

New Strategies in Lung Cancer: Epigenetic Therapy for Non–Small Cell Lung Cancer

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Abstract

Recent discoveries that non–small cell lung cancer (NSCLC) can be divided into molecular subtypes based on the presence or absence of driver mutations have revolutionized the treatment of many patients with advanced disease. However, despite these advances, a majority of patients are still dependent on modestly effective cytotoxic chemotherapy to provide disease control and prolonged survival. In this article, we review the current status of attempts to target the epigenome, heritable modifications of DNA, histones, and chromatin that may act to modulate gene expression independently of DNA coding alterations, in NSCLC and the potential for combinatorial and sequential treatment strategies. *Clin Cancer Res*; 20(9); 2244–8. ©2014 AACR.

Background

Recent advances in the treatment of advanced non–small cell lung cancer (NSCLC) have focused on the discovery that selective inhibition of driver mutations, in genes critical to tumor growth and proliferation, may lead to dramatic clinical responses. In turn, this has led to the regulatory approval of agents targeting two of these molecular aberrations, mutations occurring in the EGF receptor (*EGFR*) gene or translocations affecting the anaplastic lymphoma kinase (*ALK*) gene (1, 2). Current standard therapy for advanced nonsquamous NSCLC involves initial molecular profiling of the tumor to ascertain the presence or absence of a driver mutation. Approximately 50% of nonsquamous lung cancers and a small proportion of squamous tumors will harbor a molecular alteration that may be targeted either with an approved or investigational agent (3). Patients without recognized driver mutations are treated predominantly with systemic chemotherapy and have a median survival that ranges from 10 to 14 months (4, 5). Among strategies under investigation for the treatment of NSCLC, epigenetic therapy is of particular interest as it may have efficacy for tumors that are not addicted to traditionally targetable pathways or mutations. This review focuses on potential epigenetic targets in NSCLC and discusses results from early-phase clinical trials of agents targeting the epigenome.

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On the Horizon

Epigenetics and cancer

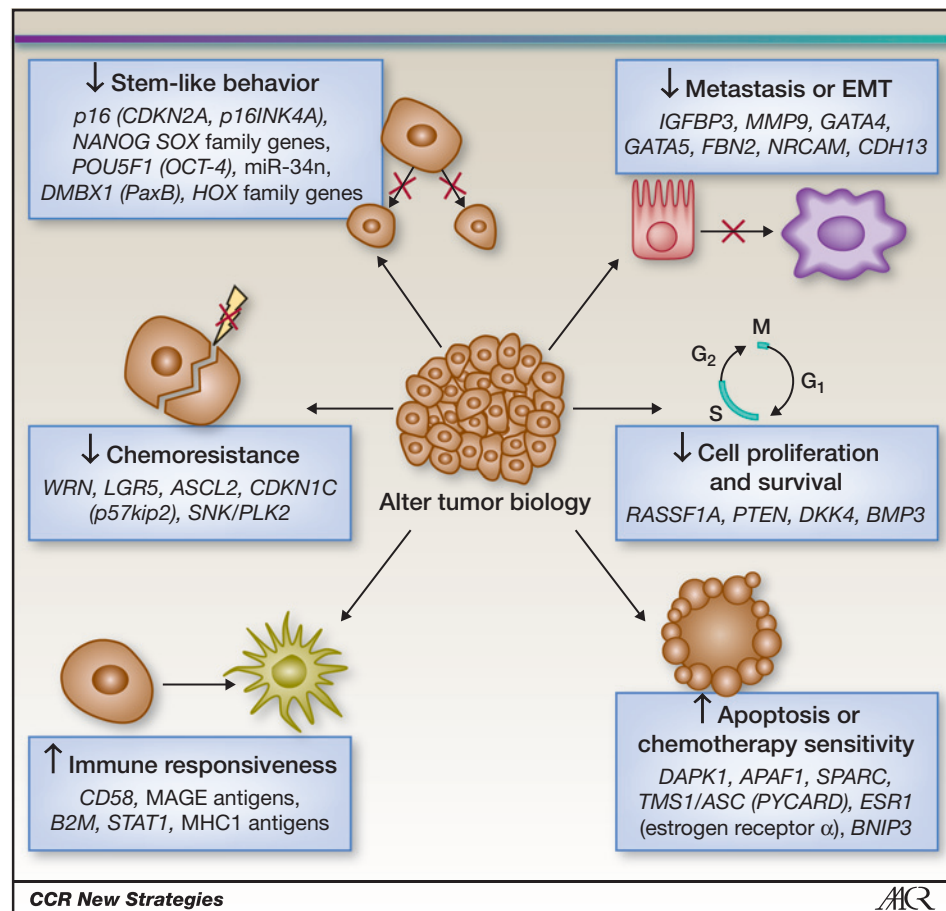
The epigenome consists of heritable modifications of DNA, histones, and chromatin that may act to modulate gene expression independently of DNA coding alterations. Epigenetic changes such as global DNA hypomethylation, regional DNA hypermethylation, and aberrant histone modification, each influence the expression of oncogenes and lead to development and progression of tumors (6). Crucially, epigenetic dysregulation, unlike genetic mutations, may be reversed by selectively targeted therapies. Epigenetic modifications that may be readily targeted with currently available therapies include regional DNA hypermethylation and histone hypoacetylation with hypomethylating agents and histone deacetylase inhibitors (HDI), respectively.

Tumor cells experience dramatic epigenetic changes, including CpG dinucleotide hypermethylation and loss of acetylation, thus downregulating tumor suppressor genes (TSG), while the converse also occurs, with pronounced hypomethylation of the promoter regions of oncogenes and microsatellite regions leading to their activation (Fig. 1; ref. 7).

Recent data suggest that modifiable epigenetic dysregulation may also mediate a drug-resistant subpopulation of cells within the heterogeneous tumor population (8).

To date, four drugs targeting epigenetic changes have achieved regulatory approval by the U.S. Food and Drug Administration (FDA), including decitabine, 5-azacytidine (both indicated for the treatment of high-risk myelodysplastic syndrome), vorinostat, and romidepsin (both indicated for cutaneous T-cell lymphoma). Although early clinical studies in NSCLC, using cytotoxic doses of these drugs as single agents, showed little evidence of activity, more recent, lower-dose combination studies of HDIs and hypomethylating agents have demonstrated signals of efficacy and more importantly suggest that the lung cancer epigenome can be modified in a clinically relevant manner (9, 10).

Figure 1. Concurrent widespread changes in gene expression with epigenetic therapy. EMT, epithelial-mesenchymal transition. Reprinted with permission from Azad et al. (44).



Epigenetic targets in NSCLC

DNA methylation. Hypermethylation of promoters and consequent silencing of TSGs in NSCLC drive oncogenesis by disrupting multiple cellular processes involved in growth and division; these include DNA repair, apoptosis, cell-cycle regulation, and regulation of both signaling pathways and invasion (8). CG dinucleotides occur at a high frequency in TSG promoters, and these CpG islands are usually unmethylated or hypomethylated in normal cells (11, 12). During malignant transformation, methylation of cytosine in CpG islands by DNA methyltransferases (DNMT) leads to repression of TSG transcription and potentiation of oncogenesis.

Histone deacetylation. Histone modification in specific gene promoter regions in turn modulates the expression of genes. Acetylation of lysine residues leads to transcriptionally active chromatin, whereas deacetylation on histone tails leads to inactive heterochromatin (12). Histone deacetylase (HDAC) enzymatic signaling leads to tightly packed DNA thus reducing access of transcription factors to coding regions, while conversely histone acetylation is controlled by histone acetyltransferases (HAT; ref. 13).

There are 18 human HDAC isoforms, and these are divided into four classes (class I–IV) with most containing metalloenzymes that require Zn^{2+} for catalytic activity. It is these metalloenzymes that are targeted by the majority of

the approved HDAC inhibitors. HDAC1 overexpression has been documented in many cancers, including NSCLC; however, several class II enzymes have also been reported to be downregulated resulting in poorer prognosis (14–16). Aberrant methylation may also lead to dysregulated HDAC function in lung cancer via interactions between methyl-binding proteins and corepressors such as mSin3A (17).

DNA methylation as a prognostic marker in NSCLC

Several studies have suggested that the presence of DNA hypermethylation in NSCLC tumor cells is associated with shorter recurrence-free survival (RFS) in stage I NSCLC (18, 19).

In a nested case-control study of 71 stage I NSCLC patients with recurrent disease and 158 control stage I patients without recurrent disease, Brock and colleagues studied methylation of six genes associated with lung cancer development and growth, including p16, CDH13, APC, RASSF1A, MGMT, ASC, and DAPK, using a multiplex methylation-specific PCR assay (18). Promoter methylation of p16, CDH13, APC, and RASSF1A was strongly associated with recurrence in apparently curatively resected early-stage patients, and patients with two or more methylated genes had a 5-year RFS of 27.3% compared with 65.3% for patients with less than two methylated genes ($P < 0.001$). In addition, methylation of both p16 and CDH13 in tumor and

mediastinal lymph nodes of patients was associated with a particularly poor prognosis when compared with unmethylated patients (5-year RFS, 0% vs. 53.3%, $P < 0.001$).

In a recent study of 587 patients with NSCLC, high-resolution DNA methylation analysis of CpG islands was used to develop a methylation signature associated with early recurrence in resected NSCLC (19). Five hypermethylated genes (HIST1H4F, PCDHGB6, NPBWR1, ALX1, and HOXA9) were found to be strongly associated with reduced RFS. The gene signature developed in this study divides patients into two arms, patients with zero to one methylated markers and longer RFS and those with two or more methylated markers and short RFS (HR = 1.95, $P 0.001$).

These findings are particularly relevant given the high rates of recurrence (30%–40%) noted in patients with resected stage I NSCLC (20). Retrospective analyses of large clinical trials have suggested that stage I patients with primary tumors ≥ 4 cm in diameter may benefit from adjuvant chemotherapy (21). Methylation analyses such as those outlined have the potential to further risk-stratify patients and guide the use of adjuvant therapy for surgically resected early-stage patients.

Translating lung cancer epigenetics into therapeutic strategies

DNA methyltransferase inhibitors—single-agent studies. Decitabine and 5-azacytidine are cytosine analogues that act to inhibit DNMT and consequently DNA methylation (22). Decitabine is a deoxyribonucleotide that is directly incorporated into DNA thus inhibiting DNA methylation, whereas azacytidine is a ribonucleotide precursor that has approximately 10% of the potency of

decitabine (22). Although their regulatory approvals to date have been in hematologic malignancies, several clinical trials of single-agent therapy have been conducted in solid tumors that included NSCLC (23).

Between 1972 and 1977, 103 patients with NSCLC received single-agent azacytidine on seven different solid tumor clinical protocols; however, efficacy proved extremely limited with an objective response rate of only 8% (23). Similarly more than 200 patients with NSCLC have been enrolled on clinical trials of single-agent decitabine with only two objective responses reported (23). These disappointing initial findings have moved the field toward investigation of combinatorial therapies in particular concurrent epigenetic therapy with HDIs.

HDAC inhibitors. Two HDIs have been FDA approved to date, vorinostat (SAHA) and romidepsin (depsipeptide), for use in cutaneous and peripheral T-cell lymphomas. It is unknown at present whether the strategy of using highly selective agents is better than broader targeting of multiple HDAC isoforms in lung cancer (24). HDIs have been demonstrated to have a multitude of anticancer effects, including causing G₁ cell-cycle arrest via activation of p21 and decreasing cyclin expression, ultimately leading to activation of apoptotic pathways (25). Additional effects include downregulation of checkpoint kinase 1, suppression of proangiogenic and matrix remodeling genes, and activation of T cells and natural killer cells by upregulating MHC class I and II, CD80/CD86 and MICA/MICB (25–27). Clinically the use of single-agent HDIs in patients with previously treated advanced NSCLC has yielded disappointing results with disease stabilization rather than objective response being the main effect (see Table 1). Although HDI monotherapy

Table 1. Selected trials investigating epigenetic therapies in NSCLC

Study design	Patients, N	Response rate (%)	PFS (months)	OS (months)	Reference
Combined epigenetic therapies					
Phase I/II of 5Aza + entinostat	45	4%	1.9	6.4	Juergens et al. (10)
Epigenetic therapy with chemotherapy					
Randomized phase II carboplatin/paclitaxel ± vorinostat (first line)	94	34% vs. 12.5%	6 vs. 4.1	13 vs. 9.7	Ramalingam et al. (33)
Randomized phase II gemcitabine ± C1-994	180	3.5% vs. 3.8%	NR	6.3 vs. 6.2	Von Pawel et al. (34)
Epigenetic therapy combined with targeted therapy					
Phase I/II erlotinib + vorinostat (patients with EGFRm progressing on erlotinib alone)	24	0%	2	10.2	Cardenal et al. (35)
Phase II vorinostat + bortezomib	18	0%	1.43	4.7	Jones et al. (36)
Randomized phase II erlotinib ± entinostat	132	3% vs. 9.2%	1.9 vs. 1.8	8.9 vs. 6.7	Witta et al. (37)
Epigenetic monotherapy					
Phase II vorinostat	16	0%	2.3	7.1	Traynor et al. (38)
Phase I/II decitabine	15	0%	NR	6.7	Momparler et al. (39)
Phase II fazarabine	23	0%	NR	8	Williamson et al. (40)
Phase II pivaloyloxymethyl butyrate	47	6.4%	1.5	6.2	Reid et al. (41)
Phase II romidepsin	19	0%	NR	NR	Schrump et al. (42)

Abbreviations: 5Aza, 5-azacytidine; EGFRm, EGFR mutant; NR, not reported; OS, overall survival; PFS, progression-free survival.

does not seem to be a successful strategy in NSCLC, there is promise that when combined with demethylating agents, the multitargeting approach may have more activity.

Dual targeted epigenetic therapy. Because of the limited activity of single-agent epigenetic therapy and the complications associated with DNMT inhibitors at cytotoxic doses, including prolonged cytopenias and consequent loss of dose intensity, the potential for combination low-dose epigenetic therapy has been explored (10). In a phase I/II study of combination azacytidine and etinostat, 45 heavily pretreated advanced NSCLC patients were enrolled (10). The recommended combination phase II dosage for the combination was azacytidine, 40 mg/m² (day 1–6 and day 8), and etinostat, 7 mg orally on day 3 and 10 on a 28-day cycle. Grade 3–4 toxicities were noted in 28% of patients, with fatigue and transient hematologic toxicity being the most common. Two patients had objective responses, including a complete response (CR) of 14-month duration and a partial response that lasted 8 months, in addition 10 patients had stable disease of at least 12 weeks in duration. Median progression-free survival (PFS) was 7.4 weeks and median survival was 6.4 months. Of interest, the patient who experienced a prolonged CR had experienced rapid tumor progression on three previous chemotherapy regimens and analysis of her tumor demonstrated candidate gene methylation (28). Despite having previously been refractory to standard systemic therapy, 25% of patients on this study had objective responses to the immediate poststudy therapy [these therapies included chemotherapy and also immunotherapy targeting the programmed death-1 (PD1) immune checkpoint], lending support to the hypothesis that combination epigenetic therapy may modify the sensitivity of tumors to systemic therapy (28).

Future directions. With the hypothesis that epigenetic therapy may epigenetically "prime" NSCLC tumors to systemic therapy, and given the interesting results noted above with standard cytotoxic therapy after epigenetic therapy in previously chemoresistant patients, we have initiated a randomized phase II study for second-line advanced NSCLC (29). Patients are randomized to either standard single-agent chemotherapy or alternatively to initial epigenetic therapy with etinostat and oral or intravenous azacytidine followed by standard single-agent chemotherapy on disease progression. PFS at 6 months is the primary endpoint for this study with secondary endpoints of traditional PFS and overall survival. We have also recently opened a randomized phase II clinical trial, in the second- and third-line advanced NSCLC setting, examining the role of initial epigenetic therapy with 5-azacytidine/etinostat or azacytidine alone for four cycles followed by the antiprogrammed death-1 antibody, nivolumab (30). Recent findings in NSCLC cell lines suggest that 5-azacytidine may upregulate several immune-related tumor genes, including programmed death-ligand 1 (PD-L1), one of the two ligands of PD1 and a target of several immune checkpoint inhibitors in clinical development (28). PD-L1 expression is a potential biomarker of response to PD1/PD-L1-directed therapeutics, hence

our interest in the potential for increased efficacy of anti-PD-1 after prior epigenetic therapy.

In each of these studies, we will assess a panel of candidate promoter methylation markers, including APC, HCAD, p16, RASSF1A, GATA4, and Actin, in serial plasma blood samples for changes induced by epigenetic therapy and subsequent chemotherapy or immunotherapy. Availability of initial tumor tissue for epigenetic analyses is a requirement for trial enrollment, and where possible patients will undergo repeat biopsies after epigenetic therapy. Promoter methylation, gene expression analysis, driver mutational status, and other candidate markers of epigenetic modulation will be evaluated in the pre- and posttreatment blood and tissue samples. By evaluating these markers in blood and tissue, we will assess the impact of epigenetic therapy on the patient and tumor and correlate this with clinical variables, including response and survival, with the aim of personalizing epigenetic therapy based on individual patient and tumor characteristics.

Other ongoing avenues of clinical investigation include a phase I study of inhaled azacytidine in patients with advanced NSCLC and a combination study of tetrahydrouridine and 5-fluoro-2-deoxycytidine for advanced solid tumors, including NSCLC (31, 32).

Translation of recent findings concerning the role of microRNAs and long noncoding RNAs in NSCLC into clinical trials is another promising avenue of investigation though beyond the scope of this article (43).

Conclusions

Epigenetic modifications play an important role in the development and progression of NSCLC. Recent data on the use of gene methylation as a prognostic marker for early-stage NSCLC are promising and may help to direct efforts toward targeted epigenetic therapy as adjuvant therapy.

The potential use of epigenetic therapy as a "priming" tool before cytotoxic or immunologic therapies for advanced NSCLC is currently being explored in prospective phase II clinical trials, and the results of these studies are awaited with interest.

Disclosure of Potential Conflicts of Interest

J.R. Brahmer reports receiving commercial research grants from ArQuele, Bristol-Myers Squibb, and MedImmune and is a consultant/advisory board member for Bristol-Myers Squibb (uncompensated), MedImmune, and Merck. R.J. Kelly is a consultant/advisory board member for Novartis. No potential conflicts of interest were disclosed by the other author.

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Development of methodology: P.M. Forde, R.J. Kelly

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.M. Forde

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.M. Forde

Writing, review, and/or revision of the manuscript: P.M. Forde, J.R. Brahmer, R.J. Kelly

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.M. Forde, R.J. Kelly

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