased aggressiveness and interestingly supporting a resistance to ROS1-TKI (Crizotinib) (Gou et al., 2018). Still regarding EMT process, our laboratory observed a more frequent mesenchymal switch in NSCLC cell lines and tumors harboring HER2 activation, classically related to aggressiveness. Interestingly, anti-HER2 therapies allowed to restore epithelial features and reduce invasiveness (Da Silva et al., 2020). Finally, c-MET–molecular alterations are also described in a minority of NSCLC and many preclinical and clinical trials have been designed in lung cancer. However, NSCLC harbor a large heterogeneity in c-MET molecular modification such as overexpression, amplification and point mutations that could explain
many controversial results to date (Drilon et al., 2017), although exon-14 skipping mutations seem promising (Pasquini and Giaccone, 2018).

In conclusion, both common and uncommon oncogenic drivers seem intrinsically linked to EMT processes. A large part of studies reported that mesenchymal features are associated with resistance to various drugs. Whether EMT is a consequence of or a prerequisite to drug resistance are two non-mutually exclusive possibilities. A better comprehension on those phenomena may lead to innovative pharmacological strategies.

c. EMT and immune profile

Among EMT-induced properties contributing to enhance metastatic potential, the ability of tumor cells to escape immune surveillance has gained major interest along with the emergence of immunotherapies. Thus, numerous studies report a positive correlation between a mesenchymal switch and the expression of immune checkpoint proteins. As a cornerstone in immunotherapy management, PD-(L)1 has been largely explored in NSCLC context. Several in vitro and preclinical studies reported an induction of PD-L1 expression by different EMT pathways and EMT-TFs, and PD-L1/EMT-TFs co-expression has been reported in human lung cancer specimens (Asgarova et al., 2018; F. Li et al., 2018; Noman et al., 2017). This co-expression has actually been observed in many histological types (Alsuliman et al., 2015; Chen et al., 2017; Ock et al., 2016) and largely reported in NSCLC, from metastatic to locally and resected lung cancers (Ancel et al., 2019; Kim et al., 2016), and in CTCs (Manjunath et al., 2019). Other reports studying PD-1 and PD-L1 expression further corroborated this association with EMT phenotypes (Kim et al., 2016; Mak et al., 2016). Additionally, EMT seems to affect other immune checkpoint systems including CTLA-4, TIM-3 but also PD-L1/2, PD-1 and B7-H3 that were also found overexpressed, suggesting a wide range effect of EMT on tumoral immune escape (Lou et al., 2016). Chae et al. observed that overexpression
of druggable immune checkpoints, such as CTLA-4 and TIM-3 (but not PD-L1 in their study context), is associated with an EMT signature in NSCLC and with a lower infiltration of CD4 T cells (Chae et al., 2018). Thus, besides a direct effect on EMT in regulating the expression of immune checkpoint protein in tumor cells, EMT also acts on immune cell infiltration, as we discuss later in the text, contributing to create an immunosuppressive TME in the vicinity of EMT+ areas.

Overall, EMT process appears as a promising biomarker intrinsically related to tumor aggressiveness in NSCLC. EMT could thus help refining tumor prognostic and help clinicians in the choice of pharmacological strategies, especially regarding targetable oncogenic drivers and immunotherapies.

**EMT process in clinical lung cancer management**

Aiming at going beyond a descriptive level, we here below report how clinicians could benefit from examining EMT in NSCLC, both in early and metastatic stages.

**a. EMT relevance as prognosis factor in early lung cancer**

As previously mentioned, 5-year survival rates in early stage NSCLC remain poor, even after a complete resection, and relapse fatally occurs in a large number of cases. Aiming at reducing this burden, adjuvant platinum-based regimens are employed, though with limited effects. Many targeted therapies are currently available in lung cancer but their use is restricted to advanced stages. For example, pre- and/or post-operative anti-PD-(L)1 cannot be employed despite over than 50% tumor specimens harboring PD-L1 positive cells. This highlights a crucial need to further refine the characterization of tumor samples in order to identify patients that could benefit from a personalized strategy such as immune checkpoint inhibitors. Identifying patient with worse outcomes is a key step to this end, and robust prognostic markers are thus needed. Considering the extensive literature bridging EMT to tumor aggressiveness, EMT has been explored, solely or in combination with other markers
through different approaches that, as we discuss later in the review, still need to be optimized and validated in order to be exploitable in a clinical context. Chikaishi et al thus described a non-informative EMT status based on vimentin, gamma-catenin, fibronectin and E-cadherin expressions in 183 resected tumors, unable to predict patient’s outcomes (Chikaishi et al., 2011). These results could reflect the incorporation in the analyzed cohort of a large number of stage IA tumors, known to display a specific better prognosis. Examining homogenous and larger cohorts, many other studies reported a positive association between EMT+ characteristics and poor outcome. The evaluation of the prognostic and predictive value of EMT in early stages of NSCLC (NCT03509779) is being examined in a prospective cohort (TWIST lung). In other studies, higher vimentin expression in tumor cells was proposed as a predictor of metastasis occurrence (Aruga et al., 2018; Dauphin et al., 2013; Tsoukalas et al., 2017; Y. Wang et al., 2019). In SCC, vimentin expression failed to establish an independent prognostic but high S100A expression and lack of intercellular E-cadherin allowed to predict patients at a high risk of recurrence and poor prognosis (Zhang et al., 2013). Both in AC and SCC, reduced membranous staining of E-cadherin and expression of vimentin were shown to be independent predictors of mortality (Aruga et al., 2018; Che et al., 2015; Shao et al., 2019). Overpassing the clinical challenge to collect tumor biopsies, CTCs in peripheral blood were also explored as a predictor of outcomes in early stages of lung cancers. Regarding CTCs, many parameters are confronted such as CTCs count, CTCs variation or CTCs employed as liquid biopsy (Cabel et al., 2017; Syrigos et al., 2018). Moreover, CTCs can be informative through their biomarker expression and assessing EMT seems promising. Indeed, CTCs with a mesenchymal switch were associated with poor outcomes (Li et al., 2017, p. 4; Liu et al., 2018; Manjunath et al., 2019; de Miguel-Pérez et al., 2019). Thus, considering EMT statuses in CTCs could allow to enhance CTC clinical relevance in lung cancer management (Jin et al., 2017; Lowes and Allan, 2018; Wu et al., 2015).
To summarize on prognosis significance, studies examining multiple EMT markers are numerous and mainly concordant, supporting an independent capacity of EMT signature to predict patient outcomes. However, it appears crucial to identify some most relevant markers to examine their expression in routine practice. In these conditions, vimentin and E-cadherin expressions seem promising.

b. EMT implication in advanced and metastatic lung cancers

In addition to promoting local and distant dissemination/recurrence, EMT was proposed by many authors to support resistance to therapies (Dudas et al., 2020). We explore here EMT interrelation with therapeutic options currently used in advanced stages of NSCLC, such as chemo/radiotherapies, targeted therapies and immune checkpoint blocking antibodies.

- Chemo/radio resistance

Extensive literature today emphasizes a role of EMT in resistance to chemotherapies currently used in clinical strategy such as cisplatin, paclitaxel, gemcitabine, and vinorelbine (Fischer et al., 2015; Han et al., 2016; van Staalduinen et al., 2018; Suda et al., 2017; Toge et al., 2015).

More particularly, mesenchymal attributes have been associated to cisplatin resistance, the major first line chemotherapy in NSCLC. This is supported by numerous in vitro and in vivo data (Chen et al., 2016; Guo et al., 2018; He et al., 2018; G.-B. Jiang et al., 2019). Similar findings also support a role of EMT in resistance to docetaxel, a cytotoxic gold-standard drug in lung cancer typically used in second line (Chen et al., 2014; Shen et al., 2014). Importantly, the majority of these reports emphasized reversible and flexible EMT-mediated resistance processes, offering targeting perspectives. Aiming at circumventing chemotherapy resistance, many interesting approaches are thus being developed to adapt combination treatment protocols and/or to block EMT and sensitize tumor cells to chemotherapy. For instance, examining different protocols of pemetrexed/cisplatin combination treatment on lung cancer cell lines, Tièche et al identified a resistant cell-subpopulation with EMT and cancer stem cell
characteristics emerging in all tested treatment settings. Interestingly, the authors observed that a pretreatment with pemetrexed, before the addition of cisplatin, reduced the emergence of this EMT/cancer stem cell phenotype and significantly enhanced the inhibitory effect of cisplatin on lung cancer cell growth (Tièche et al., 2016). Another in vitro study reported an EMT-mediated resistance to antifolate pemetrexed chemotherapy and further showed that blocking EMT signaling with the flavonoid kaempferol restored pemetrexed sensitivity (Liang et al., 2015). Using in vitro drug-resistant NSCLC cell models, Kurokawa et al. observed that acquired cisplatin resistance reduces the sensitivity of cancer cells to a subsequent treatment with gefitinib, an EGFR-TKI. Cisplatin-induced resistance to gefitinib was associated with acquisition of both EMT and induction of AXL, a now well-described EMT-associated tyrosine kinase receptor that may bypass EGFR signaling for survival and proliferation and that has become an attractive therapeutic target (Kurokawa et al., 2013).

Regarding radioresistance, some studies have examined the relationships between EMT and ionizing radiation. Radiation was thus shown to induce EMT and enhance motility and invasiveness in various lung cancer cell lines (Gomez-Casal et al., 2013; Jung et al., 2007; Lu et al., 2018; Yao et al., 2016). As observed for chemoresistance, radioresistance-mediated by EMT seemed to be a reversible and targetable process. For instance, Notch-1-regulating flavonoid compounds (Rhamnetin and Cirsiliol) were found to inhibit EMT and induce radiosensitization in different NSCLC cell lines (Kang et al., 2013). PD-L1 expression was also reported to be increased along with EMT after ionizing radiation. Down-regulating PD-L1 in radiation resistant cells was shown to alleviate radiation resistance and to decrease EMT attributes, and combined radiotherapy and anti–PD-L1 antibody synergistically enhanced antitumor immunity in a xenograft mouse model (Gong et al., 2017).

Although limited to preclinical in vitro and in vivo studies, those observations highlight the interest of monitoring EMT to refine sequential therapy, line management and drug
combination, and also to identify EMT pathways as potential targets to enhance or restore chemo/radio sensitivity.

- **EGFR-TKIs resistance**

As mentioned earlier, EMT is involved in primary and acquired resistance to anti-EGFR drugs. Additionally, clinical studies and trials confirmed a potential interest for patients of examining EMT in anti-EGFR therapies.

Only few clinical reports evaluated EMT in patients with tumors harboring activating EGFR mutations. Those observations associated mesenchymal features with EGFR-TKI resistance (Miyoshi et al., 2015; Poh et al., 2018; N. Zhang et al., 2017). As there are no validated markers of response to EGFR inhibitors in EGFR wild-type patients, more numerous clinical trials assessed EMT interest as predictor of response in this molecular context. Thus, Villalobos et al. examined E-cadherin and vimentin expression in a cohort of 104 advanced and metastatic NSCLC patients treated with erlotinib/bevacizumab or a chemotherapy regimen and unselected regarding on their EGFR genotype. It appears that tumors with mesenchymal attributes exhibited increased PFS in the chemotherapy group in comparison to the EGFR-TKI group suggesting promising better efficacity of standard chemotherapy in comparison to erlotinib/bevacizumab combination for mesenchymal-like tumors (Villalobos et al., 2019). Additionally, based on NSCLC cell lines and validated in clinical conditions in the BATTLE-1 cohort treated with erlotinib (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination), Byers et al. described an EMT signature able to predict EGFR-TKIs resistance. In this (BATTLE-1 NSCLC) cohort, the epithelial EMT signature predicted a better disease control in patients receiving erlotinib in comparison to mesenchymal-switched NSCLC. EMT signature was not associated to different response for other therapies including platinum drugs, pemetrexed, docetaxel and paclitaxel (Byers et al., 2013). As a consequence, the ability to identify tumors that have not undergone EMT may help to select patients who would most likely benefit from EGFR inhibition, particularly in
second line for patients harboring a wild type EGFR cancer. Additionally, \textit{in vitro} studies support that targeting EMT may reverse or prevent acquisition of therapeutic resistance to EGFR inhibitors. This is for instance illustrated in an \textit{in vitro} study reporting that the reversion of an epithelial phenotype through forced E-cadherin expression in NSCLC cell lines restored sensitivity to the EGFR inhibitor gefitinib (Witta et al., 2006).

In the same line of thought, examining EMT biomarkers in resistance to anti-EGFR therapy may also point to EMT-induced alternative pathways that could overcome EGFR signaling for cell survival and growth. This is very well illustrated by now numerous studies showing that, as we mentioned earlier, AXL is frequently overexpressed in EGFR inhibitor resistance (Karachaliou et al., 2018; Kim et al., 2019; Singh and Silakari, 2017; F. Wang et al., 2019; Zhang et al., 2012). AXL is indeed considered as a promising target to overcome EGFR resistance. AXL inhibitors have been generated with some of them assessed in clinical trials such as Cabozantinib, a small size TKI multi-targeting AXL, MET, RET, KIT and VEGFR2 (Neal et al., 2016; Nokihara et al., 2019; Wakelee et al., 2017). In conclusion, it appears that, in addition to be a biological marker of tumor aggressiveness, EMT signature could be a marker of non-response to EGFR-TKIs in lung cancer tumors.

- **Immune evasion**

Cancer immunotherapy, including competing antibodies, checkpoint inhibitors, vaccines, and adoptive cell transfer, is based on restoring the immune response towards the tumor. Examining the interplay between EMT and the immune system has been proposed as a promising strategy to improve immunotherapy efficiency (Horn et al., 2020; Soundararajan et al., 2019). To date, current practice solely relies on blocking antibodies that have already proven to be cornerstone options for patients with lung cancer (Doroshow et al., 2019). Despite few contradictory results (Cooper et al., 2015; Okita et al., 2017), PD-L1 expression does not seem sufficient to accurately predict response to immuno-related drugs (Duma et al., 2019; Xia et al., 2019), and examining potential companion biomarkers both in tumors and
CTCs could enhance predictive significance (Kloten et al., 2019). PD-L1 also remains a poor prognostic indicator of overall survival (Takada et al., 2018; Woodard et al., 2016).

In the PACIFIC study, PD-L1 inhibition by Durvalumab showed an improvement in PFS in a narrowly selected cohort of non-metastatic advanced-stages patients who received a chemoradiotherapy pretreatment. Even in doing so, the response rate only reached 28.4% (Antonia et al., 2018). This objective response rate (ORR) seems lower and deceiving in comparison to ORR observed in metastatic stages, showing a real need to refine predictor markers of response. With this in mind, EMT was proposed as a tandem marker with PD-L1 expression, able to predict resistance to immunotherapy (Jia et al., 2019). Thus, taking in consideration both vimentin and PD-L1 expression in primary tumor (Ancel et al., 2019) or in CTCs (Manjunath et al., 2019) allows to redefine patients with worse outcomes. This subgroup co-expressing high levels of both vimentin and PD-L1 could be associated to worse ORR with immunotherapies.

Furthermore, Funaki et al. reported an enhanced PD-L1 expression observed after a platinum-based regimen treatment via a TGF-β- induced EMT in lung cell lines (Funaki et al., 2017). More than aggressiveness substratum in lung cancer, EMT-PD-L1 strong correlation could thus explain efficacy observed for chemotherapies in combination with immunotherapies in NSCLC (Gandhi et al., 2018; Paz-Ares et al., 2018). Indeed, improved response rate observed with durvalumab after a chemo-radiation in advanced stages and with pembrolizumab-chemotherapy association in first line metastatic stages, support its relevance. Considering major role of EMT in immunosuppression exacerbating resistance to immunotherapies, many reports argue for potential interest in combination of therapies to prevent and/or overcome treatment resistance (Soundararajan et al., 2019).
Strategies in development - limitations and perspectives

In the light of its extensively documented implication in promoting tumor aggressiveness in diverse tumor types and especially in lung cancer, EMT is thus today considered both as a promising companion prognostic/predictive biomarker and as a target for anticancer therapy. Along these lines, we drew some propositions, exploring how interrogating EMT status as a companion biomarker or how inhibiting EMT could potentially affect cancer management in different contexts. These propositions are recapitulated in Figure 3.

It is nevertheless important to emphasize that a major limitation to the exploitation of EMT in the clinic resides in the fact that reliable EMT signatures/biomarkers still need to be validated in clinical settings. In order to assess EMT polarization, mRNA expression signature (Chen et al., 2019; Gordian et al., 2019; Rudisch et al., 2015; Thompson et al., 2020) or specific EMT canonical markers are often analyzed. It is important to recognize that tumor cells broadly interplay with stroma, and particularly with stromal mesenchymal cells including fibroblasts or immune infiltrative cells that express frequently analyzed EMT markers. The examination of gene expression signature on total mRNA may thus introduce critical biases. Such explorative methods thus need to be further validated with concomitant analysis of tumor specific marker expression or using other alternative methods such as single cell sequencing (Karacosta et al., 2019; Ramirez et al., 2020), which is still restricted to the preclinical field. Examining EMT by in situ approaches (immunohistochemistry in combination with epithelial markers such as cytokeratins, in situ hybridization) probably allows a more accurate analysis of EMT-associated gene expression modulations occurring in tumor cells. Nevertheless, determining thresholds and cut-off values to score and define the extend of EMT is also a thorny challenge that needs to be further evaluated in clinical trials, particularly in the context of immunostaining analyses. In this line of idea to quantify the extent of EMT in tumors, establishing a numerical EMT index using selected validated biomarkers is a promising perspective of current EMT research (Fici et al., 2017).
In addition, the tumor material to be analyzed for EMT is also a subject of discussion. In early cancer contexts, pathological examination of whole surgically resected tumors facilitates the appreciation of tumor heterogeneity in its entirety (Neelakantan et al., 2015). In metastatic stages, EMT characterization on biopsy samples is limited to fewer tumoral territories and mostly in non-pretreated condition. Furthermore, EMT being recognized as a dynamic process associated to tumor invasion and early dissemination, one can hypothesize that metastases would rather contain tumor cells that reverted to more epithelial phenotypes through mesenchymal-epithelial transitions. In order to develop a personalized medicine and to adapt treatments in a real-time manner taking EMT into account, it thus seems pertinent to propose that EMT characterization should be performed on lung primary tumor biopsies and on CTCs issued from timely and repeated liquid biopsies.

Concerning the exploitation of EMT as a therapeutic target, there are no dedicated EMT inhibitors used in the clinic. However, existing drugs impacting RTK known to be involved in EMT (such the anti TGF-β, or Notch and Snail inhibitors) have been used for this purpose in preclinical models (Feng et al., 2020). Elaborating EMT inhibitors is a very active sector and many other anti-EMT compounds are being generated, some of which have been tested in NSCLC context (Otsuki et al., 2018). Most of them are still in preclinical development and we chose here to illustrate those confronted to clinical phases. RO4929097, a gamma secretase inhibitor designed to target Notch signaling has been employed in early phases for ovarian (Diaz-Padilla et al., 2015) and pancreatic (De Jesus-Acosta et al., 2014) cancers with limited results. Phase II trials unfortunately also failed to demonstrate its efficacy on advanced and metastatic NSCLC (NCT01193868) as well as in recurrent or refractory NSCLC (NCT01070927) alone or in combination with erlotinib (NCT01193881). To date, drug production has been stopped. A well-designed and randomized phase II study of 132 patients, evaluated the outcome of erlotinib combined or not to entinostat (isoform selective
HDACi), described as a potential inhibitor of EMT. Erlotinib combined with entinostat did not improve PFS based on a 4-month follow-up in global population. Interestingly, OS was longer in patients with high E-cadherin levels assessed at the time of diagnostic, with a safety profile and demonstrating the need to identify biomarker predictive of response to improve patient stratification (Witta et al., 2012). With the aim to identify biomarker of EGFR-TKIs response in NSCLC, Reckamp et al. originally assessed EMT markers in serum-samples from 22 patients. Decreased soluble E-cadherin and MMP-9 serum levels between baseline and first evaluation were correlated with better response to erlotinib and celecoxib combination (Reckamp et al., 2008). However, this combination did not seem to improve outcomes in an unselected population on a phase II trial (Reckamp et al., 2015). TLY3039478 also designed against Notch has been tested in a phase I trial. Eight patients with advanced NSCLC were recruited, demonstrating safety with a signal of efficacy based on metabolic response or tumor necrosis (Massard et al., 2018). A further clinical trial based on this drug is still recruiting (NCT02836600). Innovative strategies such as si-mi-RNAs could also represent an interesting approach in solid tumors (Naghizadeh et al., 2019), although their vectorization process is still insufficiently developed to date (Wang et al., 2014).

Targeting EMT in NSCLC could thus be beneficial alone but more probably in combination, specifically with chemotherapies to prevent and/or overcome resistance to actual treatments. Combining anti-EMT molecules with chemotherapy may also conceptually override a suspected implication of mesenchymal-epithelial transition in metastatic outgrowth. Combination of anti-EMTs with other targeted therapies may also be beneficial. Additionally, the redundancy of EMT activation pathways (Figure 2) also constitutes a clear challenge in targeting EMTs (Yin et al., 2019; Zoni et al., 2015), and pleads in favor of multi-target TKI approaches that are being examined (Hellerstedt et al., 2019; de Jonge et al., 2019; Wheatley-Price et al., 2019). Multiple therapeutics against EMT-activating pathways (TGFβ, FAK, FGFR, PDGFR,…) have been tested with no convincing effects (Gerber et al., 2020;
Giaccone et al., 2015; Han et al., 2018; Paik et al., 2017), although researches are still ongoing with FGFR (SenthilKumar et al., 2020) or FAK inhibitors (Mak et al., 2019). Among EMT-associated targetable receptor pathways, AXL seems one of the most currently promising (C. Zhu et al., 2019). Accordingly, AXL small molecule inhibitors are currently being tested as monotherapy or in combination with chemotherapy or anti-EGFR therapy in clinical trials (Kim et al., 2020; Levin et al., 2016).

In another way, the high prevalence of PD-L1 expression in tumors with mesenchymal attributes and data suggesting resistance to immune checkpoint inhibitors would further refine patients benefiting to anti-PD-L1 therapies. Thus, among higher PD-L1 expressers, a mesenchymal switch could predict resistance to immunotherapies in comparison to tumor with an epithelial-like phenotype. As a hypothesis, patients with NSCLC harboring EMT+/PD-L1+ markers could thus benefit from combination of immunotherapy and chemotherapy. Synergistic effects of combined TGF-β inhibition and PD-L1 blockade are also explored (Lind et al., 2020; Sow et al., 2019).

Conclusion

Numerous data emphasize a narrow relationship between EMT and lung cancer, in early to advanced and metastatic stages. Examining EMT parameters as a routine biomarker is foreseen to improve personalized lung cancer management. For early-resected lung cancer, the detection of EMT traits could help identifying patients with worse outcomes and guide clinicians towards an adaptation of clinical surveillance and adjuvant strategies. For conventional therapies, immunotherapies and oncogenic drivers targeted-therapies, EMT may appear as a predictive factor and/or marker of resistance and could steer clinicians to an alternative therapeutic option. Specific EMT actors may also represent promising new therapeutic targets to be used in combination therapy. A better characterization of most
relevant EMT actors to be considered for specific purpose seems crucial and will undoubtedly facilitate and speed up the implementation of EMT consideration in clinical practice.

Disclosure Statement

The authors declare that they have no conflicts of interest.

Author Contributions

All authors, AJ, MD, GD, BNR, VD, MP and CG, participated in manuscript preparation and revision. All authors read and approved the final manuscript.

Acknowledgement

The research effort associated with this review was funded in part by the "Partenariat Hubert Curien-Tournesol". CG is a Senior Associate Researcher from the FRS-FNRS (Belgium).

Funding sources

This research received no external funding.


Gou W, Zhou X, Liu Z, Wang L, Shen J, Xu X, et al. CD74-ROS1 G2032R mutation transcriptionally up-regulates Twist1 in non-small cell lung cancer cells leading to increased...
https://doi.org/10.1016/j.canlet.2018.02.032. 813
axis reverses cisplatin resistance by downregulating drug resistance genes in cisplatin-
resistant non-small cell lung cancer cells. EBioMedicine 2018;35:204–21. 817
https://doi.org/10.1016/j.ebiom.2018.08.001. 818
patients with refractory advanced non-small-cell lung cancer: a multicentre, randomised phase
duced EMT phenotype is associated with the acquisition of cisplatin or paclitaxel resistance
He Y, Xie H, Yu P, Jiang S, Wei L. FOXC2 promotes epithelial-mesenchymal transition and
Results of a Phase II Placebo-controlled Randomized Discontinuation Trial of Cabozantinib
https://doi.org/10.1016/j.clcc.2018.10.006. 831
current therapies and new targeted treatments. The Lancet 2017;389:299–311. 837
https://doi.org/10.1016/S0140-6736(16)30958-8. 838
Horn LA, Fousek K, Palena C. Tumor Plasticity and Resistance to Immunotherapy. Trends
Ito T, Kudoh S, Ichimura T, Fujino K, Hassan WAMA, Udaka N. Small cell lung cancer,
an epithelial to mesenchymal transition (EMT)-like cancer: significance of inactive Notch
https://doi.org/10.1007/s13577-016-0149-3. 847
Jia D, Li X, Bocci F, Tripathi S, Deng Y, Jolly MK, et al. Quantifying Cancer Epithelial-
Mesenchymal Plasticity and its Association with Stemness and Immune Response. J Clin
of non-small cell lung cancer cells by promoting EMT in an AKT signaling pathway-
https://doi.org/10.26355/eurrev_201905_17811. 854
Jiang YN, Ni XY, Yan HQ, Shi L, Lu NN, Wang YN, et al. Interleukin 6-triggered ataxia-
telangiectasia mutated kinase activation facilitates epithelial-to-mesenchymal transition in
https://doi.org/10.1016/j.yexcr.2019.05.011. 858
early stage lung adenocarcinoma: a case series report and literature review. Oncotarget
Phase I Study of BI 853520, an Inhibitor of Focal Adhesion Kinase, in Patients with


Poh M-E, Liam C-K, Rajadurai P, Chai C-S. Epithelial-to-mesenchymal transition (EMT) causing acquired resistance to afatinib in a patient with epidermal growth factor receptor 35
https://doi.org/10.21037/jtd.2018.06.122.


https://doi.org/10.1002/cncr.29480.

https://doi.org/10.1016/j.lungcan.2008.05.017.


https://doi.org/10.1371/journal.pone.0124283.


https://doi.org/10.1371/journal.pone.0204186.


**Figure 1: main EMT markers and activation pathways**


**Figure 2: Inhibitors currently used in NSCL management. Inhibitors promisingly analyzed in clinical trials are also depicted.**

Drugs currently available and used in lung cancer management with potential impact on EMT are depicted in blue. Additionally, drugs in pink represent molecules in development with potential interest as EMT-inhibitors in NSCLC. EML4-ALK: Echinoderm microtubule-associated protein-like 4 / Anaplastic lymphoma kinase fusion, PD-(L)1: Programmed death-(ligand) 1.

**Figure 3: Proposal for potential refinements in NSCLC management according to the EMT status and considering that anti-EMT drugs are available.**

The propositions in (A) and (B) solely concern cancer contexts in which the EMT status has been interrogated in published studies that are detailed in the main text. These hypothetical management scenarios have been elaborated considering that robust EMT markers/signatures
can be validated in clinical practice. The figure thus does not present current therapeutic options.