Histone Deacetylase, Proteasome, and Heat Shock Protein Inhibitors for the Treatment of Lung Cancer

Suresh S. Ramalingam, MD

Systemic therapy is the mainstay of treatment of advanced stage non-small cell lung cancer (NSCLC) and is also integral to therapy for earlier stages of the disease. Combination chemotherapy regimens confer modest improvement in overall outcomes for patients with various stages of NSCLC. The emergence of molecularly targeted agents has paved the way for individualized treatment approaches for NSCLC and provides hope that newer classes of drugs can be developed successfully. This article provides an update on some novel targeted therapeutic approaches under evaluation for the treatment of NSCLC.

SUMMARY OF PRESENTATIONS

Histone Deacetylase Inhibitors

Histones are the core proteins in the double helical structure of DNA. Acetylation of histone proteins, mediated by histone acetyl transferase, leads to conformational changes in DNA that favor the transcriptional process.[1] On the other hand, histone deacetylase (HDAC) renders the DNA to a transcriptionally inactive state. The dynamic equilibrium between the acetylated and deacetylated states of histone proteins is altered in the milieu of cancer. Therapeutic inhibition of HDAC results in transcription of several important cell cycle regulatory proteins and could, therefore, be used for the treatment of cancer. A number of HDACs have been described in the literature, but the class I enzymes are considered to be the most relevant for the treatment of cancer. In addition to their effects on the histone proteins, HDAC inhibitors also affect the acetylation of nonhistone proteins that play major roles in cancer. Vorinostat, a HDAC inhibitor, has been approved by the Food and Drug Administration for the treatment of refractory cutaneous T-cell lymphoma. This led to the evaluation of vorinostat and other HDAC inhibitors in a variety of cancers. Dr. Charles Rudin explained the rationale for the use of HDAC inhibitors in cancer at this meeting.

Department of Hematology and Medical Oncology, Emory University, Winship Cancer Institute, Atlanta, Georgia.

Disclosure: Suresh S. Ramalingham, MD, has served on the ad hoc advisory board for Syndax.

Address for correspondence: Suresh S. Ramalingam, MD, Emory University, Winship Cancer Institute, 1365 Clifton Road NE, Rm C-3090, Atlanta, GA 30322. E-mail: suresh.ramalingam@emory.edu

ISSN: 1556-0864/10/0512-0433

Vorinostat

Dr. Chandra Belani provided an update on the development of vorinostat in NSCLC. This agent has demonstrated modest effects as monotherapy in patients with advanced NSCLC in a phase II study, but no responses were noted.[2] Therefore, a combination strategy involving vorinostat with carboplatin and paclitaxel was developed by Ramalingam et al.[3] The safety of this regimen was evaluated in a phase I study, which documented robust responses in patients with advanced NSCLC. This led to a placebo-controlled randomized phase II study of carboplatin and paclitaxel with or without vorinostat for first-line therapy.[4] There was a statistically significant improvement in response rate (34% versus 13%) with the addition of vorinostat and also a trend toward improved progression-free survival (6 versus 4.1 m) and median survival (13 versus 9.7 m). Certain toxicities such as thrombocytopenia, nausea, emesis, diarrhea, and fatigue were more common with vorinostat and chemotherapy. In contrast to the encouraging results of this study, a phase II/III study conducted by Merck noted no improvement in efficacy with the addition of vorinostat to chemotherapy. The two studies used a different schedule and also had differences in patient eligibility criteria. Overall, the issue regarding toxicities associated with vorinostat that led to the discontinuation of treatment in a number of patients has to be addressed before further development of this agent in NSCLC. A follow-up study to evaluate a shorter schedule of vorinostat (5 days on and 16 days off) is currently being planned.

Entinostat

Entinostat is an orally administered inhibitor of class I HDAC. It is given once every other week and has a favorable tolerability profile. Two different strategies are currently being pursued with this agent. The synergy between HDAC inhibitors and epidermal growth factor receptor (EGFR) inhibitors[5] led to a randomized phase II study of erlotinib given alone or in combination with entinostat to patients with recurrent or refractory NSCLC. This trial has completed accrual, and the results are awaited.

Dr. Rudin described another novel strategy of combining entinostat with a demethylating agent that demonstrated synergistic induction of gene expression in preclinical studies.[6] A phase I study with the combination of 5-Azacytidine and entinostat has been completed and demonstrated good tolerability. The common toxicities were injection site reactions, nausea, emesis, peripheral edema, and electrolyte abnormalities. Most of the toxicities were grade 1 or 2 in severity. One complete response and one partial response

were noted in 28 evaluable patients. Eight patients had stable disease. The responses were slow to occur, which indicates the need for continuation of the combination for at least a few cycles to achieve responses. The patient who achieved a complete response had much higher plasma levels of 5-azacytidine than the others. Analysis of the diagnostic biopsy revealed multiple methylated loci in this patient. A follow-up study testing a shorter schedule of 5-azacytidine for 8 days of each cycle is being planned. Entinostat will be given on days 3 and 10 of each cycle.

Romidepsin

Dr. David Gerber presented an update on romidepsin, a pan-HDAC inhibitor that has demonstrated activity in lung cancer cell lines. It enhances erlotinib effect on NSCLC cell lines in vivo and in vitro.[7] Inhibition of mitogen-activated protein kinase phosphorylation by romidepsin correlates with increased efficacy of erlotinib. A phase I/II study is currently ongoing to evaluate the combination of erlotinib and romidepsin in advanced NSCLC. The combination has been tolerated well without any dose-limiting toxicities to date. Romidepsin is given on days 1, 8, and 15 of each 28-day cycle. Erlotinib is given from day 4 onward. Dose escalation has been completed for the 8 mg/m^2 and 10 mg/m^2 cohorts. Preliminary correlative studies have demonstrated a near fivefold increase in histone acetylation.

In addition to these compounds, other HDAC inhibitors such as panabinostat and belinostat are also in various stages of clinical investigation.

Proteasome Inhibitors

Ramalingam et al. summarized the data with proteasomal inhibitors in NSCLC. The ubiquitin-proteasome machinery plays an important role in cellular protein degradation and thereby regulates key cellular functions.[8] Inhibition of the proteasome with bortezomib is used as a standard treatment approach for patients with multiple myeloma. Bortezomib is an irreversible inhibitor of the 26 S ribosomal activity. It has demonstrated modest single-agent activity in patients with advanced NSCLC.[9] The combination of bortezomib with standard chemotherapy has not resulted in a major improvement over the efficacy of chemotherapy alone and is unlikely to be pursued further. A phase II study by Ramalingam et al. with bortezomib in patients with advanced bronchioloalveolar carcinoma noted a promising disease stabilization of 70%. In a randomized phase II study by Lynch et al., bortezomib did not add to the efficacy of erlotinib when given as a combination. Taken together, it is unlikely that bortezomib will be developed further in NSCLC. Nevertheless, several newer proteasome inhibitors offer hope based on their favorable pharmacokinetic properties and target selectivity over that of bortezomib. Carfilzomib, an irreversible proteasome inhibitor, has demonstrated favorable anticancer effects in bortezomib-refractory cases of myeloma and is currently in phase II studies. Other proteasomal inhibitors currently under development include MLN-9708, CEP-18770, PR-047, and NPI-0052. It is hoped that these agents will demonstrate favorable anticancer effects in patients with NSCLC.

Dr. Howard West provided the rationale for the use of the ubiquitin pathway as a target for cancer therapy. Ubiquitin is a highly conserved regulatory protein that labels proteins for degradation. It controls stability, function, and cellular localization of a number of important proteins. A mutation in CBL, an E3 ubiquitin ligase, has been documented in NSCLC. CBL interacts with EGFR signaling and could serve as a therapeutic target for combined inhibition with EGFR inhibitors. PYR-41, an E1 inhibitor, is currently under investigation in preclinical studies and several other selective inhibitors are likely to be developed. This group of agents is very early in the course of development and is not currently in clinical investigation.

Heat Shock Protein Inhibitors

Heat shock proteins (HSPs) are among the most highly expressed proteins and account for 1 to 2% of the total protein in unstressed cells.[10] They play an important chaperone function and facilitate protein folding and transport. HSPs serve as client proteins for EGFR, HER2, vascular endothelial growth factor receptor 2, phosphatidylinositol 3-kinase, Akt, Src, HIF1, p53, and CDKs. Based on these observations, inhibition of HSP has emerged as a therapeutic strategy against cancer.

AUY 922

Dr. Greg Riely discussed early data with AUY922, a HSP 90 inhibitor that has demonstrated anticancer effects in xenograft models. An ongoing phase I study evaluates a weekly schedule of AUY 922 with an initial dose of 2 mg/m^2. Sixty patients have been enrolled to date. Adverse events with AUY-922 included asthenia, nausea, diarrhea, and vomiting. Visual symptoms were noted at higher doses (>40 mg/m^2). The dose-limiting toxicities were fatigue, diarrhea and atrial fibrillation. Maximum tolerated dose is 70 mg/m^2. Though no objective responses have been noted, dose-dependent induction of HSP 70 has been demonstrated. Phase II evaluation is currently underway.

STA-9090

Dr. Geoffrey Shapiro discussed the development of STA-9090, a potent second generation small molecule HSP 90 inhibitor (nongeldanamycin). It degrades oncogenic client proteins and has deep penetration into hypoxic tumors. Preclinical synergy has been noted with a variety of other anticancer agents. Phase I evaluation of STA-9090 has been completed with a 1-hour intravenous infusion given once or twice weekly. This novel agent depletes mutant EGFR in low concentrations and has demonstrated activity in EGFR resistant cell lines. It also depletes MET thereby providing another mechanism to overcome EGFR resistance. A phase II study is currently underway in patients with advanced NSCLC. Three cohorts of patients will be enrolled: EGFR mutation with failure of EGFR TKI, KRAS mutation, and KRAS wild type. A phase I study of STA-9090 in combination with docetaxel will soon be initiated.

IPI-504

Dr. Sequist discussed the promising early data with IPI-504, a potent and selective inhibitor of HSP90. It has demonstrated a response rate of 7% when given as monotherapy in a cohort of patients with heavily pretreated advanced NSCLC. This study is built on the observation that mutated EGFR is a sensitive client protein of HSP90. Both T790 mutation and MET amplification are susceptible to HSP90 inhibition, and therefore, an ongoing study is evaluating the role of IPI-504 in patients who failed prior therapy with an EGFR inhibitor. Two confirmed responses have been noted among 28 patients with wild-type EGFR, though none were seen in patients with mutated EGFR. IPI-504 was tolerated well, and the study is ongoing.

CONCLUSIONS AND FUTURE DIRECTIONS

Lung cancer is a heterogenous disease and is often not driven by a single molecular pathway. Therefore, therapeutic approaches that target multiple pathways are necessary for treatment of NSCLC. HDAC, proteasome, and HSP inhibitors have the ability to target a number of vital targets and, therefore, represent promising avenues for therapy. Further characterization of the specific molecular targets, development of novel combinations, and evaluation for predictive biomarkers are important next steps.

REFERENCES

1. Marks P, Rifkind RA, Richon VM, et al. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer* 2001;1:194–202.
2. Traynor AM, Dubey S, Eickhoff JC, et al. Vorinostat (NSC# 701852) in patients with relapsed non-small cell lung cancer: a Wisconsin Oncology Network phase II study. *J Thorac Oncol* 2009;4:522–526.
3. Ramalingam SS, Parise RA, Ramananthan RK, et al. Phase I and pharmacokinetic study of vorinostat, a histone deacetylase inhibitor, in combination with carboplatin and paclitaxel for advanced solid malignancies. *Clin Cancer Res* 2007;13:3605–3610.
4. Ramalingam SS, Maitland ML, Frankel P, et al. Carboplatin and Paclitaxel in combination with either vorinostat or placebo for first-line therapy of advanced non-small-cell lung cancer. *J Clin Oncol* 2010;28:56–62.
5. Witta SE, Gemmill RM, Hirsch FR, et al. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res* 2006;66:944–950.
6. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med* 2003;349:2042–2054.
7. Zhang W, Peyton M, Xie Y, et al. Histone deacetylase inhibitor romidepsin enhances anti-tumor effect of erlotinib in non-small cell lung cancer (NSCLC) cell lines. *J Thorac Oncol* 2009;4:161–166.
8. Mitchell BS. The proteasome—an emerging therapeutic target in cancer. *N Engl J Med* 2003;348:2597–2598.
9. Fanucchi MP, Fossella FV, Belt R, et al. Randomized phase II study of bortezomib alone and bortezomib in combination with docetaxel in previously treated advanced non-small-cell lung cancer. *J Clin Oncol* 2006;24:5025–5033.
10. Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol Med* 2002;8:S55–S61.