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ORIGINAL ARTICLE

A phase I study of 5-azacytidine and erlotinib in advanced solid tumor malignancies

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Abstract
Introduction The epidermal growth factor receptor (EGFR) is a validated target in malignancy; however, patients with wild type EGFR obtain little sustained benefit from anti-EGFR monotherapy. Epigenetic therapy to reactivate tumor suppressor genes may enhance the anti-proliferative effect of erlotinib. This phase I study evaluated the combination of erlotinib and 5-azacytidine for safety and maximal tolerated dose (MTD).
Methods Thirty patients with advanced solid tumors were treated in a standard 3 + 3 cohort design. Erlotinib was dosed at 150 mg daily, and 5-azacytidine was escalated by increasing the number of daily doses of 75 mg/m^2 per cycle. Patients were followed for dose-limiting toxicity (DLT). Efficacy was assessed by RECIST criteria.
Results Common non-hematologic toxicities included rash, diarrhea, nausea, and fatigue; the majority was $\leq$ Grade 2. DLTs included conjunctivitis in cohort 1 and infusion reaction in cohort 2. No DLTs occurred in cohorts 3, 4, or 5; however, 2 serious neutropenic infections arose in cohort 5 after cycle 1. Cohort 4 was expanded to 6 patients and was the MTD. Partial response (lung, ovarian) and stable disease occurred in 2 and 11 patients, respectively. Median progression-free survival was 2 months. Two patients with lung and larynx cancer had prolonged stable disease.
Conclusion The combination of erlotinib and 5-azacytidine was well tolerated with interesting clinical activity in lung, head and neck, and ovarian cancer. The recommended dose for phase II study is erlotinib 150 mg daily and 5-azacytidine 75 mg/m^2 daily on days 1–4 and 15–18 of a 28-day cycle.

Keywords 5-azacytidine · DNA methylation · Erlotinib · Epidermal growth factor receptor · Epigenetic · Clinical trial · Phase I

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Introduction

Cell proliferation is regulated by a complex and intersecting set of signal transduction pathways, often deregulated in human cancer. The human epidermal growth factor receptor (EGFR or HER-1) is a member of the HER (*Erb*B) family of transmembrane glycoprotein receptors, comprised of an extracellular ligand binding domain and an intracellular receptor tyrosine kinase (RTK) which initiates downstream mitogenic signaling [1]. Aberrant EGFR signaling in malig-

nancy occurs via gene amplification, protein over expression, or constitutive activation secondary to RTK mutation. Increased activation of EGFR has been associated with poor prognosis in multiple cancers including lung, head and neck, colorectal and pancreatic and accordingly, has been a target for drug development [2–5]. Two classes of anti-EGFR therapy have been validated in human malignancy: the anti-EGFR monoclonal antibodies cetuximab and panitumumab, which bind the extracellular domain of EGFR; and the small molecule RTK inhibitors erlotinib and gefitinib, which bind the intracellular ATPase domain.

Early studies in non-small cell lung cancer (NSCLC) identified a subgroup experiencing marked benefit from erlotinib: patients with acquired mutations in the ATP binding pocket of EGFR resulting in constitutive activation [6]. However, such oncogene addiction is rare in solid tumor oncology; the EGFR mutated population comprises 10–17% of NSCLC and has not been identified in colorectal, head and neck, or pancreatic cancer [7]. Most patients with wild type EGFR exposed to anti-EGFR therapy achieve little sustained benefit, raising interest in mechanisms of resistance and therapeutic combinations that may overcome it. EGFR signaling interacts with multiple downstream pathways including Ras/Raf/MAPK, PI3K/Akt, STAT, and Src Kinase. Thus, potential molecular resistance mechanisms include activation of alternative RTKs that bypass EGFR, such as PI3K, Ras, and Stat 3; and constitutive activation of the EGFR signaling cascade downstream of EGFR as seen in K-ras mutations or PTEN silencing [8].

A second initiator of human malignancy is the altered genome. DNA mutations have been long understood to cause cancer, by enhancing the function of oncogenes or by silencing tumor suppressor genes. Epigenetic silencing of tumor suppressor genes is increasingly recognized as a common event in cancer initiation and progression. Mammalian cells can undergo post-replicative modification of DNA by covalent addition of a methyl group to the 5-position of cytosine within a CpG dinucleotide by a family of enzymes called DNA methyltransferases (DNMTs) [9]. The CpG dinucleotide is over-represented in the promoter region of approximately half of human genes, occurring in clusters called CpG islands. CpG islands are usually unmethylated in normal cells; however, in cancer, hypermethylation of promoters is frequent. Promoter hypermethylation of multiple genes has been identified in most human cancers and results in transcriptional silencing of tumor suppressors whose function is to regulate cell growth and signaling [10]. Critical cellular pathways disrupted by aberrant promoter methylation of tumor suppressors include cell-cycle regulation ($p16^{INK4a}$, $p15^{INK4b}$, $p14^{ART}$, Rb, p73), DNA repair (O^5-MGMT and *h*MLH1), cell–cell adhesion (E-cadherin, VHL, APC), apoptosis (DAPK, TMS1), and growth factor response (RARβ, SOCS-1, PTEN) [9]. Unlike genetic mutations, gene silencing by promoter hypermethylation is potentially reversible. 5-Azacytidine is a cytosine analog that inhibits DNMTs, with consequent hypomethylation of DNA. 5-Azacytidine has been tested in multiple cancer cell lines and shown to re-express methylated genes. It is approved for the treatment of myelodysplastic syndromes (MDS).

We hypothesize that restoration of tumor suppressor function by demethylation will enhance the anti-proliferative and pro-apoptotic effect of EGFR blockade in solid malignancy. As a general principle, broad re-expression of tumor suppressors may restore appropriate proliferative-apoptotic balance in malignant cells. Re-expression of p15, p21, or p27, cell cycle inhibitors downstream of EGFR, or PTEN, a PI3K/Akt inhibitory protein, may have particular synergy with anti-EGFR therapy. The goal of this phase I study was to establish the safety of combining erlotinib with 5-azacytidine in patients with refractory solid tumors. Further, we sought the maximal tolerated dose (MTD) of the combination when erlotinib is administered continuously at the FDA-approved dose, and 5-azacytidine is administered at a dose and schedule associated with broad gene promoter demethylation [11], for future biomarker and phase II studies.

Materials and methods

The study was approved by the Medical Scientific Review Committee at the University of New Mexico and by the Western Institutional Review Board.

Eligibility

All patients were required to have a histologic diagnosis of a solid tumor malignancy that was either locally advanced and incurable or metastatic. All were required to have disease which had been previously treated and/or for which there was no acceptable standard treatment regimen. All were appropriate candidates for treatment, with an Eastern Cooperative Oncology Group (ECOG) performance status of $\leq$2 at the time of the initiation of therapy, adequate end-organ function, no severe co-morbidity, and ability to provide informed consent. Patients were excluded if they were pregnant or lactating women; had a myocardial infarction or unstable angina within 6 months; had uncontrolled‘ clinically significant dysrhythmia; had evidence of uncontrolled metastatic disease of the central nervous system; and had received either radiotherapy or surgery within 2 weeks. All patients were required to sign an informed consent.

Study evaluations

Within 4 weeks prior to the initiation of therapy, participants underwent the following screening assessments:

Table 1 Dosing cohorts

Dosing cohort	Erlotinib dose (mg/day)	5-Azacytidine dose (mg/m^2/day)	Route	Number of days every 2 weeks	5-Azacytidine dose per cycle (mg/m^2)	5-Azacytidine dosing on day	# Patients[a]
1	150	75	IV	1	150	1, 15	3 + 3
2	150	75	IV	2	300	1, 2, 15, 16	3 + 3
3	150	75	IV	3	450	1–3, 15–17	3
4	150	75	SC	4	600	1–4, 15–18	3 + 3[b]
5	150	100	SC	4	800	1–4, 15–18	3 + 3

[a] One patient was replaced in cohorts 1, 2, and 5 secondary to withdrawal or disease progression during cycle 1

[b] Initially, 3 patients were treated on cohort 4 without DLT, and dose was escalated. After excess late toxicity in cohort 5, cohort 4 was expanded to total of 6 and declared the MTD

baseline imaging for RECIST measurements (version 1.0); medical history and physical examination; complete blood count with leukocyte differential (CBCD); basic metabolic profile; hepatic function panel; baseline tumor marker assessment if applicable. CBCD, metabolic profile, and hepatic function were repeated with each treatment cycle, along with documentation of toxicities and an interim history and physical exam. Baseline imaging was repeated after each two cycles of therapy (every 8 weeks) while receiving protocol treatment. All toxicities were graded according to the Common Terminology Criteria for Adverse Events from the National Cancer Institute, Version 3.0 (NCI-CTCAE).

Treatment

This was a single-arm, non-randomized phase I trial of erlotinib administered at the standard U.S. Food and Drug Administration-approved dosage of 150 mg daily, in combination with escalating doses of 5-azacytidine in cohorts of 3 + 3 (see Table 1). For the first 4 cohorts, the 5-azacytidine dose was escalated by increasing the number of days of administration at 75 mg/m^2 per 2-week cycle. In cohort 5, the daily dose was increased to 100 mg/m^2 for 4 days every 2 weeks. Patients were instructed to take erlotinib on an empty stomach, either 1 h before or 2 h after a meal. There were no protocol-specific pre-medications, which were left to the discretion of the treating physician. During the first 3 cohorts, 5-azacytidine was administered intravenously; to enhance patient convenience and compliance, the protocol was amended for subcutaneous (SC) administration for cohorts 4 and 5.

Dose escalations and modifications

Doses of erlotinib were not escalated, and modifications were made in accordance with the package insert. Patients who developed severe (NCI-CTCAE Grade 3-4) diarrhea, gastroenteritis, mucositis, or rash were managed by conservative measures, including dosage interruptions and reductions, as well as the use of anti-motility agents such as loperamide. Patients who required dosage interruption could remain off protocol therapy for up to 28 days before being removed from the protocol. When dose reduction was necessary, the erlotinib dose was reduced in 50 mg/day decrements. If patients acutely developed new or progressive pulmonary symptoms, including dyspnea, cough, or fever, treatment with erlotinib was interrupted and a diagnostic evaluation to rule out interstitial lung disease (ILD) was performed. If ILD was diagnosed, erlotinib was permanently discontinued and supportive care instituted as necessary.

Dose escalation rules for 5-azacytidine followed the standard "3 + 3" cohort design. Initially, 3 patients were enrolled into each cohort, beginning with erlotinib 150 mg PO daily, days 1–28, and 5-azacytidine 75 mg/m^2 intravenously on days 1 and 15 (dose level 1). If none of the first 3 patients experienced dose-limiting toxicity (DLT) by Day 28 of Cycle 1, then the dose was escalated in the subsequent cohort to the next higher dose level. If 1 of the 3 patients experienced DLT by Day 28 of Cycle 1, then the cohort was expanded to 6 patients. If none of these 3 additional patients experienced DLT, then the dose was escalated to the next higher dose level in the subsequent cohort. If $\geq$2 of initial 3 or 6 patients at one dose level experienced DLT then the maximum tolerated dose (MTD) was exceeded and up to 9 more patients were treated at the next lower dose level to further characterize the safety, tolerability, and anti-tumor effects of the combination.

A DLT was defined as a grade 4 hematologic or non-hematologic toxicity within the first cycle of the combination and was required to be at least possibly related to treatment. Non-hematologic toxicities necessitating dose reduction during cycle 1 were also considered DLTs.

Criteria for response and toxicity

Toxicity was assessed at each dose level, and each cycle during therapy. The duration of each adverse event, and its treatment, if any, was recorded. Grade 4 non-hematologic toxicities were reported immediately to the study chair.

Response Criteria for Solid Tumors (RECIST version 1.0) [12] or Gynecologic Cancer Intergroup criteria [13] were utilized to determine antitumor response if appropriate. Progression-free survival duration was determined from the point of protocol entry until progression or death. Survival duration was determined from the point of protocol entry until death. Living patients were censored at last follow up for vital status.

Statistical considerations

This was a phase I dose escalation trial. There were 5 dosing cohorts planned with a minimum of 21 and a maximum of 36 patients enrolled. The analysis of demographic characteristics (age, gender, smoking history, and ethnicity) and baseline characteristics, including weight change, performance status, and histologic subtype, was descriptive. The primary endpoint of this study was to document the toxicities and their reversibility of the combination of 5-azacytidine and erlotinib and to define maximal tolerable dose of the combination. The secondary endpoints of this study were to describe any potential anti-tumor effects, as determined by the objective tumor response (complete and partial responses), clinical benefit (complete and partial responses, plus stable disease), progression-free survival (PFS), and overall survival (OS).

Results

Patient demographics

A total of 30 subjects were enrolled from August 2008 through October 2010. All were evaluable for protocol toxicity and OS, 26 for response, and 29 for PFS. There were 14 women and 16 men; the median age was 58.5 years (range 31–79 years). Baseline characteristics are presented in Table 2.

Toxicity

A total of 91 cycles of protocol therapy were delivered, with a median number of two cycles (range 0.5–9 cycles). Six patients did not complete the first cycle of protocol therapy. One withdrew consent, one was hospitalized for a serious adverse event (SAE) unrelated to protocol therapy, and 4 were taken off study due to clinical evidence of rapid disease progression.

Eight SAEs occurred during protocol therapy. Three SAEs were considered at least possibly related to protocol therapy: Neutropenic pneumonia; neutropenic sigmoid diverticulitis and *Escherichia coli* bacteremia; dehydration. Patients fully recovered from all three treatment-related SAEs. The remaining 5 events were related to progression of underlying disease.

Treatment-related toxicities are reported in Table 3. The most common non-hematologic toxicities were acneiform rash (70%), diarrhea (43%), nausea/vomiting (40%), and fatigue (37%); the vast majority were Grade 1 or 2. One participant was observed to have asymptomatic pneumonitis on restaging CT scan after two cycles and was removed from the study. The most common hematologic toxicity was neutropenia, with eight patients experiencing Grade 3–4 neutropenia; 2 neutropenic events were complicated by infection and recorded as SAEs. There were no treatment-related deaths.

Dose-limiting toxicity and maximal tolerated dose

In cohort 1, one patient experienced grade 2 conjunctivitis during the first cycle of protocol therapy. Delay and subsequent dose reduction of erlotinib were required, and consequently the cohort was expanded to 6 patients without further DLT. In cohort 2, one patient developed a Grade 2 infusion reaction to 5-azacytidine. She was successfully re-challenged with 50% dose reduction, and experienced PR. The cohort was expanded to 6 patients without further DLT. No DLT was encountered in cohort 3, 4, or 5. When cohort 5 was expanded to confirm MTD, two SAEs occurred (neutropenic pneumonia; neutropenic diverticulitis) in patients beyond cycle 1 of therapy. Consequently, this dose was not considered acceptable for continuous administration in phase II studies, despite the absence of DLT in 6 evaluable patients. Three additional patients were subsequently enrolled in cohort 4, and the recommended dose for phase II study is erlotinib 150 mg daily and 5-azacytidine 75 mg/m^2 daily on days 1–4 and 15–18 of a 28-day cycle.

Clinical benefit

Among response-evaluable patients, the rate of clinical benefit was 50% (13/26 subjects including 2 PR and 11 SD). Two partial responses were observed: a confirmed response lasting 6 months in a patient with previously treated adenocarcinoma of the lung, and an unconfirmed response lasting 3 months in a patient with serous ovarian carcinoma. Disease control lasting >4 months was observed in 8 patients with the following disease histologies: lung adenocarcinoma (2), HPV-negative head and neck squamous cell carcinoma (SCC) (2) cervix SCC (1), vulvar SCC

Table 2 Patient characteristics

Subject #	Histology[a]	Age	Gender	Race/ Ethnicity[b]	PFS (months)	Number of prior systemic therapies	Prior exposure to anti-EGFR therapy	Best response
1	Cervix (SCC)	53	F	NHW	5.3	2	N	SD
2	GIST (Sarcoma)	72	F	H	1.6	2	N	PD
3	Prostate	58	M	NHW	1	2	N	PD
4	Kidney (Wilm's Tumor)	30	M	H	Inevaluable	3	N	Inevaluable
5	Ovary (Undifferentiated)	55	F	H	1.1	2	N	PD
6	Vulva (SCC)	68	F	H	5.6	1	N	SD
7	Thyroid (Anaplastic sarcomatoid)	73	M	NHW	1.8	2	N	PD
8	Skin (Melanoma)	67	M	NHW	1.9	1	N	PD
9	Uterine (AdenoCA with squamous differentiation)	50	F	NHW	0.5	3	N	PD
10	Ovary (Serous)	46	F	H	3	2	N	PR
11	Base of tongue (SCC)	67	M	NHW	3.9	1	Y (cetuximab)	SD
12	Ovary (Papillary serous)	46	F	NHW	5.4	6	Y (lapatinib)	SD
13	Cervix (SCC)	48	F	NA	5.2	2	N	SD
14	Ureter (Papillary urothelial)	58	M	H	1.7	2	N	PD
15	Kidney (Clear cell)	61	M	H	1	4	N	PD
16	Prostate (AdenoCA)	53	M	NHW	2.1	3	N	SD
17	Ovary (Serous)	55	F	NA	4	4	N	SD
18	Base of tongue (SCC)	53	M	NHW	1.3	2	Y (cetuximab)	PD
19	Lung (AdenoCA)	68	F	NHW	6.9	4	Y (gefitinib, erlotinib)	SD
20	Lung (Small cell)	53	M	AA	1.4	2	N	PD
21	Lung (AdenoCA)	69	M	NHW	5.9	2	N	PR
22	Breast (Ductal)	52	F	NHW	8.3	1	N	SD
23	Larynx (SCC)	65	M	NHW	8.9	2	Y (cetuximab)	SD
24	Ovary (AdenoCA)	50	F	H	1.2	4	Y (lapatinib)	Inevaluable
25	Parotid (ACC)	69	M	NHW	1.8	0	N	PD
26	Sarcoma (Myxoid)	48	M	NHW	2	5	N	PD
27	Ovary (Serous)	58	F	NHW	2.5	2	N	SD
28	Esophagus (AdenoCA)	72	M	NA	3.9	3	N	Inevaluable
29	Colon (AdenoCA)	66	M	NA	2	3	N	PD
30	Ovary (Papillary serous)	74	F	NHW	1.9	3	N	Inevaluable

[a] *Histology, abbreviations*: *SCC* Squamous cell carcinoma, *adenoCA* adenocarcinoma, *ACC* adenoid cystic carcinoma

[b] *Race/ethnicity abbreviations*: *NHW* Non-hispanic white, *H* Hispanic, *NA* Native American, *AA* African American

(1), ovarian serous carcinoma (1), breast ductal carcinoma (1). Two patients experienced disease control lasting longer than 6 months: a patient with platinum and cetuximab-refractory head and neck SCC for 8.9 months, and a patient with EGFR wild type, erlotinib-resistant adenocarcinoma of the lung for 6.9 months. The median PFS for the 29 progression-evaluable patients was 2 months (range of 0.5–8.9 months). The median OS was 7.5 months (range 1–20 months). At the time of data analysis, 5 subjects were still alive.

Discussion

Despite significant progress in chemotherapy, the majority of patients with advanced malignancy die within 2 years of diagnosis. Recently, in an effort to increase efficacy and therapeutic index, new therapies seek to exploit molecular differences between normal somatic cells and their malignant counterparts. EGFR has been extensively investigated and validated as a therapeutic target in lung, colorectal, pancreatic, and head and neck cancers. However, few

Table 3 Toxicities

	Grade 1	Grade 2	Grade 3	Grade 4	Total (%)
Non-hematologic toxicities					
Acneiform rash	7 (23%)	13 (43%)	1 (3%)		70
Alopecia	2 (7%)				7
Diarrhea	7 (23%)	5 (17%)	1 (3%)		43
Dry skin	2 (7%)	1 (3%)			10
Fatigue	2 (7%)	8 (27%)	1 (3%)		37
Infection		4 (13%)	2 (7%)		20
Injection site reaction	3 (10%)				10
LFT abnormality	4 (13%)	2 (7%)			20
Mouth Sores/Mucositis	3 (10%)				10
Nausea/Vomiting	9 (30%)	3 (10%)			40
Peripheral neuropathy			1 (3%)		3
Pneumonitis		1 (3%)			3
Taste alteration	3 (10%)	3 (10%)			20
Hematologic toxicities					
Anemia	2 (7%)	4 (13%)	1 (3%)		23
Neutropenia	1 (3%)	2 (7%)	5 (17%)	3 (10%)	37
Thrombocytopenia	4 (13%)	1 (3%)			17

patients with wild type EGFR experience prolonged benefit from EGFR monotherapy. We hypothesize that the efficacy of erlotinib will be enhanced by concurrent hypomethylating therapy with 5-azacytidine, secondary to re-expression of tumor suppressors interacting with the EGFR signaling cascade.

The first objective of this phase I study was to ensure the safety of combining erlotinib and 5-azacytidine in patients with refractory solid tumors. The regimen was well tolerated, with an additive rather than synergistic toxicity profile. Common, reversible toxicities of erlotinib include acneiform rash and diarrhea [14]. In addition, the potentially serious toxicity of pneumonitis occurs in up to 1% of cases worldwide, more commonly in the Asian experience [15]. Here, the rates of rash, diarrhea, and pneumonitis were numerically similar to trials evaluating erlotinib monotherapy. In MDS, myelosuppression is the most common adverse event associated with 5-azacytidine, while the most common non-hematologic toxicities include mild injection site reaction and nausea [16]. In this study, significant hematologic toxicity was uncommon until cohort 5, when two serious neutropenic infections occurred in patients beyond cycle 1. The propensity for bone marrow suppression is lower in solid tumors than in MDS, where the malignant cell itself is a myeloid precursor. Nonetheless, at the dose of 100 mg/m^2 daily for 4 days, administered every 2 weeks, the antimetabolite function of 5-azacytidine resulted in unacceptable myelotoxicity for uninterrupted dosing.

The second objective of this study was to identify the MTD of the combination of erlotinib, administered at 150 mg daily, and 5-azacytidine when administered at doses associated with broad gene demethylation. 5-Azacytidine is an azanucleoside originally developed as an antimetabolite, however, later recognized as an inhibitor of DNA methylation. In 2004, 5-azacytidine was FDA approved in MDS, a pre-leukemic bone marrow disorder where methylation load at diagnosis correlates with poor prognosis, and reduced methylation on therapy correlates with clinical response [11]. 5-Azacytidine is first activated by conversion into the active drug, 5-aza-2′-deoxycytidine-5′-triphosphate. This false nucleotide is incorporated into DNA in place of cytosine, and then traps DNA methyltransferase (DNMT) in a covalent complex [17]. The mechanism depends upon incorporation of drug into DNA during S phase and in cell culture leads to maximal demethylation at 48 h [18]. However, without ongoing pharmacologic repression, the hypermethylated state will revert [19]. Thus, in our study we selected the daily dose of 5-azacytidine associated with demethylation in MDS, 75 mg/m^2. Because demethylation is S-phase dependent, the dose escalation design increased the number of daily doses administered per cycle, with the goal of achieving 4 days of 5-azacytidine exposure per 2-week cycle. This schedule imitates our orthotopic model of lung adenocarcinoma, where administration of 5-azacytidine in 4-day pulses resulted in broad promoter demethylation, and re-expression of multiple genes involved in cell cycle regulation, DNA damage, apoptosis, and tissue remodeling [20]. A two-week interval, rather than the MDS interval of 28 days, was selected to increase chronicity of the hypomethylation

stimulus. Ultimately, a classic DLT was not defined in cycle 1 for cohort 5; however, treatment break followed by dose reduction was required in later cycles due to severe neutropenic infection. Given uninterrupted administration is the goal of epigenetic therapy, the recommended dose and schedule for continuous administration in phase II studies are erlotinib 150 mg daily and 5-azacytidine 75 mg/m^2 daily on days 1–4 and 15–18 of a 28-day cycle.

This phase I study has several important limitations. Because the primary goals of the study were safety and definition of MTD, rather than efficacy, no effort was made to select a patient population that may respond more favorably by EGFR gene copy number or mutation analysis, nor by assessment of a specific panel of methylated genes. The study would have been strengthened by incorporation of a pharmacodynamic surrogate of epigenetic reprogramming, such as demethylation changes in circulating tumor cells, to further justify recommended dose and schedule of 5-azacytidine. Finally, accumulating evidence indicates that a lower daily dose of 5-azacytidine may be sufficient to induce epigenetic changes, particularly when paired with the histone deacetylase inhibitor, entinostat [21]. For example, current studies of this combination in hematologic malignancies and NSCLC are evaluating 5-azacytidine at 40–50 mg/m^2 for 8–10 total daily doses per 28-day cycle. A lower dose of 5-azacytidine combined with erlotinib may result in similar hypomethylation stimulus, presumably with lower toxicity, a question deferred to the phase II arena.

The tolerability and clinical activity of the regimen presented here justify the design of disease-specific phase II studies with biomarker endpoints. In particular, interesting activity was seen in a patient with erlotinib-resistant lung adenocarcinoma (EGFR wild type), and in a patient with cetuximab-resistant laryngeal squamous cell carcinoma. Epigenetic therapy is of unique interest in NSCLC, where loss of p16^{INK4a} activity by gene methylation is one of the earliest initiation events, and additional investigations have shown acquired promoter methylation in dozens of tumor suppressors including PTEN, DAPK, APC, MGMT, RASSF1A, ECAD, p15, and p27 [22–26]. Epigenetic silencing of PTEN, p15, and p27 is uniquely relevant to combined therapy with erlotinib and 5-azacytidine. PTEN is epigenetically silenced in up to 60% of NSCLC cases, and in vitro re-expression with a demethylating strategy restores sensitivity to EGFR inhibition by gefitinib [27]. The cell cycle regulatory proteins p15 and p27 are downstream of EGFR, and downregulate proliferative signaling induced by EGFR activation. A phase II study testing the efficacy of erlotinib and 5-azacytidine in the salvage therapy of advanced, EGFR wild type NSCLC is under development, and will incorporate biomarker endpoints including baseline PTEN methylation status and serial assessment of sputum and circulating tumor cells for methylation changes of tumor suppressor genes. These will include PTEN, cell-cycle regulatory genes downstream of EGFR, and a panel of methylated genes associated with poor prognosis in NSCLC [28].

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