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In vitro and in vivo effects of the conformationally restricted polyamine analogue CGC-11047 on small cell and non-small cell lung cancer cells

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Abstract

Purpose—Polyamines are essential for normal growth; however, the requirement for, and the metabolism of, polyamines are frequently dysregulated in cancer. Polyamine analogues have demonstrated promising preclinical results in multiple model systems of cancer, but their clinical utility has been limited by apparent toxicity. A representative compound of a new generation of short chain, conformationally restricted polyamine analogues, CGC-11047 has been synthesized and ongoing phase I clinical trials indicate it to be well tolerated at weekly doses of 610 mg (dose escalation is still in progress). Therefore, studies were designed to gain a better understanding of its effects on cellular polyamine biochemistry and efficacy in the treatment of human lung cancer models in vitro and in vivo.

Methods—Human lung cancers cell lines representing non-small cell and small cell lung cancers were investigated for their growth and biochemical response to CGC-11047. Effects of in vitro treatment with CGC-11047 on cell growth, the activity of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC), and the expression and activity of the polyamine catabolic enzymes spermidine/spermine *N*¹-acetyltransferase (SSAT) and spermine oxidase (SMO) were measured. Additionally, the overall effects on intracellular polyamine pools were monitored. Finally, the in vivo efficacy of CGC-11047 in the treatment of a nude mouse model of human non-small cell lung cancer was evaluated.

Results—CGC-11047 effectively inhibited the growth of both small cell and non-small cell lung cancer cells in vitro. The greatest biochemical effects were observed in the non-small cell lung cancer cells where in addition to a profound down regulation of ODC activity, there was a significant increase in polyamine catabolism leading to a greater degree of polyamine pool depletion and greater accumulation of CGC-11047 when compared with the changes observed for

the small cell lines. Importantly, CGC-11047 was found to be highly significant ($P < 0.0001$) in delaying the progression of established tumors in an in vivo model of human non-small cell lung cancer.

Conclusion—CGC-11047 represents a promising new polyamine analogue that warrants further preclinical and, potentially, clinical evaluation in lung cancer.

Keywords

Polyamines; Analogues; Lung cancer; SSAT; SMO; ODC

Introduction

The natural polyamines, putrescine, spermidine, and spermine are small polycationic alkylamines that have been shown to be essential for cell growth. Polyamine concentrations are increased and polyamine metabolism dysregulated in multiple tumor cell types, thus making this pathway a rational target for antineoplastic therapies [10, 20, 28, 34]. Targeting the key enzymes involved in the polyamine biosynthetic pathway, ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) has been demonstrated to be successful as a means of targeting polyamines and regulating cell growth, but has had limited clinical success [2, 33, 38]. In an attempt to overcome the shortcomings of the inhibitors of polyamine biosynthesis, several groups have synthesized and tested polyamine analogues that were designed to take advantage of the self-regulatory properties of the natural polyamines [4, 18, 26, 55].

In addition to having the expected results of down-regulating biosynthesis, several analogues of spermine were found to have the unusual property of super-inducing the polyamine catabolic enzyme spermidine/spermine N^1 -acetyltransferase (SSAT) which results in the depletion of intracellular polyamines, inhibits cell growth, and in a tumor type-specific manner, induces apoptotic cell death [11, 15, 23, 29, 35, 41]. N^1 . N^{12} -Bisethylspermine (BESpm), a symmetrically substituted spermine analogue has been previously shown to induce SSAT to very high levels in numerous tumor cell types, leading to a rapid cytotoxic response of the cells.[16]. Despite impressive in vitro activity, BESpm was not effective in xenograft models of various tumor types [6]. A closely related analogue, N^1 , N^{11} -diethylnorspermine (DENSpm), demonstrated very similar in vitro effects with respect to induction of SSAT and cytotoxicity, and also demonstrated impressive in vivo activity against multiple solid tumors [7, 21, 42]. As a result, phases I and II clinical trials have been completed with DENSpm [24, 30, 48, 56]; however, to date DENSpm has not been effective as a single agent. One of the potential problems limiting the effectiveness of DENSpm may have been the daily administration schedule in most of its clinical trials. Such a schedule may have contributed to the observed dose-limiting toxicities, thus impeding its clinical utility [30]. Therefore, the search has continued for a polyamine analogue with similar antineoplastic characteristics as the symmetrically substituted analogues, BESpm and DENSpm, but whose dosing schedule could be adjusted to allow efficacy in the absence of serious dose-limiting toxicities.

CGC-11047 is a second-generation analogue of BESpm that is also symmetrically substituted, but that is conformationally restricted with a cis double bond between the central carbons (Fig. 1a). CGC-11047 has shown positive results in the treatment of non-Hodgkin's lymphoma and is currently in several clinical trials. Treatment with CGC-11047 has demonstrated significant growth inhibition in numerous tumor cell lines with ID_{50} s as low as 50 nM in DU145 cells [43]. The growth inhibition was concurrent with analogue accumulation and decreased natural polyamines. However, the basis for this decrease in polyamine pools was not determined [43]. Consequently, despite some very promising

results with CGC-11047, further studies on its biochemistry and potential mechanism of action are needed. Here we report on the effects of CGC-11047 on four human lung cancer cell lines that represent the major forms of lung cancer, with an emphasis on biochemical mechanisms that may contribute to its antiproliferative effects. Importantly, we demonstrate impressive antitumor effects *in vivo* against a human xenograft model of lung adenocarcinoma that suggests that a clinical trial of CGC-11047 in lung cancer may be warranted.

Materials and methods

Chemicals and reagents

[(1*N*),(12*M*)]Bis(Ethyl)-*cis*-6,7-dehydrospermine (CGC11047) was obtained from Progen Pharmaceuticals (Redwood City, CA). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), Invitrogen/Life Technologies, Inc. (Rockville, MD), BioRad (Hercules, CA), Aldrich Chemical Co. (Milwaukee, WI), Hyclone (Logan, UT), and J.T. Baker Inc. (Phillipsburg, NJ).

Cell culture and analysis of growth inhibition of human lung cancer cell lines

The non-small cell cancer lines, NCI A549, NCI H157, and the small cell lung carcinoma lines, NCI H82 and NCI H69, were cultured as previously reported [15]. Cells were treated for the indicated times and concentrations. Cell growth was determined by standard trypan blue exclusion.

RNA purification and Northern blot analysis

Extraction of total cellular RNA was performed using Trizol Reagent (Invitrogen) according to the manufacturer-supplied protocol. For Northern blotting, total RNA (20 μ g) was separated on a denaturing 1.5% agarose gel containing 6% formaldehyde and transferred to Zetaprobe membrane (Bio-Rad) and hybridized to random primer-labeled PAOh1/SMO or SSAT cDNAs to determine SMO and SSAT expression [14, 52]. Blots were stripped and reprobed with a labeled 18S ribosomal cDNA as a loading control. Hybridized membranes were analyzed and images generated on a PhosphoImager using Image Quant software (Molecular Dynamics).

Analysis of polyamine content, SSAT, SMO, and ODC activity

Intracellular polyamine concentrations were determined using the precolumn dansylation labeling, reverse phase high-pressure liquid chromatography method as reported by Kabra et al. [32] using 1,7-diaminoheptane as an internal standard. Polyamine concentrations were reported as nmol/mg protein for each sample, where lysate protein content was measured by the method of Bradford [9]. SSAT activity of cellular extracts was measured as previously published [11]. The SMO enzyme activity in the cell lysates was assayed as previously reported [53]. ODC activity was measured by the methods of Seely and Pegg [45].

Western blot analysis

Total cell extracts were obtained by lysing cells on ice in RIPA buffer (PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 30 μ g/ml aprotinin, 100 μ M sodium orthovanadate, 10 μ g/ml PMSF) and insoluble material was pelleted at 12,000*g* for 20 min at 4°C. Thirty μ g of total protein was loaded per lane and separated on a 10% SDS-PAGE gels transferred electrophoretically to Immunoblot PVDF membrane (BioRad) for immunoblotting. Briefly, membranes were blocked for 1 hour in Odyssey blocking buffer, per manufacturer's instructions. The primary antibodies, an affinity purified antisera to human SMO [3], SSAT [17] and a mouse anti- β -actin purchased from Santa Cruz (Santa Cruz, CA) were all used at

dilutions of 1:1000 with 0.1% Tween 20 in blocking buffer for 1 h at room temperature. Following washes, blots were incubated with appropriate fluorescent dye-conjugated secondary antibodies (1:4000 each, 0.1% Tween 20, in blocking buffer protected from light, for 45 min). Western blot results were quantified using the LICOR immunofluorescence system (LI-COR Biosciences, Lincoln, NE).

In vivo studies

A549 non-small cell lung cancer cells (1.0×10^7) suspended in Hank's Balance Salt Solution (HBSS) were injected subcutaneously into the right flank of 4–6-week-old female Athymic nude *nu/nu* mice (Harlan, Indianapolis, IN). Tumors were allowed to grow for 13 days resulting in an average volume of $>0.3 \text{ cm}^3$. Tumor-bearing animals were then randomly sorted into two groups, control ($n = 15$) and treatment ($n = 10$). The treatment group received 100 mg/kg of CGC-11047 in 200 μl HBSS by IP injection once a week for 3 weeks on and 1 week off for a total of 14 weeks by intraperitoneal injection (IP). Control animals were injected with 200 μl HBSS. Mice were weighed and tumor volumes were measured weekly. A Kaplan–Meier time to progression model was used to analyze the data with time to progression is defined as time for the individual tumor to increase fourfold after the initiation of treatment. The log-rank test was used to determine statistical significance.

Results

Inhibition of cell growth by CGC-11047

We have previously demonstrated differential sensitivity of human lung cancer phenotypes to specific polyamine analogues. Specifically, human non-small cell lung cancer cells were found to be more sensitive to non-conformationally restricted analogues than small cell lung cancer cells and this differential sensitivity was associated with differential induction of polyamine catabolism [12, 15, 19]. To determine if a similar differential sensitivity was observed with the conformationally restricted CGC-11047, we compared cellular responses of two representative lines of small cell (H69 and H82) to two representative lines of non-small cell lung cancers (A549 and NCI-H157). CGC-11047 effectively inhibited cell growth in both small cell and non-small cell lung cancers in a concentration-dependent manner after 96 h of treatment (Fig. 1b). The IC_{50} values for all cell lines ranged from 0.1 to 0.5 μM , with the order of sensitivity $\text{A549} > \text{H69} = \text{H157} > \text{H82}$. Although there appears to be some differences in sensitivity between the different cell lines, these data indicate that CGC-11047 effectively inhibits in vitro cell growth of multiple human lung cancer lines regardless of their phenotype.

Effects of CGC-11047 on polyamine metabolism

Several classes of polyamine analogues alter polyamine metabolism through multiple regulatory pathways [20]. The original basis for the synthesis of polyamine analogues for the treatment of cancer was based on the self-regulating nature of polyamine metabolism [39, 40]. Therefore, the effects of CGC-11047 on polyamine metabolism were examined in the multiple lung cancer cell lines. The response to treatment with respect to alterations in polyamine metabolism were greater in the non-small cell phenotype than observed in the small cell phenotype, consistent with previous findings with other analogues [19]. In H157 and A549 cells, 24-h treatment with 10 μM CGC-11047 up-regulated the polyamine catabolic enzyme spermidine/spermine *N*¹-acetyltransferase (SSAT) and down-regulated the polyamine biosynthetic enzyme ornithine decarboxylase (ODC). The induction of SSAT occurred at the level of mRNA (Fig. 2a), protein (Fig. 2b, c), and activity (Fig. 3), with SSAT enzyme activity increasing $>1,000$ -fold in both A549 cells and H157 cells (Fig. 2). By contrast, SSAT enzyme activity was not altered in H82 and H69 after 24-h treatment exposure to 10 μM of CGC-11047. Spermine oxidase (SMO) was significantly induced only

in the A549 cells (Figs. 2, 4), as has been previously observed with other polyamine analogues [25], demonstrating an approximate threefold increase in SMO activity after 24 h exposure to 10 μ M CGC-11047 (Fig. 4).

The activity of one rate-limiting polyamine biosynthetic enzyme, ODC, demonstrated a reduction with CGC-11047 treatment to nearly undetectable levels in the H69, H157, and A549 cells, and to ~20% of control in H82 cells. The decreases observed were more prominent in H157 and A549 cells since they have a higher basal level of ODC activity as compared to H69 and H82 cells (Fig. 5).

The changes in polyamine concentrations resulting from analogue treatment are consistent with the observed changes in polyamine metabolic enzyme activities in the representative cell lines (Table 1). Specifically, the non-small cell lung cancer lines H157 and A549 demonstrated the greatest depletion of all three polyamines, consistent with their profile of decreased ODC activity and increased polyamine catabolism (Figs. 2, 3, 4), whereas the small cell line H69 was intermediate in its depletion of polyamine and H82 cells were the least affected, again consistent with their profile of polyamine metabolic enzyme activity in response to analogue treatment (Figs. 2, 3, 4). It is also important to note that analogue accumulation by the individual cell lines was inversely proportional to the level of natural polyamine depletion (Table 1).

In vivo efficacy of CGC-11047

The in vivo therapeutic effect of CGC-11047 was assessed using A549 xenografts in athymic nude *nu/nu* mutant mice. The A549 cell line was chosen for use in nude mice since in the in vitro setting CGC-11047 induced the greatest increase in polyamine catabolic enzyme activity, the greatest depletion of intracellular polyamines, and the largest growth inhibitory effects of all the cell lines studied. Thirteen days after implantation of 1.0×10^6 A549 cells the average tumor volume was 0.3 cm³ (SD = 0.18) ($n = 25$) and average weight of the mice was 22.5 g (SD = 1.96). Mice were randomized into treated ($n = 10$) and control ($n = 15$) groups. Animals received intraperitoneal injections of 100 mg/kg of CGC-11047 in 200 μ l of Hanks balanced salt solution or of 200 μ l of Hanks alone. The treatment schedule was 100-mg/kg dose once weekly for 3 weeks followed a week of rest, for a total of 14 weeks. The once weekly dosing $\times 3$ every 4 weeks was selected since previous xenograft studies with other polyamine analogues have demonstrated once or twice weekly schedules are equivalent to the 5 \times per week dosing, but with greater tolerability [31]. Treatment of established tumors with a weekly dose of CGC-11047 resulted in a significant delay in progression ($P = 0.0001$, Fig. 6a). By week seven all control animals had progressed (>fourfold increase in tumor starting volume), whereas only 30% of the treated animals had progressed. Even after 14 weeks of treatment, 30% of the treatment group had still not progressed and one mouse was found to be completely tumor free. Importantly, there were no overt signs of drug-induced toxicities and all treated animals continued to feed and gain weight normally (Fig. 6b).

Discussion

The frequent dysregulation of polyamine metabolism in tumor cells make polyamine metabolism and function a rational target for antineoplastic therapy [20, 46, 47, 50, 51]. Although inhibitors of virtually all of the biosynthetic enzymes in polyamine metabolism have been synthesized, none has demonstrated clinical efficacy as a single agent in clinical trials for cancer (see [20]). In an attempt to overcome the limitations encountered with specific inhibitors of enzyme function, we and others have exploited the self-regulatory properties of polyamine metabolism for therapeutic advantage through the use of structural analogues of the natural polyamines [4, 5, 8, 18, 39, 43, 44, 49]. The initial studies with

polyamine analogues, particularly with BENSpm, were promising, but clinical trials with a number of analogues in lung, breast, and other tumors did not reveal efficacy as single agents. This might be due in part to less-than-optimal dosing schedules and resulting dose-limiting toxicities, but probably also due to the inherent properties of the analogues utilized. Here, we report on the effects of a conformationally restricted polyamine analogue, CGC-11047, that is effective in inhibiting the growth of four human lung cancer cell lines that are representative of the major forms of lung cancer. Further, we demonstrate that CGC-11047 is well tolerated and effective in delaying tumor progression in established tumors *in vivo*.

Our laboratory originally demonstrated a phenotype-specific response to polyamine analogues that was linked to increased polyamine catabolism, a finding that was subsequently corroborated by several other laboratories [11, 13–15, 22, 41]. Specifically, analogues that were most effective in inducing SSAT were found to be selective in their cytotoxic effects against non-small cell lung cancer cells. As these studies were performed with the first generation symmetrically substituted and second generation asymmetrically substituted polyamine analogues, we sought to determine if the symmetrically substituted, conformationally restricted polyamine analogue, CGC-11047 would demonstrate similar selectivity *in vitro*. Consistent with previous polyamine analogue studies, the small cell line H82 was the most resistant to CGC-11047 treatment [11, 12, 25]. However, at higher concentrations, it too was significantly growth inhibited. The most sensitive line, the non-small cell line, A549 was also the line that demonstrated the greatest depletion of intracellular polyamines and the greatest accumulation of the analogue. Similarly, the other non-small cell line, H157, also demonstrated greater overall polyamine pool depletion and analogue accumulation than the two representative small cell lines. This greater perturbation of polyamine pools in the non-small cell lines is consistent with previous analogue studies and is most likely the result of the significantly greater increase in polyamine catabolism induced by analogue treatment of the non-small cell lines as compared to the small cell lines, which demonstrate little or no induction of either SSAT or SMO in response to analogue treatment [11, 12, 25, 54]. Although analogue treatment of all cell types reduced ODC activity, most likely due to an increase in ODC antizyme production as previously demonstrated by Mitchell and colleagues [36, 37], it is clear that the combination of decreased polyamine synthesis and increased polyamine catabolism leads to the greatest depletion of intracellular polyamines. This phenotypic difference in total polyamine depletion may have more significance *in vivo* than *in vitro*, since in the *in vivo* setting the availability of circulating polyamines may be able to overcome the simple down regulation of ODC exhibited by the small cell lung cancer phenotype. The availability of circulating polyamines apparently caused decreased efficacy in the clinical trial of 2-difluoromethylornithine (DFMO) in small cell lung cancers [1, 2]. By contrast, in the non-small cell phenotype the increased polyamine catabolism may result in a greater growth inhibitory response *in vivo* by responding to analogue treatment with both a decrease in biosynthesis and an increase in catabolism. This may be particularly true for tumor types represented by the A549 cell line, in which nearly completed down regulation of ODC activity combined with highly induced catabolic enzymes, SSAT and SMO [25]. However, further *in vivo* testing in both phenotypes, with an analysis of the *in vivo* effects of CGC-11047 on polyamine metabolism, will be required to determine if differential sensitivity based on phenotype-specific changes in polyamine catabolism exists *in vivo*.

Although preclinical studies with the first generation, symmetrically substituted polyamine analogue BENSpm were encouraging [6, 7, 17, 27], clinical trial results were less so [24, 30, 48, 56]. As previously mentioned, the less than desirable clinical results may have been a result of the inherent properties of BENSpm, and/or the daily dosing schedule used in those trials that showed GI and CNS dose-limiting toxicities. We have previously demonstrated

that daily or continuous administration of polyamine analogues is not necessary to achieve significant tumor growth inhibition *in vivo* [31]. Therefore, to reduce the potential for toxicity, we utilized a single weekly dose of 100 mg/kg CGC-11047. This treatment schedule demonstrated efficacy comparable to that reported for BENSpm given three times a day at 80 mg/kg for six consecutive days [7]. The single weekly dose of CGC-11047 significantly delayed tumor progression without observable toxicity. As the single dose schedule was highly effective in delaying tumor progression and even caused the complete regression of tumor in one of ten mice, and since there was no observable toxicity, alternative dosing schedules might be considered in future studies.

In summary, CGC-11047 represents a new conformationally restricted, short chain, symmetrically substituted polyamine analogue that demonstrates significant growth inhibitory activity in multiple human cells lines representative of the major forms of human lung cancer. Interestingly, although there is clearly a phenotype-specific response with respect to the induction of polyamine catabolism exhibited between the non-small cell lung cancer phenotype (high induction) and the small cell (not induced), this differential response did not translate into significant difference in growth response *in vitro*. However, as the non-small cell lines demonstrated greater polyamine depletion and greater accumulation of analogue, likely a result of increased catabolism, they may be expected to be more sensitive to analogue effects in the *in vivo* setting. Importantly, CGC-11047 demonstrated highly significant growth inhibition in a nude mouse model of human non-small cell lung demonstrating that further pre-clinical and, potentially, clinical trials in lung cancer are warranted.

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Abbreviations

ODC	Ornithine decarboxylase
SMO	Spermine oxides
SSAT	Spermidine/spermine <i>N</i> ¹ -acetyltransferase

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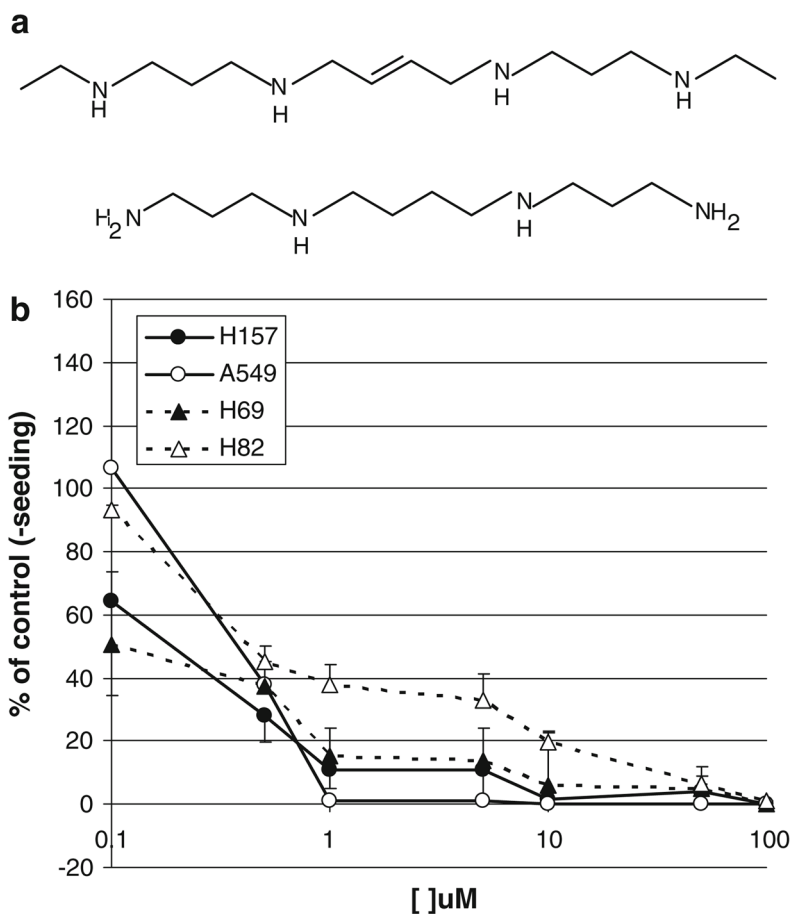


Fig. 1. Effects of the conformationally restricted polyamine analogue, CGC-11047 of growth of human lung cancer cells in vitro. **a** Structures of CGC-1047 and the natural polyamine spermine. **b** CGC-11047 inhibits growth in human non-small cell and small cell lung cancer lines in vitro. Growth inhibition induced by increasing concentrations of CGC-11047 in the non-small cell (H157, A549) and small cell (H82, H69) lung cancer lines was determined by trypan blue exclusion cell after 96-hour treatment. Each point represents three independent experiments \pm SE

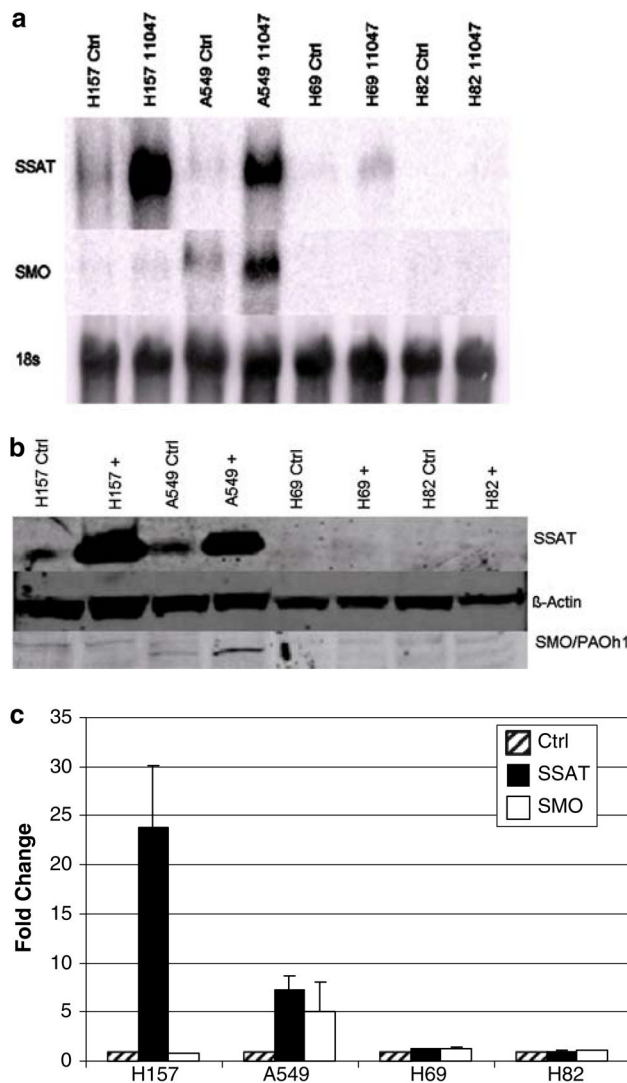


Fig. 2. Induction of polyamine catabolic enzymes at the level of mRNA and protein. **a** Northern blot analysis of CGC-11047 human lung cancer cell lines. Cells were exposed to 10 μ M CGC-11047 for 24 h, where indicated. Each lane was loaded with 10 μ g of total RNA and blots were sequentially probed with labeled SMO and SSAT cDNAs with 18s rRNA serving as a loading control. The illustrated blot is representative of three independent experiments. **b** Western analysis of CGC-11047-treated human lung cancer cells. Cells were exposed to 10 μ M CGC-11047 for 24 h, where indicated and 15 μ g of total cellular protein was loaded per lane. After transfer, membranes were sequentially incubated with SSAT, SMO, or β -actin antibodies, as indicated. The illustrated blot is representative of three independent experiments. **c** Quantified values of protein expression normalized to β -actin. Values represent the mean of three independent experiments (\pm SD) and are shown as the fold change over the control

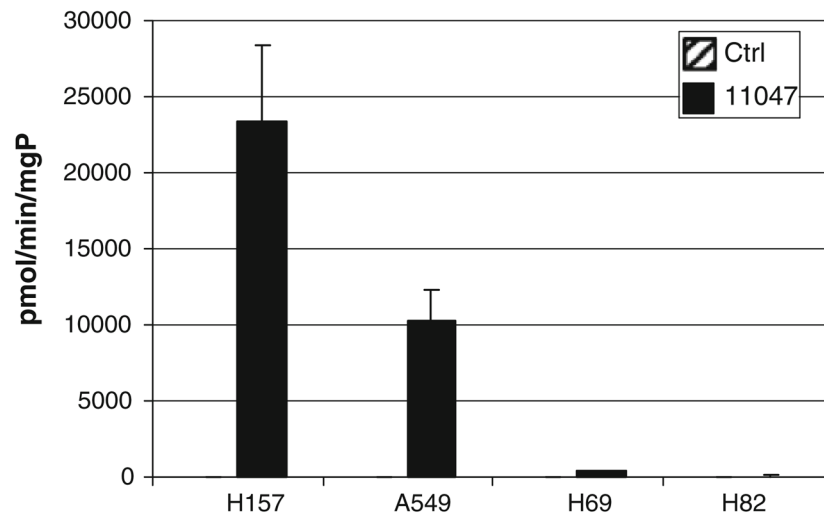


Fig. 3. CGC-11047 induces SSAT enzyme activity in non-small cell lung cancer cell lines. Cells were treated with 10 μ M CGC-11047 for 24 h and SSAT activity was determined. *Striped bars* represent control levels and *solid bars* represent treated cells. The activity values are shown in pmols/min/mg protein. Each bar represents three independent experiments performed in quadruplicate (\pm SE)

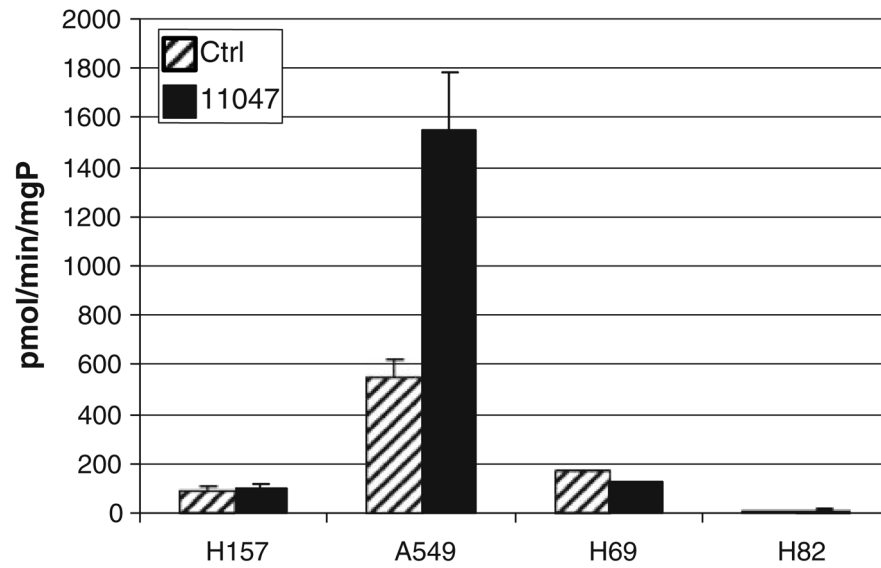


Fig. 4. CGC-11047 induces SMO activity in the A549 non-small cell lung cancer cell line. Cells were treated with 10 μ M CGC-11047 for 24 h and assayed for SMO activity. *The striped bars* represent controls and *solid bars* represent treated cells. The activity values are shown in pmols/min/mg protein. Each bar represents three independent experiments performed in triplicate (\pm SE)

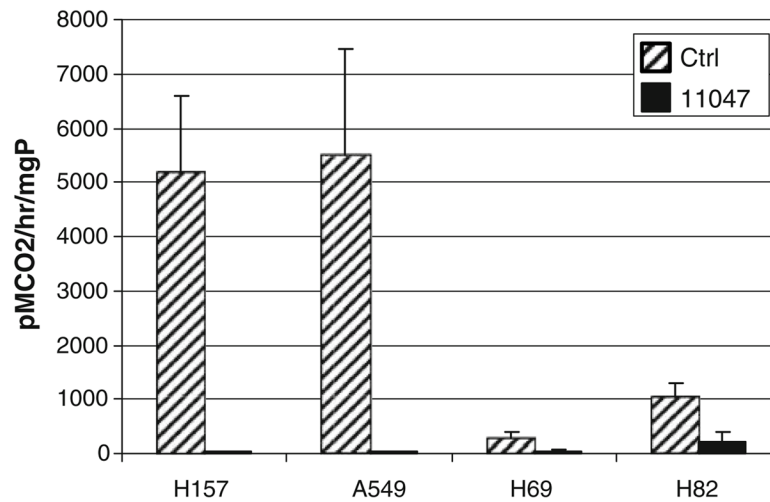


Fig. 5. CGC-11047 treatment decreases ODC activity in human lung cancer cells. Cell lines were treated with 10 μ M CGC-11047 for 24 h and ODC activity in treated and control cells was determined. The *striped bars* represent controls and *solid bars* represent treated cells. The activity values are shown in pmols CO₂/hour/mg protein. Each bar represents a minimum of two independent experiments performed in quadruplicate (\pm SE)

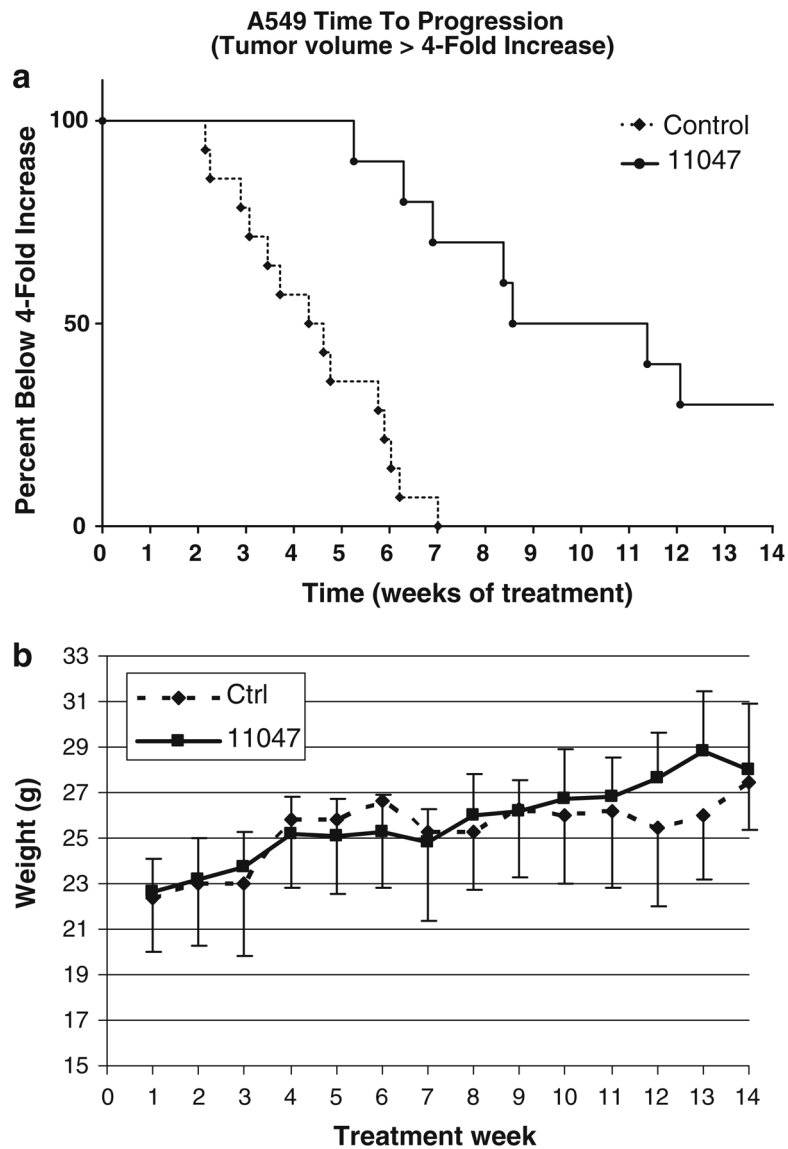


Fig. 6. **a** Treatment of CGC-11047 on A549 cells in vivo increases the time to progression. Tumor cells were implanted in mice and allowed to grow until they reached a minimum of 200 mm³ prior to treatment. Animals with established tumors were then treated once a week as indicate in “Materials and Methods” with either 100 mg/kg CGC-11047 or HBSS. Tumors were measured weekly as described and the fold-increase over week one was calculated. Kaplan–Meier Analysis indicates that CGC11047-treatment significantly slows in vivo tumor growth compared to untreated controls ($P = 0.0001$, using the Mantel–Cox log rank test). **b** CGC11047 does not effect normal weight gain of treated animals

Table 1

Effects of CGC-11047 on intracellular polyamine pools in human lung cancer cells

Cell type and treatment	Polyamines (nmol/mg protein)			
	Putrescine	Spermidine	Spermine	CGC-11047
H157 Control	1.5 ± 0.9 ^a	4.7 ± 1.7	5.1 ± 1.4	ND
H157 Treated	ND	ND	0.24 ± 0.12	24.2 ± 5.1
A549 Control	3.2 ± 1.7	11.6 ± 3.7	3.25 ± 1.5	ND
A549 Treated	0.1 ± 0.1	ND	0.24 ± 0.13	22.3 ± 2.8
H69 Control	10.4 ± 1.3	7.5 ± 3.5	7.7 ± 2.4	ND
H69 Treated	1.0 ± 0.7	1.9 ± 1.9	3.9 ± 2.1	15.4 ± 4.7
H82 control	6.2 ± 2.1	8.3 ± 2.2	5.7 ± 1.2	ND
H82 Treated	1.8 ± 0.7	4.6 ± 1.5	4.9 ± 1.3	11.6 ± 2.1

Indicated cells were exposed to 10 μM CGC-11047 for 24 h and intracellular polyamine pools and analogue accumulation were measured

ND indicates values below the 0.05 nmol/mg protein limit of detection

^aPolyamine pool values represent the mean of three independent experiments performed in duplicate ± SE