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

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Disclosure of Potential Conflicts of Interest

J.R. Brahmer reports receiving commercial research grants from Bristol-Myers Squibb, MedImmune, and ArQuele and is a consultant/advisory board member for Bristol-Myers Squibb (uncompensated), Merck, and MedImmune.

R.J. Kelly is a consultant/advisory board member for Novartis.

No potential conflicts of interest were disclosed by the other author.
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 has 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.

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 epidermal growth factor receptor (EGFR) gene or translocations affecting the anaplastic lymphoma kinase (ALK) gene(1)(2). Current standard therapy for advanced non-squamous NSCLC involves initial molecular profiling of the tumor to ascertain the presence or absence of a driver mutation. Approximately 50% of non-squamous 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 10-14 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.

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
hypomethylating agents and histone deacetylase inhibitors (HDIs) respectively.

Tumor cells experience dramatic epigenetic changes, including CpG dinucleotide hypermethylation and loss of acetylation thus downregulating tumor suppressor genes (TSGs), while the converse also occurs, with pronounced hypomethylation of the promoter regions of oncogenes and microsatellite regions leading to their activation (Figure 1)(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 United States Food and Drug Administration including decitabine, 5-azacytidine (both indicated for the treatment of high risk myelodysplastic syndrome), vorinostat and romidepsin (both indicated for cutaneous T cell lymphoma). While 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).

Epigenetic Targets in NSCLC

DNA Methylation

Hypermethylation of promoters and consequent silencing of TSGs in NSCLC drives oncogenesis by disrupting multiple cellular process 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 leads to re 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 while 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 (HATs)(13).

There are 18 human HDAC isoforms and they are divided into 4 classes (classes 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 over-expression has
been documented in many cancers including NSCLC however several class II enzymes have also been reported to be down-regulated resulting in poorer prognosis(14)(15)(16). Aberrant methylation may also lead to dysregulated HDAC function in lung cancer via interactions between methyl binding proteins and co-repressors 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 et al 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, patients with two or more methylated genes had a 5-year RFS of 27.3% compared with 65.3% for patients with fewer than two methylated genes, p<0.001. Additionally 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 NSCLC patients, 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 ≥4cm 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 DNA methyltransferase (DNMT) and consequently DNA methylation(22). Decitabine is a deoxyribonucleotide that is directly incorporated into DNA thus inhibiting DNA methylation while azacytidine is a ribonucleotide precursor that has approximately 10% of the potency of decitabine(22). While
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 NSCLC patients received single agent azacytidine on 7 different
solid tumor clinical protocols however efficacy proved extremely limited with an objective
response rate of only 8%(23). Similarly over 200 NSCLC patients 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.

134 HDAC inhibitors

Two HDIs have been FDA approved to date; vorinostat (SAHA, Zolinza®) and romidepsin
depsipeptide, Istodax®) for use in cutaneous and peripheral T cell lymphomas. It is unknown at
present if 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 down-regulation of checkpoint kinase 1, suppression of pro-angiogenic and matrix
remodeling genes and activation of T-cell and natural killer cells by upregulating MHC class I
and II, CD80/CD86 and MICA/MICB(25)(26)(27). Clinically the use of single agent HDIs in
patients with previously treated advanced NSCLC have yielded disappointing results with
disease stabilization rather than objective response being the main effect (see Table 1). While
HDI monotherapy does not appear to be a successful strategy in NSCLC there is promise that
when combined with demethylating agents the multi-targeting approach may have more activity.

Dual Targeted Epigenetic Therapy

Due to 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 40mg/m2 (day 1-6 & day 8) and etinostat 7mg po 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 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 post-study 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 chemo-resistant 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 (OS). We have also recently opened a randomized phase II clinical trial, in the 2nd and 3rd line advanced NSCLC setting, examining the role of initial epigenetic therapy with 5-azacytidine/etinostat or azacytidine alone for 4 cycles followed by the anti-programmed 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 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 post-treatment 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-flouro-2-deoxycytidine for advanced solid tumors including NSCLC(31)(32).
Translation of recent findings concerning the role of microRNAs and long non-coding 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 is promising and may help to direct efforts towards targeted epigenetic therapy as adjuvant therapy. The potential use of epigenetic therapy as a “priming” tool prior to 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.

References


29. Available at www.clinicaltrials.gov: Identifier NCT01935947

30. Available at www.clinicaltrials.gov: Identifier NCT01928576

31. Available at www.clinicaltrials.gov: Identifier NCT02009436

32. Available at www.clinicaltrials.gov: Identifier NCT00978250


Table 1. Selected trials investigating epigenetic therapies in NSCLC

<table>
<thead>
<tr>
<th>Study Design</th>
<th>No. of patients</th>
<th>Response Rate (%)</th>
<th>PFS (m)</th>
<th>OS (m)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined epigenetic therapies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase I/II of 5Aza + Entinostat</td>
<td>45</td>
<td>4%</td>
<td>1.9m</td>
<td>6.4m</td>
<td>Juergens et al (10)</td>
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<tr>
<td>Epigenetic therapy with chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Randomized Phase II Carboplatin/Taxol +/- Vorinostat (first line)</td>
<td>94</td>
<td>34% Vs 12.5%</td>
<td>6m Vs 4.1m</td>
<td>13m Vs 9.7m</td>
<td>Ramalingam et al(33)</td>
</tr>
<tr>
<td>Randomized Phase II Gemcitabine +/- C1-994</td>
<td>180</td>
<td>3.5 % Vs 3.8%</td>
<td>NA</td>
<td>6.3m Vs 6.2m</td>
<td>Von Pawel et al(34)</td>
</tr>
<tr>
<td>Epigenetic therapy combined with targeted therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase I/II Erlotinib + Vorinostat (Pts with EGFRm progressing on Erlotinib alone)</td>
<td>24</td>
<td>0%</td>
<td>2m</td>
<td>10.2m</td>
<td>Cardenal et al(35)</td>
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<tr>
<td>Phase II Vorinostat + Bortezomib</td>
<td>18</td>
<td>0%</td>
<td>1.43m</td>
<td>4.7m</td>
<td>Jones et al(36)</td>
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<tr>
<td>Randomized Phase II Erlotinib +/- Entinostat</td>
<td>132</td>
<td>3% Vs 9.2%</td>
<td>1.9m Vs 1.8m</td>
<td>8.9m Vs 6.7m</td>
<td>Witta et al(37)</td>
</tr>
<tr>
<td>Epigenetic monotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phase II Vorinostat</td>
<td>16</td>
<td>0%</td>
<td>2.3m</td>
<td>7.1m</td>
<td>Traynor et al(38)</td>
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<tr>
<td>Phase I/II Decitabine</td>
<td>15</td>
<td>0%</td>
<td>NR</td>
<td>6.7m</td>
<td>Momparler et al(39)</td>
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<tr>
<td>Phase II Fazarabine</td>
<td>23</td>
<td>0%</td>
<td>NR</td>
<td>8m</td>
<td>Williamson et al(40)</td>
</tr>
<tr>
<td>Phase II Pivaloyloxymethyl Butyrate (Pivanex)</td>
<td>47</td>
<td>6.4%</td>
<td>1.5</td>
<td>6.2</td>
<td>Reid et al[41]</td>
</tr>
<tr>
<td>Phase II Romidepsin</td>
<td>19</td>
<td>0%</td>
<td>NR</td>
<td>NR</td>
<td>Schrump et al(42)</td>
</tr>
</tbody>
</table>
Figure 1. Concurrent widespread changes in gene expression with epigenetic therapy

Reprinted with permission from Azad et al. (44).
Stem-like behavior
- p16 (CDKN2A, p16INK4A), NANOG
- SOX family genes, POU5F1 (OCT-4), miR-34n, DMBX1 (PaxB), HOX family genes

Immune responsiveness
- CD58, MAGE antigens, B2M, STAT1, MHC1 antigens

Apoptosis or chemotherapy sensitivity
- DAPK1, APAF1, SPARC, TMS1/ASC (PYCARD), ESR1 (oestrogen receptor α), BNIP3

Metastasis or EMT
- IGFBP3, MMP9, GATA4, GATA5, FBN2, NRCAM, CDH13

Cell proliferation and survival
- RASSF1A, PTEN, DKK4, BMP3

Immune responsiveness
- CD58, MAGE antigens, B2M, STAT1, MHC1 antigens

Chemoresistance
- WRN, LGR5, ASCL2, CDKN1C (p57kip2), SNK/PLK2

Alter tumor biology
- IGFBP3, MMP9, GATA4, GATA5, FBN2, NRCAM, CDH13
Clinical Cancer Research

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Clin Cancer Res Published OnlineFirst March 18, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-2088

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