Role of epigenetics in lung cancer heterogeneity and clinical implication

Nian Dong 1#, Lin Shi 2#, Diane C. Wang 1#, Chengshui Chen 1*, Xiangdong Wang 1,2*

1 Department of Pulmonary Medicine, The First affiliated Hospital, Wenzhou Medical University, Wenzhou, 325000, China

2 Zhongshan Hospital, Shanghai Institute of Clinical Bioinformatics; Fudan University Center for Clinical Bioinformatics; Zhongshan Hospital Institute of Clinical Science of Fudan University, Shanghai, China

# Authors contributed to this paper equally as the first authors

*Correspondence to:

Chengshui Chen MD, Prof
wzchencs@163.com

Xiangdong Wang MD, PhD, Prof
xiangdong.wang@clintransmed.org
Abstract

Lung cancer, as a highly heterogeneous disease, can be initiated and progressed through the interaction between permanent genetic mutations and dynamic epigenetic alterations. However, the mediating mechanisms of epigenetics in cancer heterogeneity remain unclear. The evolution of cancer, the existence of cancer stem cells (CSCs) and the phenomenon of epithelial-mesenchymal transition (EMT) have been reported to be involved in lung cancer heterogeneity. In this review, we briefly recap the definition of heterogeneity and concept of epigenetics, highlight the potential roles and mechanisms of epigenetic regulation in heterogeneity of lung cancer, and summarize the diagnostic and therapeutic implications of epigenetic alterations in lung cancer, especially the role of DNA methylation and histone acetylation. Deep understanding of epigenetic regulation in cancer heterogeneity is instrumental to the design of novel therapeutic approaches that target lung cancer.

Keywords: Epigenetics, heterogeneity, lung cancer, clinical implication
1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide with 220,000 estimated new diagnosis and 160,000 estimated deaths per year [1]. Non-small cell lung cancer (NSCLC) accounts for 85% of lung cancers, including adenocarcinoma (AD), squamous cell carcinoma (SCC), and large cell carcinoma (LCC) subtypes [2]. NSCLC was considered as a heterogeneity disease due to histological and molecular heterogeneity. First-line treatment of advanced disease remains platinum-based doublet chemotherapy. Recently, the recognition of tumor-driven genetic mutations led to the development of molecule-targeted drugs, including those targeting epidermal growth factor receptor (EGF), vascular endothelial growth factor, insulin-like growth factor I signaling and others [3-6]. However, the majority of NSCLC are diagnosed at a late stage resulting in a poor prognosis with the 5-year survival for localized, regional and widely disseminated NSCLC being 55%, 27 and 4% respectively [7], also due to the lack of early detection and disease-specific biomarkers [8]. The initiation and progression of lung cancer is a result of the combination of permanent genetic mutations as well as dynamic epigenetic alterations [9], which exhibits intertumoral/intratumoral heterogeneity [10] (Figure 1).

Intertumoral heterogeneity, based on the genetic mutations, commonly refers to the heterogeneity among different cases [11-14]. In the past decade, there is sound data and compelling evidence for intertumoral heterogeneity with clinical implication. For example, the efficacy of EGFR inhibitors is different because of the different cases
with or without EGFR mutations [15]. Although such molecule-targeted drugs on the basis of intertumoral heterogeneity together with the traditionally histologic-guided chemotherapy provides more therapeutic choices for clinicians. However, lung cancer has a high incidence coupled with poor a 5-year survival rate of less than 17%, which is partly influenced by intratumoral heterogeneity in an individual case or in an individual tumor [11, 16-18]. It has been reported that epigenetic modulators indirectly contribute to the unscheduled expression of epigenetic mediators, facilitate the mediator-induced reprogramming of cell phenotypes of intratumoral heterogeneity, or transduce signals from internal or external stimuli [19]. Such intratumoral heterogeneity can be observed in distinct regions or individual cells of solid tumors. An increasing number of studies have demonstrated that aberrant epigenetics lead to cell-to-cell variability, which is thought to be responsible for treatment failure for cancer [20].

As mentioned above, epigenetics mainly contribute to the intratumoral heterogeneity. Several epigenetic defects, such as NSD1 and SETD2, are also associated with genetic mutations, which further induce intertumoral heterogeneity [21]. The present review will overview recent discoveries in the field of epigenetics in tumor heterogeneity, and summarize the potential diagnostic and therapeutic implications of epigenetic alterations in lung cancer. With the advent of the era of precision medicine, elucidating tumor heterogeneity as well as its potential regulatory mechanism will benefit the discovery and development of new diagnostics and therapies for lung
cancer.

2. Roles of epigenetics in lung cancer development and metastasis

Cancer development and metastasis is the major cause of mortality and morbidity in lung cancer. A growing body of evidence suggests that the epithelial-to-mesenchymal transition (EMT) plays a central role in lung cancer development and metastasis. Early changes in cell morphology occur with epithelial cells losing their polarity (e.g. E-cadherin) and acquiring new features of mesenchyme (e.g. Vimentin, N-cadherin). The induction of EMT is accompanied by a dynamic reprogramming of the epigenome involving changes in histone modification and ncRNAs [22]. The loss of E-cadherin expression or function by genetic or epigenetic aberrations is a common phenomenon in lung cancer [23]. Smoking may induce EMT by HDAC-mediated downregulation of E-cadherin in NSCLC, and HDAC inhibitor MS-275 may reverse the CSC-induced EMT[24]. Recently, numerous HDAC-containing complexes appear to play distinct roles in the regulation of E-cadherin during EMT, including the Mi2/nucleosome remodeling and deacetylase (Mi2/NuRD) complex, which is identified to interact with Twist, a major regulator in EMT [25]. TGF-β1 acts as a critical switch in the induction of EMT, which is also influenced by histone acetylation. The Smad complex, directly downstream of TGF-β, translocated into the nucleus and regulated the transcription by directly binding to the promoter of its downstream and the specific transcriptional co-activators or co-repressors, such as p300/CBP and HDAC [23]. Some epigenetic regulatory mechanisms of EMT and
CSC of lung cancer are listed in Table 1.

Additionally, the HDACs may regulate the E-cadherin expression through ncRNAs. MiRNAs are involved in the regulation of EMT of lung cancer through regulation of various signaling pathways, such as TGF-\(\beta\), EGF, and HGF signaling pathway (Figure 2). Of those, MiR200b and miR200c were reported to have an effect on H3 acetylation at E-cadherin promoter site [26]. The miR-200 plays an essential role in EMT suppression through targeting Zeb, which is linked to lung cancer [27]. Gregory et al. have reported low miR-200 levels in cells that had undergone EMT in response to TGF-\(\beta\), while enforced miR-200 expression was justified to prevent TGF-\(\beta\)-induced EMT. Moreover, the lack of miR-200 expression was positively correlated with absent E-cadherin [28]. The metastasis suppressive role of the miR-200 was observed in NSCLC cell lines with mutant K-ras and p53 as well. The TGF-\(\beta\)-induced EMT of NSCLC cell lines were entirely miR-200 dependent [29]. The re-expression of miR-200 also downregulated genes that are involved in metastasis signaling and proliferation, such as DLC1, HFE, ATRX, HNRNPR3, and so on [30]. There is increasing evidence of an autocrine TGF-\(\beta\)/Zeb/miR-200 signaling regulatory network that controls the plasticity of NSCLC cells. Strong correlation between Zeb1/2 and TGF-\(\beta\) was revealed and negative correlations between miR-200 and TGF-\(\beta\) as well. The crosstalk between the Zeb/miR-200 axis in NSCLC is a characteristic feature of epigenetic regulation in cancer heterogeneity of lung cancer [31]. Zeb1/2 and miR-200 are involved in a double-negative feedback loop, that is to
say, the miR-200 members target and suppress Zeb1/2 and promote epithelial differentiation and Zeb1/2 knockdown can enhance miR-200. The inversely related expression levels between miR-200 and Zeb1/2 in NSCLC tissue arrays and the upregulated miR-200 expression was found to increase E-cadherin and suppress the Wnt/β-catenin pathway by targeting Zeb1/2 [32]. The feedback loop was reported to play a pivotal role in the stabilization of cellular differentiation in response to prevalent extracellular cues, notably TGF-β. The TGF-β-containing autocrine loop leads to the permanence of the mesenchymal phenotype of NSCLC, which contributes to the heterogeneity of NSCLC [31]. Therefore, the interconnection among TGF-β, miR-200 and Zeb can explain the reversibility of the mesenchymal phenotype. With the demonstration of the Zeb/miR-200 loop in TGF-β-induced EMT of NSCLC, it reminds us of the epigenetic regulation in the process of EMT of NSCLC and shows epigenetic abnormality as a promising target in NSCLC.

The epigenetic regulatory mechanisms of histone modification and ncRNAs employed by lung cancer during EMT provide new opportunities which may be harnessed for improved and individualized cancer therapy based on defined molecular mechanism [33]. NSCLC differs in the basal level of E-cadherin, a hallmark of cancer heterogeneity, which is predominantly down-modulated through HDACs with HDAC inhibitors reversing the expression of E-cadherin of NSCLC. In NSCLC, E-cadherin expression is modulated mainly through zinc finger proteins, including transcriptional repressors Snail, Slug, and Zeb. Cooperative interactions between zinc finger proteins
and CtBP (a co-repressor complex) promote the recruitment of epigenetic enzymes, notably HDACs, to negatively regulate E-cadherin expression [34].

3. Epigenetics contribute to the heterogeneity of lung cancer

3.1 Epigenetics-major driver to cancer mutagenesis

Promoter CpG islands are normally unmethylated, or relatively hypomethylated, for transcription of DNA during tumorigenesis [35-38]. The context of promoter methylation in lung cancer include p16INK4a, CDKN2A, ASSF1A, FHT, TSLC1, APC, RARβ, CDH1, CDH13, DAPK, and MGMT, of which p16INK4a was deleted or methylated in about 60% NSCLC and <10% SCLC [39-42]. During lung cancer progression, the methylation of CDKN2A, which could be induced by tobacco-specific carcinogen (4-methyl nitrosaminino-1-(3-pyridyl)-1-butanone), occur from lung airway basal cell hyperplasia (17%) to squamous metaplasia (24%) or carcinoma in situ (50%) in a rat model. Additionally, the degree of hypomethylation of genomic DNA correlated with the severity of the lung cancer, such that genome-wide DNA methylation decreased as the tumor progressed from a benign proliferating mass to metastatic invasive cancer [43]. FHT was methylated, mutated, or deleted in 40–70% of NSCLC and 50–80% of SCLC, RASSF1 in 30–40% NSCLC and 70–100% of SCLC, and TSLC1 in 85% of NSCLC [44-46]. Promoter methylations of RASSF1A, APC, ESR1, ABCB1, MT1G, or HOXC9 in NSCLC were associated with Stage I or CDKN2A together with the early genesis of lung cancers. Hypermethylated hDAB2IP, H-cadherin, DAL-1, or FBN2, were closely associated
with advanced stages and later phases of NSCLC.

Modifications of specific residues on histones, besides DNA methylation, by secondary binding proteins affect chromatin conformation and transcription, or cellular transformation and malignant growth. It was reported that lung cancer cells displayed an aberrant pattern of histone H4 modifications with a loss of H4K20 trimethylation. H4K20 trimethylation was present in normal lung epithelium, somewhat lower in early squamous precursors lesions, and strongly decreased with progression [47]. Aberrant acetylation of histone tails was closely linked to carcinogenesis, and HDACs overexpressed in lung cancer were correlated with tumor size. HDAC1, HDAC2, and HDAC3 were upregulated in human lung cancer [48, 49]. HDAC3 was upregulated in patients with lung SCC and correlated with a poor prognosis for lung AD. HDAC6 may interfere with the maturation of heat shock protein 90 and mediate cell cycle regulation. Additionally, the miR-196a and miR-200b are overexpressed in lung cancer with more than 23- or 37-fold changes, respectively [50]. DNA methylation status of Wnt antagonist SFRP5 can predict the response to the EGFR-TKIs therapy in NSCLC, demonstrating the underlying correlation between genetic mutation and epigenetic alteration in NSCLC cancer mutagenesis [51]. The present study indicated that the expression of thyroid hormone receptorβ1 located at 3q24.2 was frequently lost in both SCLC (61%) and NSCLC (48%) cell lines and TRβ1 expression status was closely correlated with TRβ1 methylation in the promoter region [52].
3.2 Epigenetics- major contributor to cancer cell stem like behavior

Cancer cells, carrying the oncogenic and tumor suppressor mutations, initiate tumor and drive forward tumor progression. CSCs are thought to be located at the top of a hierarchical differentiation model and maintain themselves by self-renewal, which further forms a heterogenous cancer population [53]. It was found that the tumor suppressor genes and oncogenes, Bim-1, Notch, Wnt, and Sonic hedgehog pathways, are involved in regulation of self-renewal of both normal and cancer stem cells [54, 55]. Overexpression of the Wnt genes and hypermethylation negatively regulator genes of Wnt (e.g. WIF) have been documented in NSCLC and are thought to play an important role in cancer stem cell maintenance [56]. IL-6 enriched lung cancer stem cell subpopulations could lead to the hypermethylation of p53 and p21 through DNMT1 upregulation [57]. Oxidative stress was reported to repress the expression of HDAC8, following the subsequent histone 3 acetylation and increased the expression of HOXA5 and SOX2, to promote plasticity of lung cancer stem-like cells [58]. SOX2 is the predominant downstream target of EGFR signaling and plays a major role in the modulation of self-renewal and expansion of stem-like cells from NSCLC [59, 60], while histone acetyltransferase inhibitor CPTH6 could inhibit lung cancer stem-like cells [61].

There is growing evidence suggesting that CSCs play a critical role in tumor progression, metastasis, and drug resistance. Similar to normal tissue stem cells, CSCs exhibit significant phenotypic and functional heterogeneity. Current
anti-cancer therapies fail to eradicate CSC clones and instead favor the expansion of
the CSC pool and select forresistant CSC clones thereby resulting in
treatment resistance and subsequent relapse in these patients. More and more studies
suggest that the epigenetic events, such as DNA methylation and histone modification,
are closely linked to CSCs, although the regulatory mechanisms of CSCs in NSCLC
remain elusive. The identification of CSC-specific markers and regulatory
mechanisms induced by epigenetics and the targeted therapeutic destruction of CSCs
remains a significant challenge.

4. The clinical implication of epigenetic heterogeneity in lung cancer

Chromatin modifiers may act as potential targets for drug discovery and development
for cancer on basis of the reversibility of epigenetic modifications. Epigenetic therapy
holds great promise for clinical treatment given the prevalence of epigenetic
abnormalities in various types of disease. Two main classes of epigenetic therapies are
DNA methyltransferase inhibitors and HDAC inhibitors, which act globally by
promoting a more-open chromatin structure and subsequently promote gene
expression. A reversible type of drug resistance could be reversed by low doses of
histone deacetylase inhibitors and correlate with selection and/or induction of CSCs
from heterogeneous tumor cell populations [62].

4.1 The potential epigenetic biomarkers in lung cancer

As mentioned above, cancer cells have an aberrant methylation signature, including
global hypomethylation and hypermethylation of tumor suppressor genes [63, 64]. Global hypomethylation is usually associated with genomic instability and aberrant overexpression of oncogenic gene isoforms during the late phase in the progression of lung cancer [65]. In contrast, the DNA hypermethylation usually occurs at an early stage in lung tumorigenesis. The well-studied example of early hypermethylation are p16, RASSF1A, APC, RARβ, and MGMT, which can be regarded as candidate diagnostic biomarkers in lung cancer [66-68]. The promoter methylation of BRMS1 was demonstrated to correlate with smoking history and poor survival in NSCLC [69].

Currently, DNA methylation appears as one of the most promising epigenetic biomarkers, which can improve early detection and subsequent management of patients with lung cancer. DNA methylation could be detected in a number of body fluids of patients with lung cancer, such as sputum, bronchoalveolar lavage, and plasma or tissue biopsy and paraffin-embedded tissues [70, 71]. However, for the vast majority of the selected candidate biomarkers, the data is only available at the proof-of-principle level with inadequate retrospective validation. The potential predictive and prognostic epigenetic biomarkers in lung cancer are listed in table 2.

4.2 Epigenetic modifications therapy for lung cancer

DNA methylation is considered to be a powerful therapeutic target in lung cancer, e.g. DNMT inhibitors. The most widely studied DNA methyltransferase inhibitors are azacytidine (5-azacitidine, Vidaza) and decitabine (5-aza’-2-deoxycytidine, Dacogen),
which have entered in several phase I/II trials in solid tumors, including NSCLC [72]. Azacytidine is activated through phosphorylation and is integrated into RNA to inhibit DNMT activity and regulate histone modification patterns, while decitabine is integrated into DNA, which makes it a more potential inhibitor [73]. However, studies documented that the clinical use of azacytidine or decitabine alone appears to not be completely effective in lung cancer.

The first generation of HDAC inhibitors consists of examples such as trichostatin A (TSA), valproic acid, sodium phenyl butyrate and romidepsin. Romidepsin could induce cell cycle arrest and down-regulate the expression of MMP2 and MMP9, as well as increase the efficacy of erlotinib in NSCLCs [74, 75]. As the latest HDAC inhibitors, etinostat specifically inhibits HDAC1 and HDAC3, which are predominantly localized in the nucleus [76]. Vorinostat can reduce the expression of human telomerase reverse transcriptase in lung cancer cell by inducing DNA demethylation at the first exon of human telomerase reverse transcriptase [77]. The combination of entinostat and azacytidine inhibits mutant K-ras/Tp53 lung AD and targets epigenetic modifications of existing tumors to prevent progression. The present ongoing clinical trials involving epigenetic drugs in lung cancer are listed in table 3.

Taken together, current DNMT inhibitors and HDAC inhibitors have been proved to have clinical benefits in diseases that arise from repressive chromatin-mediated gene
silencing. However, these non-specific drugs should be carefully examined to determine whether the therapeutic benefits outweigh the potential adverse effects. That is to say, epigenomic profiling is being fostered in the era of pharmacoepigenetics and pharmacoepigenomics. These are fields that involve the study of the relationship between the epigenome and optimal drug dosage and/or response, with a goal of optimizing individualized treatment and discovering new drug targets.

5. Conclusions

The importance of epigenetics is highlighted by, but not limited to, its pivotal role in the heterogeneity of lung cancer. Although still in its infancy, epigenetics holds substantial promise in helping to explain the complex pathogenesis of heterogeneity of lung cancer. Epigenetic modifications, including DNA methylation, histone modification and ncRNAs, regulate gene expression and in turn determine the dynamic molecular and cellular events in the evolution of lung cancer, notably EMT and CSCs during disease initiation and progression. However, the abovementioned epigenetic types should be considered not as an isolated event but rather as a network of crosstalk and cooperation that contributes to the disease pathophysiology. Thus, a greater understanding of the role of epigenetics in lung cancer heterogeneity will lead to a better knowledge of gene regulation and will also translate into more effective disease treatments.
Acknowledgements

The work was supported by Shanghai Leading Academic Discipline Project (B115), Zhongshan Distinguished Professor Grant (XDW), The National Nature Science Foundation of China (91230204, 81270099, 81320108001, 81270131, 81400035, 81570075, 81500058, 81500025), The Shanghai Committee of Science and Technology (12JC1402200, 12431900207, 11410708600), Zhejiang Provincial Natural Science Foundation (Z15H010002), Zhejiang Provincial Science Technology Department Foundation (WKJ-ZJ-1526).

Reference


[26] Tryndyak VP, Beland FA, Pogribny IP. E-cadherin transcriptional
down-regulation by epigenetic and microRNA-200 family alterations is related to mesenchymal and drug-resistant phenotypes in human breast cancer cells.


[33] Bedi U, Mishra VK, Wasilewski D, Scheel C, Johnsen SA. Epigenetic plasticity:


Wnt proteins are lipid-modified and can act as stem cell growth factors. Nature 2003;423:448-52.


[62] Easwaran H, Tsai HC, Baylin SB. Cancer epigenetics: tumor heterogeneity,


gene promoter methylation signature in sputum for lung cancer risk assessment.


**Figure legends**

Figure 1 Genetic and epigenetic contributions to the heterogeneity of lung cancer. (A) Intratumoral heterogeneity was observed in individual cell of lung cancer through biopsy at distinct regions. (B) During tumorigenesis of lung cancer, smoking, aging and so on may promote clonal expansion of founder tumor cells or initiate cells with permanent genetic or dynamic epigenetic abnormalities. The formation of cancer heterogeneity forms as lung cancer develops. In an established tumor, the parental subclone may acquire new driver or passenger mutations (genetic subclone) or undergo epigenetic alterations such as DNA methylation, histone modification or ncRNAs.

Figure 2 Schematic illustration of the miRNA-regulating signaling pathways associated with EMT program in lung cancer. MiRNAs have effect on EMT through
targeting ligands, receptors, signaling pathways and transcription factors. (A) miR-200 and miR-192 regulate the EMT inducing EGF/VEGF/TGF-β signaling pathways and the core EMT regulators ZEB1/2. (B) miR-198 regulates the FGF signaling pathway. (D) miR-7 and miR-221 regulates the IGF/PDGF signaling pathways and the core EMT regulators Snail. Sharp arrows denote activation and blunt arrows indicate inhibition.

Table 1 Epigenetic regulation mechanism of EMT and CSC of lung cancer

Table 2 Predictive and prognostic epigenetic biomarks in lung cancer

Table 3 Present ongoing clinical trials involving epigenetic drug in lung cancer
Table 1 Epigenetic regulatory mechanism of CSC and EMT of lung cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Epigenetic regulation</th>
<th>Regulatory mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epigenetic regulation of CSC of lung cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD44</td>
<td>miRNA</td>
<td>CD44 expression was regulated by miR34 during differentiation, meanwhile miR-373 as well as miR520 is responsible for the downexpression of CD44</td>
</tr>
<tr>
<td>ALDH1+</td>
<td>miRNA</td>
<td>Let-7 negatively regulates ALDH1+ lung cancer cells</td>
</tr>
<tr>
<td>Oct4,SOX2 and Nanog</td>
<td>miRNA</td>
<td>miR-134, miR-296 and miR-470 represses the expression of these stemness markers</td>
</tr>
<tr>
<td>Notch</td>
<td>miRNA</td>
<td>Repression of miR-200/miR-205 activates Notch signaling</td>
</tr>
<tr>
<td>Bmi-1</td>
<td>miRNA</td>
<td>miR-200c regulates the silencing of Bmi-1</td>
</tr>
<tr>
<td><strong>Epigenetic regulation of EMT of lung cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Promotor methylation</td>
<td>Hypermethylation at the promoter of E-cadherin results in the decreased E-cadherin expression</td>
</tr>
<tr>
<td>Vimentin</td>
<td>miRNA</td>
<td>The expression of Vimentin could be regulated by miRNA-138</td>
</tr>
<tr>
<td>Twist1</td>
<td>Methylation</td>
<td>Through interacting with SET8, Twist induces N-cadherin expression and suppressing E-cadherin</td>
</tr>
<tr>
<td>Snail1</td>
<td>Histone modification</td>
<td>By mediating demethylation of Lys-4 of histone H3, Snail1 leads to the transcriptional repression of E-cadherin</td>
</tr>
<tr>
<td>EZH2</td>
<td>miRNA</td>
<td>miR-138 targets EZH2 and modulates the gene silencing effects on E-cadherin and other related downstream genes</td>
</tr>
<tr>
<td>HDAC1</td>
<td>Histone modification</td>
<td>Interrelation with HDAC1 and the co-repressor mSin3A governs the epigenetic silencing of E-cadherin promoter</td>
</tr>
<tr>
<td>HDAC2</td>
<td>Histone modification</td>
<td>Interrelation with HDAC2 and the co-repressor mSin3A governs the epigenetic silencing of E-cadherin promoter</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Methodology</td>
<td>Tissue/body fluid</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>DNA methylation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFPI-2</td>
<td>MSP</td>
<td>Primaty tumor</td>
</tr>
<tr>
<td>CHFR</td>
<td>MSP</td>
<td>Serum</td>
</tr>
<tr>
<td>RGC32</td>
<td>MSP</td>
<td>Primaty tumor</td>
</tr>
<tr>
<td>HTLF</td>
<td>Methylation-specific ligationdependent probe amplification assay</td>
<td>Primaty tumor</td>
</tr>
<tr>
<td>CALCA</td>
<td>Illumina Goldengate bead array</td>
<td>Primaty tumor</td>
</tr>
<tr>
<td>RASSF1</td>
<td>Bisulphite sequencing</td>
<td>Paraffin-embedded primary tumour</td>
</tr>
<tr>
<td>MGMT</td>
<td>Bisulphite sequencing</td>
<td>Paraffin-embedded primary tumour</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Bisulphite sequencing</td>
<td>Paraffin-embedded primary tumour</td>
</tr>
<tr>
<td>PTEN</td>
<td>Bisulphite sequencing</td>
<td>Paraffin-embedded primary tumour</td>
</tr>
<tr>
<td><strong>miRNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Let-7 pri-miRNA NA</td>
<td>qRT-PCR</td>
<td>Snap frozen</td>
</tr>
<tr>
<td>Has-mir-155</td>
<td>Microarray</td>
<td>Lung Tissue</td>
</tr>
<tr>
<td>miR-34a</td>
<td>qRT-PCR</td>
<td>FFEP</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>miR-146b</td>
<td>MirVana miRNA Bioarrays</td>
<td>Snap Frozen tissue</td>
</tr>
<tr>
<td>miR-486</td>
<td>Solexa</td>
<td>Serum</td>
</tr>
<tr>
<td>miR-30d</td>
<td>Solexa</td>
<td>Serum</td>
</tr>
<tr>
<td>miR-1</td>
<td>Solexa</td>
<td>Serum</td>
</tr>
<tr>
<td>miR-499</td>
<td>Solexa</td>
<td>Serum</td>
</tr>
<tr>
<td>miR-96</td>
<td>qRT-PCR</td>
<td>Snap Frozen tissue</td>
</tr>
<tr>
<td>miR-182</td>
<td>qRT-PCR</td>
<td>Snap Frozen tissue</td>
</tr>
<tr>
<td>miR-183</td>
<td>qRT-PCR</td>
<td>Snap Frozen tissue</td>
</tr>
<tr>
<td>Clinical trial</td>
<td>Phase</td>
<td>Protocol</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------</td>
<td>-------</td>
<td>------------</td>
</tr>
<tr>
<td>Vorinostat-irresa combined therapy on resistance by BIM polymorphysim in EGFR mutant lung cancer</td>
<td>I</td>
<td>NCT02151721</td>
</tr>
<tr>
<td>HDAC inhibitor vorinostat with chemotherapy and radiation therapy for treatment of locally advanced NSCLC</td>
<td>I</td>
<td>NCT01059552</td>
</tr>
<tr>
<td>CC-486 with nab-paclitaxel</td>
<td>II</td>
<td>NCT02250326</td>
</tr>
<tr>
<td>Entinostat with pembrolizumab</td>
<td>IB/2</td>
<td>NCT02437136</td>
</tr>
<tr>
<td>5-fluoro-2-deoxycytidine (FdCyd) with tetrahydrouridine (THU)</td>
<td>II</td>
<td>NCT00978250</td>
</tr>
<tr>
<td>Treatment</td>
<td>Phase</td>
<td>NCT Number</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-------</td>
<td>------------------</td>
</tr>
<tr>
<td>Oral 5-AZA (CC-486) combined with romidepsin (expansion cohort for NSCLC)</td>
<td>I</td>
<td>NCT01537744</td>
</tr>
<tr>
<td>Chemotherapy with or without epigenetic priming in NSCLC</td>
<td>II</td>
<td>NCT01846897</td>
</tr>
<tr>
<td>Azacitidine and entinostat before (priming) chemotherapy in treating patients with advanced NSCLC CC-486 with MK-3475</td>
<td>II</td>
<td>NCT01846897</td>
</tr>
<tr>
<td>Epigenetic priming prior to nivolumab</td>
<td>II</td>
<td>NCT01928576</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>