

A Proof-of-Principle Clinical Trial of Bexarotene in Patients with Non – Small Cell Lung Cancer

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Abstract Purpose: Bexarotene is a rexinoid (selective retinoid X receptor agonist) that affects proliferation, differentiation, and apoptosis in preclinical studies. The relationship between bexarotene levels and biomarker changes in tumor tissues has not been previously studied.

Experimental Design: BEAS-2B human bronchial epithelial (HBE) cells, retinoid-resistant BEAS-2B-R1 cells, A427, H226, and H358 lung cancer cells were treated with bexarotene. Proliferation and biomarker expression were assessed. In a proof-of-principle clinical trial, bexarotene tumor tissue levels and intratumoral pharmacodynamic effects were assessed in patients with stages I to II non – small cell lung cancer. Bexarotene (300 mg/m²/day) was administered p.o. for 7 to 9 days before resection.

Results: Bexarotene-induced dosage-dependent repression of growth, cyclin D1, cyclin D3, total epidermal growth factor receptor (EGFR), and phospho-EGFR expression in BEAS-2B, BEAS-2B-R1, A427, and H358, but not H226 cells. Twelve patients were enrolled, and 10 were evaluable. Bexarotene treatment was well tolerated. There was nonlinear correlation between plasma and tumor bexarotene concentrations ($r^2 = 0.77$). Biomarker changes in tumors were observed: repression of cyclin D1, total EGFR and proliferation in one case; repression of cyclin D3, total and phospho-EGFR in another. The cases with multiple biomarker changes had high tumor bexarotene (107-159 ng/g). A single biomarker change was detected in one case with low tumor bexarotene.

Conclusion: Bexarotene represses proliferation and biomarker expression in responsive, but not resistant HBE and lung cancer cells. Similar biomarker changes occur in lung tumors when therapeutic intratumoral bexarotene levels are achieved. This proof-of-principle trial approach is useful to uncover pharmacodynamic mechanisms *in vivo* and relate these to intratumoral pharmacokinetic effects.

Lung cancer is the major cause of cancer-related death in the United States and worldwide (1). More effective strategies for treating and preventing lung cancer are needed (2). Retinoids are natural and synthetic derivatives of vitamin A that have therapeutic and chemopreventive activity against a range of malignancies (3). Retinoids can cause cell cycle arrest in human epithelial malignancies, including mammary, prostate, and lung, as reviewed (4). However, clinical trials using classic retinoids that activate the retinoic acid receptors (RAR) for the treatment or chemoprevention of lung cancer have shown

mostly negative results (5). Greater knowledge of the mechanisms of action of classic retinoids and especially of nonclassic retinoids such as rexinoids that transcriptionally activate the retinoid X receptors (RXR) should lead to improved design of clinical studies with these agents.

We have previously identified a retinoid and rexinoid cell cycle arrest mechanism that prevents carcinogenic transformation of human bronchial epithelial (HBE) cells (6, 7). This mechanism was uncovered using all-*trans*-retinoic acid (RA) treatment of cultured HBE cells. Retinoids signal G₁ arrest

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through induction of ubiquitin-dependent proteolysis of cyclin D1 and cyclin E (6, 8, 9). This RA-triggered G₁ arrest is hypothesized to permit repair of carcinogenic damage to the genome (10). We have shown that retinoid treatment of HBE cells leads to transcriptional repression of the epidermal growth factor receptor (EGFR; ref. 11). We have previously reported that deregulation of cyclin D1, cyclin E, and EGFR is frequent in the preneoplastic bronchial epithelium and in lung cancer (12, 13). This highlights a likely role of these G₁ cyclins and EGFR in lung carcinogenesis and potentially as molecular pharmacologic targets in the lung.

Rexinoids and classic retinoids activate distinct nuclear receptors, but can engage related pathways (14). RAR β is frequently transcriptionally repressed in non-small cell lung cancer (NSCLC), and this has been proposed as a mechanism of resistance to classic retinoids (2, 15). A rexinoid can bypass this RAR β repression and still activate downstream pathways (16). Bexarotene (Targretin, LGD1069) is a rexinoid that is Food and Drug Administration approved for the treatment of cutaneous T-cell lymphoma (17–19). It has shown activity in NSCLC in early trials (16, 20–23). However, two phase III trials of first-line treatment for advanced NSCLC failed to show survival advantage from the addition of bexarotene to chemotherapy, although a subset of patients may have benefited (24, 25). There is a need to understand better the pharmacodynamic and pharmacokinetic properties of bexarotene in lung cancer because this permits a mechanistic approach to clinical trial design, especially when combination regimens are used. There is a clinical need to learn whether pathways engaged *in vitro* are also activated *in vivo*. If an antineoplastic agent affects pharmacodynamic targets in treated tissues, further single-agent clinical trials or combination regimens are warranted.

We recently reported results from a proof-of-principle trial where the EGFR-tyrosine kinase inhibitor erlotinib was administered as short-term therapy in patients with aerodigestive tract tumors (26). Cyclin D1 expression was found as a biomarker of intratumoral response. Findings from that trial and from a bexarotene and erlotinib combination trial, as well as *in vitro* studies, directly implicated cyclin D1 as a key rexinoid target in lung cancer therapy or chemoprevention (27, 28). Prior *in vitro* work has highlighted the EGFR signaling pathway as targeted by classic retinoids, but rexinoid effects were not examined (11).

Results of a short-term proof-of-principle phase II trial of bexarotene administered in the preoperative period to patients with resectable clinical stage I or II NSCLC are reported here. The primary objective was to evaluate the concentration of bexarotene in lung tumor tissue and to relate intratumoral to plasma drug levels. The secondary objectives were to study in post- versus pretreatment tumor biopsies effects of bexarotene on proliferation as measured by Ki-67 and on cyclin D1, cyclin D3, EGFR, and phospho-EGFR immunohistochemical expression. Biomarker responses were also examined in bexarotene-sensitive or bexarotene-resistant HBE and lung cancer cells. Findings reported here indicate that a strong nonlinear relationship existed between lung tumor tissue and plasma bexarotene levels. Substantial biomarker responses were observed *in vivo* only in NSCLC cases with high intratumoral bexarotene concentrations. The results obtained from this proof-of-principle trial provide a mechanistic basis for the

design of bexarotene combination regimens with chemotherapy or targeted agents. This trial platform will be useful in the early clinical evaluation of other novel anticancer agents.

Patients and Methods

Cell culture. BEAS-2B cells were derived from normal HBE cells by immortalization with an SV40 hybrid virus, as previously described (29). Retinoid-resistant BEAS-2B-R1 cells were established by exposure of BEAS-2B cells increasing concentrations of RA (6). These cells were passaged in LHC-9 media, as previously described (7, 9, 10). Bexarotene (Ligand, Inc., San Diego, CA) was dissolved in the vehicle, DMSO. The A427, H226, and H358 lung cancer cell lines were passaged in RPMI containing L-glutamine and 10% fetal bovine serum as recommended by the American Type Culture Collection (Manassas, VA). Cell culture media were supplemented with penicillin, streptomycin, and antifungal agents and kept in a humidified incubator with 5% CO₂.

Proliferation assays. Cellular proliferation was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide growth assay, as described previously (26). Cells were plated for assays with four to six replicates per experiment. Cells were then treated with bexarotene at various clinically achievable dosages or with the vehicle, DMSO, for 0 to 10 days. Growth was determined by measuring absorbance, and results were normalized to the absorbance measured in vehicle-treated cells that served as controls.

Immunoblot assays. BEAS-2B, BEAS-2B-R1, A427, H226, and H358 cells were independently treated with bexarotene or vehicle alone. Cells were harvested and subjected to immunoblot analyses, as previously described (6–8, 10, 26). Antibodies were purchased that recognized cyclin D1 (M-20, Santa Cruz Biotechnology, Santa Cruz, CA), cyclin D3 (C-16, Santa Cruz Biotechnology), EGFR (1005, Santa Cruz Biotechnology), phospho-EGFR (PY-20, MP Biomedicals, Irvine, CA), or actin (C-11, Santa Cruz Biotechnology).

Patients. Eligible patients had a pathologic diagnosis of NSCLC, clinical stage I or II, were older than 18 years, and were medical candidates for resection. Prior chemotherapy or radiotherapy was not allowed. Effective contraception or sexual abstinence was required for patients of child-bearing potential.

Exclusion criteria included hepatic dysfunction, renal dysfunction, or a serious medical disorder that would have impaired ability to receive study treatment. Concurrent use of other approved or investigational anticancer agents was not allowed. Patients with known hypersensitivity to bexarotene or with risk factors for pancreatitis were excluded.

All inclusion and exclusion criteria were assessed within 14 days before initiation of therapy. Radiographic studies were done within 28 days of screening. This clinical study was conducted after approval by the Committee for the Protection of Human Subjects at Dartmouth College and the Institutional Review Board. Informed consent was obtained from each patient enrolled onto this study.

Study drugs. This was an open-label, single-institution clinical and pharmacologic study of bexarotene in patients with resectable NSCLC (Fig. 1A). Subjects received bexarotene capsules 300 mg/m²/day p.o. as a regular single daily dose for 7 to 9 days before surgical resection and on the day of surgery. No dose modifications were permitted. Hypolipidemic therapy was not administered.

Pharmacokinetic analyses. A modified high-performance liquid chromatography method (30) was used to determine bexarotene concentrations in plasma and tumor tissue. An analogue of bexarotene, LG 100268 (provided by Ligand Pharmaceuticals), was used as the internal standard (31). Complete description of the assay is provided in the Supplementary data. In brief, an approximate 50-mg tumor tissue specimen was homogenized, digested, and then treated similar to a plasma sample. Detection was via a Rainin Dynamax Fluorescence Detector FL-1. The assay recovery of bexarotene from plasma and tissue was 88% and 95%, respectively. The assays were linear over the range of

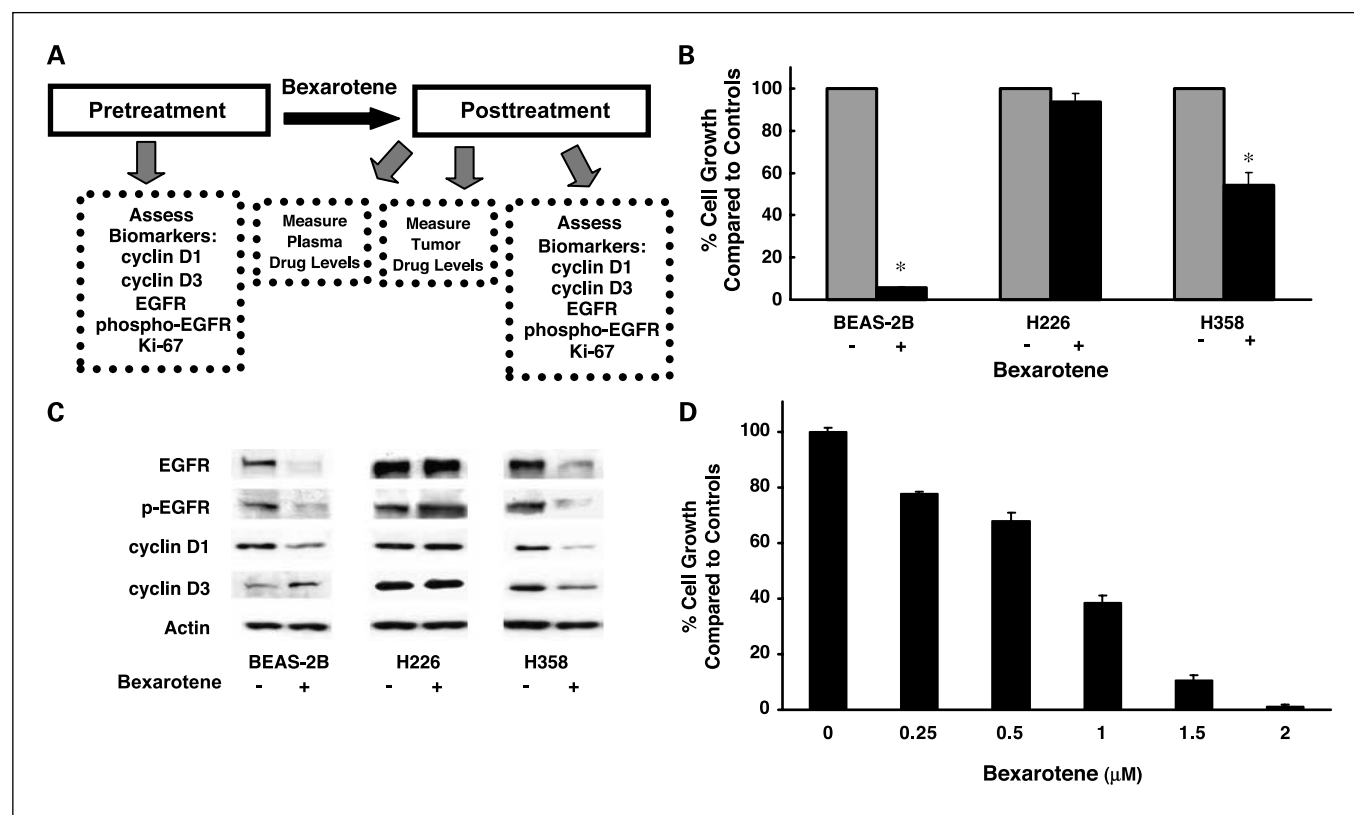


Fig. 1. The bexarotene proof-of-principle clinical trial design and bexarotene effects on growth and gene expression in HBE and lung cancer cells. **A**, in this proof-of-principle clinical trial design, resectable clinical stage I and II NSCLC cases were accrued to this 7- to 9-d bexarotene clinical trial conducted in the preoperative period. The molecular pharmacodynamic targets assessed in the post- and pretreatment biopsies were cyclin D1, cyclin D3, total EGFR, phospho-EGFR, and Ki-67 using optimized immunohistochemical assays, as described in Materials and Methods. Plasma and intratumoral bexarotene levels were measured. This study design permitted the assessment of the relationship between bexarotene pharmacokinetic and pharmacodynamic effects. This determines whether therapeutic drug levels in targeted tumor tissues affect the expected pharmacodynamic target(s). **B**, growth effects of bexarotene in BEAS-2B, H226, and H358 cells. BEAS-2B HBE cells and H226 as well as H358 lung cancer cells were independently treated with the vehicle DMSO or 1 μmol/L bexarotene for 10 d. These findings reveal that bexarotene treatment suppresses growth of BEAS-2B and H358, but not H226 cells. *, $P < 0.05$, significant growth suppression occurred as compared with vehicle controls. **C**, effects of bexarotene on immunoblot expression profiles. These HBE and lung cancer cell lines were treated as described in (B), and immunoblot analyses were done for the indicated species. Findings reveal in the bexarotene-sensitive (BEAS-2B and H358) but not bexarotene-resistant (H226) cells that repression of total EGFR, phospho-EGFR (p-EGFR), and cyclin D1, as well as cyclin D3 (data not shown at 24 h for BEAS-2B cells) occurs. No appreciable repression of cyclin D2 expression was observed in these cell lines (data not shown). **D**, dose-response relationship in growth inhibition of BEAS-2B cells by bexarotene. Significant suppression of proliferation is noted at bexarotene concentrations as low as 0.25 μmol/L.

the standard curve of 3 to 1,200 ng/mL. The lower limit of quantitation of the assays was 3 ng/mL.

Tissue collection and immunohistochemical methods. A portion of the tumor tissue harvested at the time of resection was fixed in formalin and processed for routine immunohistochemistry (13, 26). Another portion of the resection specimen was immediately snap-frozen in liquid nitrogen and used to assay for bexarotene. The formalin-fixed tissues were assayed for cyclin D1, cyclin D3, EGFR, phospho-EGFR, and Ki-67 expression using standard immunohistochemical techniques (26, 32). Quantification of immunohistochemical staining was similar to that described in prior work (26, 32). The following immunohistochemical scoring system was used: -, <5% of tumor cells staining; +, 5% to 20% of tumor cells staining; 2+, 20% to 50% of tumor cell staining; 3+, 50 to 80% of tumor cells staining, and 4+, > 80% of tumor cells staining. The percentage of tumor cells staining was based on evaluation of the entire tumor specimen. The specimens were assessed by pathologists who were unaware of the pharmacokinetic findings.

Statistical analyses. Response to bexarotene in this study was defined as a concentration of bexarotene in the tumor detected above the lower limit of the assay and a 50% decrease in the levels of cyclin D1, cyclin D3, EGFR, phospho-EGFR, or Ki-67 immunohistochemical expression comparing the posttreatment with the pretreatment specimens. The primary statistical analysis consisted of the estimation of the response rate of tumor tissue positive for the presence of bexarotene.

Changes in growth-suppressive effects *in vitro* were compared using the two-sample *t* test with significance defined as a two-sided $P < 0.05$ using Microsoft Excel software. Statistical correlation between plasma and tumor bexarotene levels was determined using the nonparametric Spearman correlation test with the Number Cruncher Statistical System statistical software (Kaysville, UT). Significance was defined as a $P < 0.05$.

Results

Bexarotene growth effects on HBE and lung cancer cells. Bexarotene suppressed growth of BEAS-2B HBE cells (Fig. 1B). The effect was dose dependent with a statistically significant inhibition observed beginning at 0.25 μmol/L and maximal growth inhibition at 1-2 μmol/L (Fig. 1B and D and data not shown). Proliferation of H358 lung cancer cells was inhibited at bexarotene dosages as low as 0.25 μmol/L (data not shown) and at 1 μmol/L dosage (Fig. 1B). Similar inhibition of proliferation by bexarotene was observed in the retinoid-resistant BEAS-2B-R1 cells and the A427 lung cancer cell line (data not shown). Appreciable growth inhibition of the H226 lung cancer cell line was not observed at these bexarotene dosages. Pharmacokinetic analyses from this bexarotene clinical

trial indicated that the bexarotene dosages used in these *in vitro* studies were within clinically achievable ranges.

Bexarotene effects on gene expression. To determine changes in the indicated growth and cell cycle regulatory proteins, immunoblot analyses were done. These revealed (Fig. 1C) that bexarotene treatment repressed cyclin D1 protein expression in the BEAS-2B HBE cell line in a dose-dependent manner (data not shown) and at a clinically achievable dosage, as compared with vehicle-treated cells. In addition, repression of cyclin D3 (at 24 h, data not shown), EGFR, and phospho-EGFR expression occurred, but no appreciable repression of cyclin D2 (data not shown) expression was observed (Fig. 1C).

Changes in expression of these biomarkers of bexarotene response were also examined after bexarotene treatments of the retinoid-resistant BEAS-2B-R1 HBE cells, as well as A427, H226, and H358 lung cancer cell lines. Immunoblot analyses of BEAS-2B-R1, A427, and H358 cells showed (Fig. 1C and data not shown) repression of cyclin D1, cyclin D3, EGFR, and phospho-EGFR at the same dosage that inhibited proliferation. No appreciable change in these examined species was noted after bexarotene treatment of H226 cells, and growth was also not appreciably affected in these cells.

Patient characteristics and treatment. Twelve patients with clinical stage I or II NSCLC were enrolled between September 3, 2002, and January 14, 2004. Clinical characteristics are shown in Table 1. One patient withdrew consent before completing the study treatment, and another patient had a solitary metastasis from colon cancer diagnosed at the time of resection. Twelve patients were evaluable for toxicity, 10 patients were available for pharmacokinetic and pharmacodynamic analyses. The patient with metastatic colon adenocarcinoma was excluded from these analyses.

Patients received a median of 9 days of bexarotene therapy (range, 7-9 days). Bexarotene was clinically well tolerated. One patient developed grade 2 rash, and another developed grade 2 headache and grade 2 chest pressure. No cases of pancreatitis occurred. Two patients underwent pneumonectomy, and 10 had lobectomy. One patient died on postoperative day 14 from pulmonary embolism that was not considered treatment related. Four patients subsequently developed recurrent NSCLC, of whom two have died.

Bexarotene tumor and plasma pharmacokinetics. Bexarotene levels in the resected lung tumors were measured in the 10 evaluable patients. Pharmacokinetic analyses confirmed plasma

Table 2. Bexarotene concentrations

Patient Number	Plasma		Tumor	
	(ng/mL)	(μ mol/L)	(ng/g)	(μ mol/L)
1	28.5	0.08	10.4	0.03
2	24.8	0.07	NE	NE
3	54.6	0.16	39.7	0.11
4	45.7	0.13	30.0	0.09
5	157.1	0.45	120.8	0.35
6	64.2	0.18	27.7	0.08
7	84.1	0.24	74.2	0.21
8	377.7	1.08	159.2	0.46
9	520.1	1.49	106.8	0.31
10	6.4	0.02	3.7	0.01

Abbreviation: NE, nonevaluable.

and tumor concentrations above the detection limits of the assay in all patients. Tumor tissue concentrations above 100 ng/g (0.29 μ mol/L) were noted in only three cases, with lower concentrations noted in the remaining cases (Table 2). There was considerable interindividual variation in the plasma bexarotene concentrations [range, 6.4-520.1 ng/ml; coefficient of variation (CV), 120.2%], and similar variability was observed in intratumoral bexarotene concentrations (range, 3.7-159.2 ng/g; CV, 86%). There was a strong correlation between the plasma and tumor tissue bexarotene concentrations (Spearman's $r = 0.9$; $P = 0.002$). These data best fitted a nonlinear relationship ($r^2 = 0.77$; Fig. 2).

Biomarker changes in resected NSCLCs. Biomarkers of bexarotene response were assessed by performing and comparing immunohistochemical assays in posttreatment and pretreatment samples (Fig. 1A). Seven paired post- and pretreatment specimens were available for immunohistochemical analyses for Ki-67, cyclin D1, cyclin D3, EGFR, and phospho-EGFR expression (Table 3). In two of these pretreatment biopsies, sufficient tumor cells were not available for immunohistochemical assays. In five paired samples, adequate staining of tumor specimens was achieved. Notably, the two cases with the highest bexarotene tumor tissue levels exhibited changes in expression of several biomarkers. The histopathology of the responding cases was squamous cell carcinoma. One case had repression of cyclin D1, EGFR, and Ki-67 expression (Fig. 3), whereas another case exhibited repression of cyclin D3, EGFR, and phospho-EGFR expression (data not shown). One patient with low intratumoral bexarotene had decreased phospho-EGFR expression without changes in other biomarkers.

Relationship between tumor bexarotene concentrations and biomarker response. The relationship between observed biomarker changes and intratumoral bexarotene measurements was displayed in Table 3. Changes in multiple biomarkers were observed only in cases with the high tumor bexarotene levels (range, 106.83-159.2 ng/g). Only one biomarker change was detected in a single tumor of all examined cases having low tumor tissue bexarotene levels (range, 3.7-10.4 ng/g).

Discussion

Retinoids have shown activity against a range of malignancies, both *in vitro* and in clinical trials, as reviewed (14, 16).

Table 1. Patient and tumor characteristics

Median age (y)	64 (range 56-84)
Gender	
Female	2
Male	10
Histology	
Adenocarcinoma	3
Squamous cell carcinoma	7
Adenosquamous carcinoma	1
Metastasis from colon adenocarcinoma	1
Pathologic stage	
IA	6
IB	2
IIA	1
IIB	2
IV	1

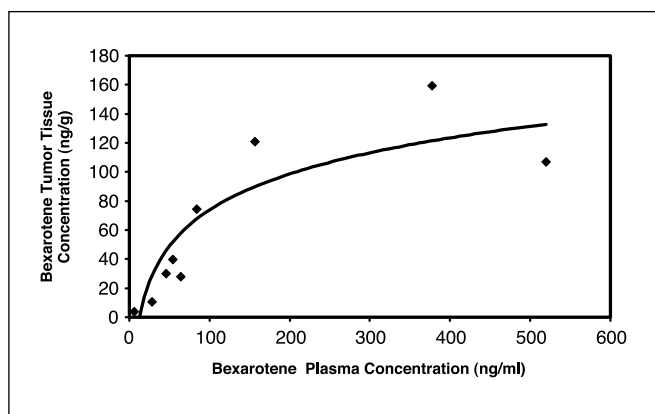


Fig. 2. Relationship between plasma and intratumoral bexarotene levels in this proof-of-principle trial. Bexarotene levels were measured in plasma collected at the time of surgical resection. Bexarotene was measured in lung tumor tissue harvested and immediately snap-frozen in liquid N₂ at the time of resection. Coefficient of determination $r^2 = 0.767$ revealed a nonlinear relationship between plasma and tumor bexarotene concentrations.

Classic and nonclassic retinoids inhibit growth of many human epithelial cells, including normal and malignant lung epithelial cell lines (4, 16). Encouraging results were initially reported for classic and nonclassic retinoids in phase I and II trials in patients with NSCLC, as reviewed (16, 22). However, phase III trials, to date, have failed to show significant benefits from a classic retinoid used for lung cancer chemoprevention or treatment (2). The discrepancy between documented *in vitro* activity and the current lack of lung cancer clinical efficacy underscores the need to understand better the mechanisms of action of this class of agents and how to overcome clinical resistance to classic retinoids.

We previously reported that in the BEAS-2B HBE cell line, retinoids and rexinoids inhibit cell cycle progression and induce cyclin D1 proteolysis through proteasome-dependent degradation (6, 7, 9). The present study advances this prior *in vitro* work by showing that bexarotene treatment not only inhibits growth of BEAS-2B cells, but also of the retinoid-resistant BEAS-2B-R1 cells and the A427 and H358 lung cancer cell lines, whereas H226 cells are resistant to bexarotene treatments. In the sensitive cell lines, bexarotene treatment also repressed expression of cyclin D1, cyclin D3, total EGFR, and phospho-EGFR. An early decline of cyclin D3 expression at 24 h of bexarotene treatment of BEAS-2B cells was followed by a subsequent increase in cyclin D3 expression. Of note, cyclin

D1 is also a key downstream signal in the EGFR pathway, and transcriptional repression of cyclin D1 was previously reported as a biomarker of clinical response to EGFR inhibition in lung cancer (26).

Translational research is needed to learn whether pathways affected by anticancer agents *in vitro* are also affected in the clinical setting. The proof-of-principle clinical trial platform described here reveals that important molecular pharmacologic pathways regulated *in vitro* are also engaged in the clinical setting of bexarotene-treated NSCLC. A novel aspect of this study is that it evaluates bexarotene levels in lung tumors following treatments, thereby permitting a correlation to be made between plasma and tumor tissue bexarotene levels. Changes in multiple biomarkers were observed in posttreatment tumor specimens, but only at the higher tumor bexarotene levels, indicating that a tight relationship exists between tumor tissue pharmacokinetics and tumor biomarker pharmacodynamic response. A change in a single biomarker was observed in only one case with low intratumoral bexarotene. This underscores the value of using several markers, especially downstream of rexinoid receptors signaling, such as G₁ cyclins. Preliminary findings from gene expression profiles (33) done in bexarotene-treated NSCLC are consistent with these results.

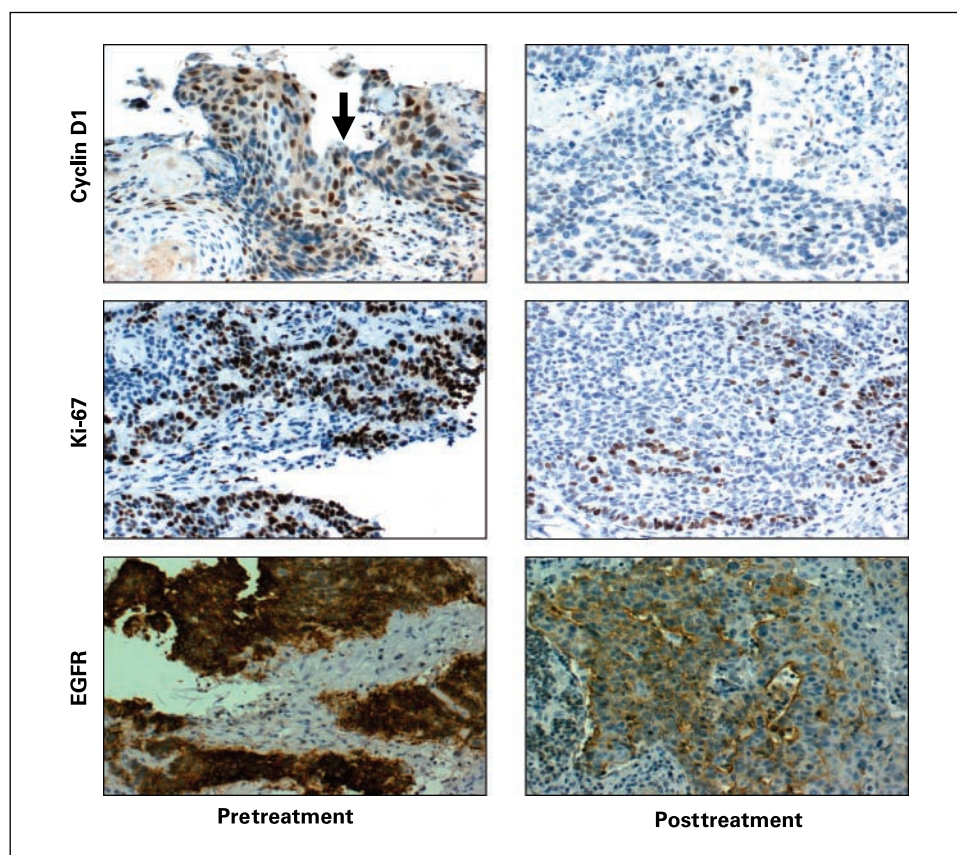
There was interpatient variability in the plasma and tumor bexarotene concentrations. Specimens were obtained at the time of surgery, whereas bexarotene was typically administered several hours before surgical resection. Although the duration of treatment was sufficient to achieve steady-state drug levels, some of the interindividual variability may be due to the different sampling times. In a prior phase I study of bexarotene (21), there was marked intra- and interpatient variability noted, both in peak concentrations and areas under the curve. These investigators also calculated time to peak concentrations in plasma to be 2.8 \pm 1.6 h. Based on this, ongoing proof-of-principle trials are evaluating both plasma areas under the curve and tumor bexarotene levels.

Two recent phase III clinical trials in patients with advanced NSCLC undergoing first-line treatment showed no overall survival advantage from the addition of bexarotene to chemotherapy, although some subsets of patients may have benefited (24, 25). Consistent with the close relationship between bexarotene intratumoral levels and biomarker responses observed in this study, subset analyses revealed that only patients who developed grade III and IV hypertriglyceridemia had benefited from bexarotene therapy (24, 25). Similar findings are described by others (34) in a trial of single-agent

Table 3. Biomarker responses

Patient	Plasma		Tumor		Biomarker response	Histology
	(ng/mL)	(μ mol/L)	(ng/g)	(μ mol/L)		
1	28.5	0.08	10.38	0.03	pEGFR (3+/1+)	Adenocarcinoma
4	45.7	0.13	30.04	0.09	None	Squamous cell carcinoma
8	377.7	1.08	159.2	0.46	Cyclin D3(2+/1+) EGFR (2+/1+)	Squamous cell carcinoma
9	520.1	1.49	106.83	0.31	pEGFR (3+/1+) Cyclin D1 (2+/1+) EGFR (3+/2+)	Squamous cell carcinoma
10	6.4	0.02	3.65	0.01	Ki-67 (4+/2+) None	Squamous cell carcinoma

Fig. 3. Biomarker immunohistochemical expression profiles in a case having high intratumoral bexarotene levels. Cyclin D1, EGFR, and Ki-67 immunohistochemical expression profiles were repressed in this representative responding case. Arrow, cyclin D1 immunostaining within cancer cells. There was a decrease in the number of tumor cells staining positive for EGFR as well as a decrease in the intensity of EGFR staining.



bexarotene in relapsed NSCLC. Patients with hypertriglyceridemia and rash had a clear survival advantage. Bexarotene concentrations in plasma were not measured. As lipid elevation is a known bexarotene effect, perhaps these responding cases had achieved higher blood levels of the drug than those cases without observed clinical benefit. Of note, the current study is the first to measure bexarotene levels in tumor tissue. Multiple biomarker changes in tumors were observed when high intratumoral bexarotene was measured. This finding underscores the need to achieve therapeutic intratumoral dosages following bexarotene treatment. Of course, other factors might also affect the clinical response to this drug. A clinical trial with dose escalation until a marker change is observed represents an alternative study design. Bexarotene treatment in the current trial was not sufficiently long to lead to appreciable lipid changes. Future clinical trials are warranted, especially to assess the effects of agents combined with bexarotene on pharmacokinetics and pharmacodynamic responses using findings presented in this proof-of-principle trial as a basis for that work.

Based on prior work, it was hypothesized that combining a retinoid and an EGFR inhibitor would confer at least additive clinical benefits by cooperatively repressing cyclin D1 and the

EGFR pathway (11, 35). Erlotinib and bexarotene have a shared downstream therapeutic target, cyclin D1, which was already highlighted as a biomarker of clinical erlotinib response (26). Indeed, a trial of patients with advanced aerodigestive tract cancer recently established that bexarotene and erlotinib treatment represents an active targeted combination regimen, which also affects cyclin D1 expression in an examined surrogate tissue (27). Whether this combined regimen cooperatively affects D-type cyclins or the EGFR pathway in tumor tissues is the subject of an ongoing proof-of-principle trial.

Taken together, these findings strongly support the view that therapeutic intratumoral levels of bexarotene are needed to achieve desired pharmacodynamic effects within clinical lung tumors, such as repression of either D-type cyclins or the EGFR. The results presented here have implications beyond those reported for bexarotene. The proof-of-principle clinical trial platform used in this study underscores the value of this approach for confirming expected pharmacodynamic and pharmacokinetic effects in target tumor tissues. This translational approach should prove useful in future work to learn whether novel pharmacologic pathways targeted at the bench are also affected in the clinic.

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