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Eribulin inhibits the growth of small cell lung cancer cell lines alone and with radiotherapy

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Abstract

Objectives—Small cell lung cancer (SCLC) patients of all stages are treated with etoposide and cisplatin or carboplatin with or without surgery or chest radiotherapy. Initial response rates are ≥70% however the majority of patients relapse and are resistant to additional therapies due to pan-resistance to these salvage therapies. Therefore, new treatments are urgently needed. The non-taxane microtubule inhibitor eribulin has produced responses in heavily pretreated breast cancer patients. We evaluated the efficacy of eribulin alone and in combination with radiation in a panel of SCLC cell lines established from patients prior to or after receiving chemotherapy and or radiation.

Material and methods—Growth inhibition by eribulin alone, radiation alone and the combination was assessed by MTS assay and clonogenic survival. Eribulin induced cell cycle arrest was evaluated by FACS. Apoptosis was evaluated by using the Caspase-GLO 3/7 luminescent plate assay and by the Vybrant apoptosis assay with analysis by FACS.

Results—Eribulin mesylate inhibited the growth of all 17-SCLC lines at concentrations of ≤10 nM which is a clinically achievable dose. Growth inhibition was not significantly different between cell lines established prior to or after chemotherapy ($p = .5$). Concurrent eribulin + radiation induced a greater G2-M arrest, an increase in apoptotic cells and increased growth inhibition over radiation alone.

Conclusions—Eribulin was highly active alone and in combination with radiation in treatment naïve SCLC lines and lines established from previously treated patients. *In vivo* pre-clinical

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studies of eribulin alone and in combination with radiation should be considered in SCLC cell lines.

Keywords

SCLC; Eribulin; Non-taxane microtubule inhibitor

1. Introduction

Small cell lung cancer accounts for about 15% of all new lung cancer cases in the U.S [1–3]. The overall 5-year survival for both men and women is less than 5%. Patients with good performance status are offered treatment with systemic chemotherapy usually etoposide and a platinum (EP) compound. Patients with early stage may have a surgical resection followed by adjuvant EP and those with stage IIB and III usually receive chest radiotherapy in addition to chemotherapy [3]. These patients are sometimes cured with initial combined modality therapies, but the majority relapse and are resistant to subsequent systemic therapies. For stage 4 (extensive stage, ES), despite initial response rates to first-line chemotherapy of 70–90%, nearly all patients progress within six months and infrequently respond to additional systemic therapies due to cross-resistance to salvage therapies [4]. Therefore, there is an urgent need to develop novel therapies including those that could be used after EP as maintenance therapy, second line therapy or combined with radiotherapy.

The rapid doubling time and high mitotic rate of SCLC makes it susceptible to antimitotic drugs. Antimitotic chemotherapeutic drugs target tubulin the central protein of the microtubule cytoskeleton altering microtubule polymerization and dynamics. Microtubule-targeted agents (MTAs) inhibit microtubule dynamics by altering the addition and loss of heterodimers of α- β-tubulin. Microtubule activity is regulated by two types of non-equilibrium polymerization dynamics and both are critical to mitosis and cell division. In dynamic-instability the microtubule ends switch between growing and shortening [5]. The second dynamic microtubule behavior termed treadmilling involves growth at one microtubule end with a commensurate shortening at the opposite end [5]. Within a given cell the microtubules can undergo predominantly dynamic- instability, treadmilling or a combination [5]. Disruption of microtubule dynamics prevents or delays satisfaction of the spindle assembly checkpoint arresting cells in mitosis resulting in mitotic catastrophe which may lead to cell death through senescence, apoptosis or regulated necrosis [6].

The vinca alkaloids such as vincristine and taxanes such as paclitaxel are mitotic inhibitors with established activity in SCLC and different mechanisms of mitotic inhibition as described below. Vincristine was a mainstay of first line therapy in combination with cyclophosphamide and adriamycin. This 3-drug combination was equally efficacious as etoposide/cisplatin. However its higher rates of toxicity led to a decline in usage. Paclitaxel had considerable activity alone, however a first line trial adding it to etoposide/platinum in SCLC failed to show a superior outcome [7].

Eribulin is a completely synthetic non-taxane non-vinca alkaloid microtubule inhibitor. The effects of eribulin on microtubule dynamic-instability are unique. Whereas both

vincristine and paclitaxel suppress dynamic-instability by suppressing both the shortening and growth phases of the microtubules, eribulin suppresses dynamic-instability by inhibiting the microtubule growth phase while having no effect on the shortening phase [8,9]. Furthermore, eribulin sequesters tubulin into nonfunctional aggregates. The combined outcome of these effects on microtubules by eribulin lead to an irreversible mitotic arrest followed by cell death [10]. In clinical trials eribulin improved survival in breast cancer patients with metastatic or locally advanced disease who were previously treated with chemotherapy [11,12]. Radiobiological studies have demonstrated that cells in the G2-M phase of the cell cycle are less efficient at repairing radiation induced DNA damage. We therefore evaluated the effects of eribulin alone on cell growth in a panel of 17 SCLC cell lines and in combination with radiation in a subset of these lines. The SCLC lines were established from patients before and after exposure to chemotherapy and/or radiation therapy.

2. Material and methods

2.1. Reagents SCLC cell lines

Eribulin was provided by Eisai Co., Ltd. Tokyo, Japan [13]. The following SCLC lines were obtained from Drs. Adi Gazdar and John Minna (UTSouthwestern, Dallas, TX) H82, H146, H187, H211, H345, H378, H446, H524, H748, H774, H841, H889, H1963, H2081, and H2171. The DMS114 and H69 lines were obtained from American Type Culture Collection. Authentication of the cell lines was by short tandem repeats DNA profiling performed through the UCCC DNA Sequencing and Analysis Core. The DNA profile for each cell line matched the profile provided by Drs. Minna and Gazdar and American Type Culture Collection (Manassas, VA). Cell lines were used within 6 months of resuscitation. The lines were mycoplasma free and maintained in RPMI–1640+5% or 10% FBS at 37C in 5% CO2. Clinical characteristics of the patients from which the lines were derived is shown in Table 1 [14].

2.2. Cell proliferation assays

SCLC lines were seeded in 96-well plates at 4000–10,000/well in growth medium. At 24 h post-plating eribulin (0–10 nM) was added and the plates were incubated for 5 days at 37 °C. MTS (Promega, Madison, WI) was added and the formazan complex was allowed to develop for up to 4 h. The absorbency of each well was measured at 490 nm using an automated plate reader (Molecular Devices, Sunnyvale, CA).

2.3. Irradiation

Cells were irradiated with 2–4 Gy using a RS-2000 (Rad Source Technologies, Inc) using a 160 KVp source, at 25 mAmp, and at a dose of 1.43 Gy/minute. Radiation dose and rate were calculated empirically.

2.4. Cell cycle analysis

Cell cycle analysis was performed as follows; 5×10^5 cells were treated with 0 nM–5 nM eribulin for 24–48 h. Treated cells were stained with propidium iodine solution overnight.

Analysis was on a Beckman Coulter Cytomics FC500. Modfit software (Verity Software House, Topsham, MN) was used to calculate the cell cycle distributions.

2.5. Apoptosis analysis

Induction of apoptotic caspases was evaluated using the Caspase-Glo 3/7 assay (Promega, Madison, WI). Cell lines were plated at 4000/ well in a 96-well plate. Eribulin was added 24 h later. The Caspase-Glo 3/7 reagents were added at 24 h post-treatment. The plates were read on a luminometer (Synergy2, Biotek, Winooski VT). The % apoptotic cells was evaluated by FACS using the Vybrant Apoptosis Assay Kit 4 (Molecular Probes, Eugene, OR). Briefly, 5×10^5 cells were incubated with eribulin alone, radiation alone or the combination for 48 h. The cells were harvested, stained with the DNA binding dyes YOPRO and propidium iodine according to manufacturer's directions and analyzed by FACS (Beckman FC500).

2.6. Clonogenic growth assays

To determine clonogenic survival, cells were plated in growth media in 6 well plates at 2000 cells/well and allowed to attach overnight. The effects of 0.31 nM eribulin alone and in concurrent combination with 2 Gy and 4 Gy radiation were evaluated. At 10 days post treatment the colonies were washed with PBS, fixed with 10% formalin and stained with 0.5% crystal violet. Following digital photography, the total colony area was quantified using MetaMorph imaging (Molecular Devices, Downingtown PA).

2.7. Statistical analysis

A two-group t-test was used to determine if there was a correlation between the eribulin IC_{50} concentrations and whether the SCLC line had been established prior to the patient receiving chemotherapy treatment or disease stage. The two-group t-test was also used to determine if eribulin alone or in combination with radiation induced a greater G2-M arrest, apoptosis and growth inhibition compared to control cells.

3. Results

3.1. Eribulin inhibited proliferation of SCLC cell lines

Eribulin inhibited the growth of all 17 SCLC lines with clinically achievable GI_{50} concentrations of ≤10 nM [15]. In 9 lines, the IC_{50} concentration was < 1.5 nM and 6 lines had sub-nanomolar IC_{50} values of < 1 nM (Fig. 1A). Growth inhibition at 10 nM eribulin in these 9 SCLC lines was 76%–98%. Three of these highly sensitive lines were established from SCLC patients with LS disease and six were from patients with ES disease (Table 1). Six had received prior chemotherapy while three had not (Table 1). Growth inhibition by eribulin in the remaining 8 lines is shown in Fig. 1B. The eribulin IC_{50} concentrations ranged from 3 nM–6 nM and growth inhibition at 10 nM eribulin was 53%–80%. Three of these lines were established from SCLC patients with LS disease, 4 lines from patients with ES disease (Table 1). The stage is unknown in the DMS114 line. Five lines were established from patients that had received chemotherapy while 3 lines were from untreated patients (Table 1). Although the sample size was small, eribulin induced growth inhibition (IC_{50}) was not significantly different between cell lines established prior to (N=5, mean =2.3 nM ± 1.4

nM) or after chemotherapy (N= 12, mean = 2.4 nM ± 2.2 nM) (p =.50). There was also no difference in eribulin induced growth inhibition in cell lines established from patients with LS (N = 6, mean 2.83 ± 2.2) and ES disease (N = 10, 2.0 nM ± 1.94 nM) (p=.47). The doubling times of the cell lines also did not correlate with eribulin IC_{50} concentrations. As shown in Table 1, the reported doubling times of the cell lines in Fig. 1A ranged from 25 to 93 h (mean 52 ± 23 h) and in Fig. 1B the cell line doubling times ranged from 38 to 65 h (mean 54 ± 10 h) [16].

3.2. Eribulin induced a mitotic cell cycle block in SCLC lines

The induction of a G2-M mitotic arrest is a hallmark of eribulin. Therefore, we investigated the cell cycle effects of eribulin in 4 of the most sensitive SCLC lines (eribulin IC_{50} < 1.5 nM), and 4 of the less sensitive lines eribulin (IC_{50} of > 3 nM). The cell lines were treated for 24 h stained with propidium iodine and analyzed by flow cytometry.

The %G2-M arrest in the 4 most sensitive cell lines is shown in Fig. 1C. The H446 and H841 lines were established from LS patients and the H378 and H187 were established from ES patients. The cell lines were treated with 1.25 nM and 5 nM eribulin. In the untreated control cells the %G2-M in the 4 lines was 10%–13% (mean = 11.2% ± 1.5%). The %G2-M arrest ranged from 28%–34% (mean = 31% ± 2.5%) in the 1.25 nM treated cells and 52%–65% (mean = 59% ± 6.5%) in the 5 nM treated cells in these 4 lines and was significantly greater than the paired %G2-M arrest of the untreated control cells (p = .02; p = .001 respectively).

The 4 less sensitive lines evaluated were H146 and H524, established from LS patients, and the H69 and H748 lines, established from ES patients. The %G2-M at 24 h in the untreated control cells in these lines ranged from 12%–20% (mean = 16.5% ± 3.7%). In the 1.25 nM eribulin treated cells, the %G2-M arrest (Fig. 1C) and was not significantly different from the paired untreated control cells (p = .27). However, post 5 nM eribulin the %G2-M in the H146, H524 and H748 lines was 43%–62% (mean = 51%, ± 9.8%) and significantly greater than the paired %G2-M arrest of the untreated cells (p = .033; Fig. 1C). In the H69 line the %G–2M at 24 h post 5 nM eribulin was 26% compared to 20% in the untreated control cells.

3.3. Induction of apoptotic caspases by eribulin

We evaluated the induction of apoptotic caspases 3/7 in the H378, H446 and H841 cell lines where the eribulin IC_{50} concentrations were < 1.5 nM. The cell lines were treated with 1.25 nM eribulin for 24 h. Eribulin induced the apoptotic caspases 3/7 in all 3-cell lines (Fig. 1D). The relative luminescent units in the eribulin treated cells were 1.5-2.6 fold higher than in the untreated control cells and the differences were statistically significant (p values < .05). The greatest increase in relative luminescent units compared to the untreated control cells was observed in the H446 line.

3.4. Effects of eribulin with radiotherapy on the growth of LS and ES SCLC cell lines

We evaluated concurrent eribulin with 2 Gy radiation in eight lines. Growth inhibition by radiation alone and in combination with eribulin was evaluated in 5 day MTS assays.

Growth inhibition by 2 Gy radiation alone in these 8 lines was 47%–73% at 5 days post treatment (Fig. 2A–D). The concurrent growth inhibition by eribulin +2 Gy radiation was evaluated in the LS-lines H446, H841, H146 and H524. The H841 line was established following chemotherapy and radiation (Table 1). There was no radiation treatment in the remaining three LS lines, however, all had received chemotherapy (Table 1). Growth inhibition by 2 Gy radiation + eribulin concentrations of 0.15–2.5 nM were evaluated in the H446 and H841 lines (Supplemental Fig. 1A–B). As shown in Fig. 2A, growth inhibition induced by concurrent 2 Gy radiation + 0.625 nM eribulin was significantly greater than that observed with radiation alone and eribulin alone. In the H446 line, growth inhibition in the combination was 87% ± 2% compared to 50% ± 0.7% induced by radiation alone (p=.02) and 45% ± 2% induced by eribulin alone (p = 0.04). In the H841 line growth inhibition with concurrent 2 Gy radiation and 0.625 nM eribulin was 76% ± 3% and significantly different from radiation alone 47% ± 5% (p =.04) and eribulin alone 12% ± 4% (p = .004).

In the H146 and H524 lines the growth inhibition by concurrent 2 Gy radiation + eribulin 1.25–10 nM was evaluated (Supplemental Fig. 1C–D). A concentration of 5 nM eribulin was necessary to significantly increase growth inhibition above that observed with radiation alone (Fig. 2B). In the H146 line growth inhibition by 2 Gy radiation + 5 nM eribulin was 76% ± 7% and significantly different from radiation alone 58% ± 5% (p = .004) and eribulin alone 64% ± 8% (p =.03). In the H524 line the 86% ± 0.7% growth inhibition in the combination was also significantly different from radiation alone (73% ± 3%, p=.003) and greater but not significantly different from eribulin alone (53% ± 7%, p = .1).

The growth inhibition by eribulin + radiation was evaluated in the ES lines H378, H187, H69 and H748. The H378, H69 and H748 lines were established following chemotherapy and radiation (Table 1). The H187 was established prior to the patient receiving chemotherapy or radiation (Table 1). In the H378 and H187 lines, 2 Gy radiation + eribulin concentrations of 0.15–2.5 nM were evaluated (Supplemental Fig. 2A–B). Growth inhibition in these 2 lines following concurrent 2 Gy radiation + 0.625 nM eribulin was significantly greater than that observed with radiation alone (Fig. 2C). In the H378 line growth inhibition following the combination of 2 Gy radiation + 0.625 nM erbulin was 65% ± 3% and significantly greater than the 40% ± 2% induced by radiation alone (p =. 01) but not greater than that induced by eribulin alone (66% ± 6% p = .5). In the H187 cells (Fig. 2C), growth inhibition in the combination was 66% ± 2% and significantly different form 2 Gy radiation alone 43% ± 4% (p= .002) and eribulin alone 19% ± 8% (p=.006).

In the H69 and H748 lines, growth inhibition by concurrent 2 Gy radiation + eribulin 1.25–10 nM was evaluated (Supplemental Fig. 2C–D). In the H69 line, growth inhibition by 2 Gy + 5 nM eribulin was 70% ± 5% and not significantly different from growth inhibition by radiation alone 50% ± 2% (p = .2) or eribulin alone 76% ± 0.7% (p=.3) (Fig. 2D). In the H748 line, the 70% ± 4% growth inhibition in the combination of 2 Gy radiation + 5 nM eribulin was significantly different from 2 Gy radiation alone 56% ± 4% (p = .02) but not eribulin alone 51% ± 13% (p = .3) (Fig. 2D).

In summary, the 4 SCLC lines established from patients with LS disease and in 3 of the 4 lines established from patients with ES disease, the growth inhibition by eribulin in

combination with radiation was significantly greater then radiation alone (p values < .05). Increased growth inhibition by the combination compared to eribulin alone was also superior in 6 of the above 7 lines but was significant only in the H446, H841, H146 and H187 lines (p values < .05).

3.5. Concurrent eribulin + radiation reduced clonogenic survival

We also evaluated clonogenic survival at 10 days post concurrent eribulin + radiation in the adherent H841 cell line. As shown in Fig. 3A, concurrent treatment of H841 cells with eribulin 0.31 nM and 2 Gy or 4 Gy radiation significantly reduced clonogenic survival compared to radiation alone and eribulin alone. The results are shown graphically in Fig. 3B and the differences were statistically significant (p values < .05).

3.6. Effects of eribulin on radiation induced G2-M cell cycle block

We evaluated the effects of eribulin on radiation induced G2-M arrest at 24 and 48 h post-treatment as cells arrested in G2-M are less efficient at repairing radiation induced DNA damage. Radiation doses were 2 Gy or 3 Gy that alone induced a G2-M arrest of >30% but < 70% at 24 h post radiation. The eribulin dose was 1.25 nM in the H446, H841, H378 and H524 lines and 5 nM in the H146, H187, and H748 lines. These eribulin doses alone induced a G2-M arrest that was significantly greater than that observed in the untreated control cells at 24 h post-treatment (Fig. 1C–D). Although in the H69 line 5 nM eribulin alone did not induce a G2-M arrest greater than that observed in the untreated control cells, the line was included in the study.

The %G2-M cells in the LS lines at 24 h post radiation + eribulin are shown in Table 2. In the H446 line the %G2-M induced by 1.25 nM eribulin +3 Gy radiation was 70% compared to 44% by radiation alone and 34% by eribulin alone. The %G2-M in the combination of 1.25 nM eribulin +3 Gy in the H841 treated cells was 59% compared to 37% by radiation alone and 32% by eribulin alone. In the H146 line the %G2-M induced by 5 nM eribulin + 2 Gy radiation was 83% compared to 66% by radiation alone and 48% by eribulin alone. The concurrent combination of 5 nM eribulin + 2 Gy radiation induced a 68% G2-M arrest in the H524 line compared to 48% by radiation alone and 43% by eribulin alone. The %G2-M induced by the concurrent combination of eribulin + radiation in the above 4 LS lines was significantly greater than the paired%G2-M induced by radiation alone (p= .0015) and eribulin alone (p = .0016). At 48 h post-treatment the fraction of cell in%G2-M were similar to the fraction observed at 24 h (data not shown).

In the 4 SCLC lines established from patients with ES disease, the %G2-M cells at 24 h post concurrent eribulin + radiation was not significantly different from the %G2-M induced by radiation alone (Table 2). However, at 48 h post 1.25 nM eribulin + 3 Gy radiation, the %G2-M induced in the H378 line was 93% compared to 41% induced by radiation alone and in the H187 line the %G2-M induced by the combination was 78% compared to 30% induced by radiation alone. The %G2-M induced by 5 nM eribulin +3 Gy radiaiton in the H748 line was 79% compared to 58% G2-M induced by radiation alone. The %G2-M induced by the combination of eribulin + radiation in these three lines compared to the paired%G2-M arrest induced by radiation alone was significant (p = .05) but was not

significantly greater than the %G2-M induced by eribulin alone (p= .18). In the ES line H69 even at 48 h post treatment the %G2-M arrest by the combination of 5 nM eriublin + radiation was not significantly greater than radiation alone or eribulin alone (Table 2).

3.7. Eribulin enhanced the fraction of radiation induced apoptotic cells

We evaluated the H446 cell line for the % apoptotic cells at 48 h post concurrent eribulin and radiation as this line showed the greatest induction of caspases 3/7 following 24 h of 1.25 nM eribulin alone. A radiation dose of 3 Gy was required to induce apoptosis at 48 h. In the untreated control cells 10% of the cells were apoptotic (Fig 4A). Three Gy radiation alone induced apoptosis in 36% of the treated cells (Fig 4B) and 1.25 nM eribulin alone induced apoptosis in 42% of the cells (Fig 4C). In the concurrent combination of 1.25 nM eribulin + 3 Gy radiation 60% of the cells were apoptotic (Fig 4D). Correspondingly the %viable in the untreated control was 90% (4A) versus 39% in the combination of eribulin + radiation (Fig 4D).

4. Discussion

New agents for the treatment of SCLC are sorely needed as none have been approved since topotecan in 2007 despite the many new agents approved in non-small cell lung cancer. Eribulin is a novel antimitotic agent approved in breast cancer and worthy of further testing in SCLC. In this report we demonstrated pre-clinical *in vitro* efficacy of single agent eribulin in a panel of 17 SCLC lines. All lines were significantly growth inhibited by clinically achievable concentrations. Nine lines were extremely sensitive with eribulin IC_{50} concentrations of < 1.5 nM and six had sub-nM IC_{50} concentrations. Eight SCLC lines were inhibited by eribulin IC_{50} concentrations of 3–6.2 nM. Eribulin induced growth inhibition was not significantly different between cell lines established from patients prior to or after receiving chemotherapy (p = .5). There was also no difference in growth inhibition in cell lines established from patients with LS and ES disease (p = .47). These results are encouraging as the majority of patients present with ES disease at the time of diagnosis and response rates to standard of care (EP) are short lived with the development of chemotherapy resistant disease in both patients with ES and LS disease [2].

Our *in vitro* results in SCLC lines are similar to those found in two recent publications encompassing 22-breast cancer cell lines where 20 lines had eribulin IC_{50} concentrations below 5 nM [17,18]. Eribulin has been FDA approved for the treatment of breast cancer patients with metastatic disease who have had at least two treatment regimens with an anthracycline and a taxane. Furthermore, the response of our panel of 17 SCLC to the MTAs docetaxel, paclitaxel and vincristine, which have established activity in SCLC, are publicly available through the National Cancer Institute Developmental Therapeutics Program [19]. As shown in Supplemental Table 1, for our panel of 17 SCLC lines the IC_{50} concentrations for docetaxel, paclitaxel and vincristine were generally but not always higher than the eribulin IC_{50} concentration.

A recent publication investigated the *in vivo* effects of eribulin on human cancer xenografts including the SCLC line H82 [20]. The H82 cell line was established from a SCLC patient with ES disease following chemotherapy and radiation [15]. The H82 xenografts were dose

q4dx3 and the dose range was 0.54–1.7 mg/kg (10 mice/group). Tumor stasis was observed at the 0.54, 0.71 and 1.27 mg/kg doses. One to 3 mice in each of these treatment groups became tumor free and remained so through the end of the study (Day 60). Treatment with 0.96 and 1.7 mg/kg eribulin resulted in tumor regression and 3 mice in the 0.96 mg/kg group became tumor free and remained so through the end of the study as did 2 mice in the 1.7 mg/kg group.

In SCLC patients who present with LS disease, concurrent chest radiotherapy is given with chemotherapy but this fails to cure the majority of patients. Thus it is important that new chemotherapies have synergy with RT as eribulin does. In addition, in ES patients consolidative thoracic radiation therapy following induction chemotherapy has had modest effects on increasing survival [21]. In the 4 LS lines evaluated in this report, the concurrent combination of eribulin + radiation, induced significantly greater growth inhibition than radiation alone. Furthermore, we demonstrated *in vitro* efficacy of concurrent eribulin plus radiation in 3 of 4 SCLC lines established form patients with ES. Eribulin also enhanced the accumulation of irradiated cells in the G2-M phase of the cell cycle where radiation induced damage is less efficiently repaired.

Our results provides the bases for further pre-clinical *in vitro* and *in vivo* evaluation of eribulin alone and in combination with radiation. Furthermore, the *in vivo* efficacy of single agent eribulin on H82 ES-SCLC tumor xenografts provides an *in vivo* model to evaluate eribulin in combination with radiation (20).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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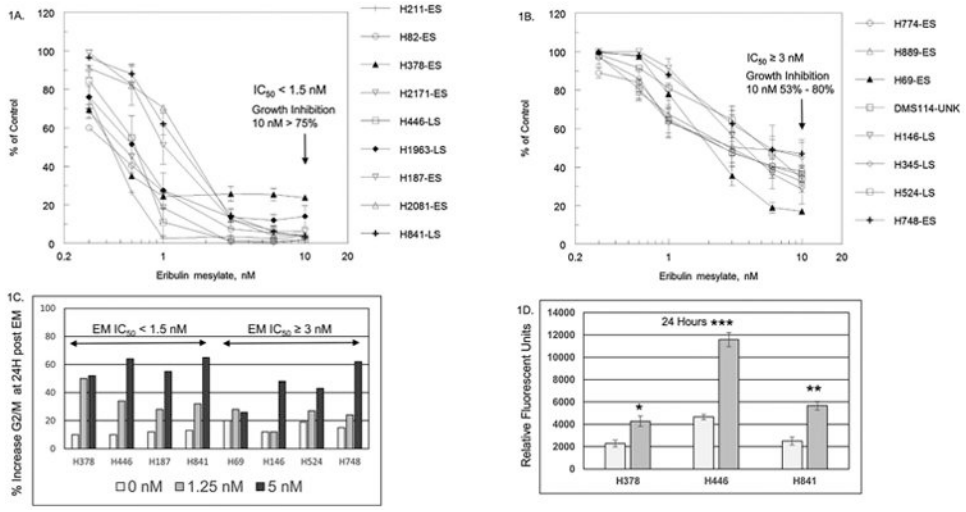

Fig. 1.

The effects of single agent eribulin on cell line growth, G2-M arrest and induction of the apoptotic caspases 3/7. Growth Inhibition: A. In these 9 SCLC lines the eribulin IC_{50} concentrations were < 1.5 nM and growth inhibition at 10 nM was > 75%. B. In these 8 SCLC lines the eribulin IC_{50} concentrations were 3–6.2 nM and growth inhibition as 10 nM was 53%–80%. LS = cell lines established from SCLC patients with limited stage disease and ES = cell lines established from SCLC patients with extensive stage disease. G2-M Arrest: The cell lines were treated with 1.25 or 5 nM EM (eribulin) for 24 h, and analyzed by flow cytometry for the %G2-M arrested cells. Apoptosis: SCLC lines with IC_{50} concentrations of < 1.5 nM were treated with eribulin at 0 & 1.25 nM for 24 h and analyzed for the induction of apoptotic caspases 3/7. Data is presented as relative fluorescent units. The increases over untreated control were statistically significant (* $p < .05$, ** $p < .005$, ***$p < .0005$).

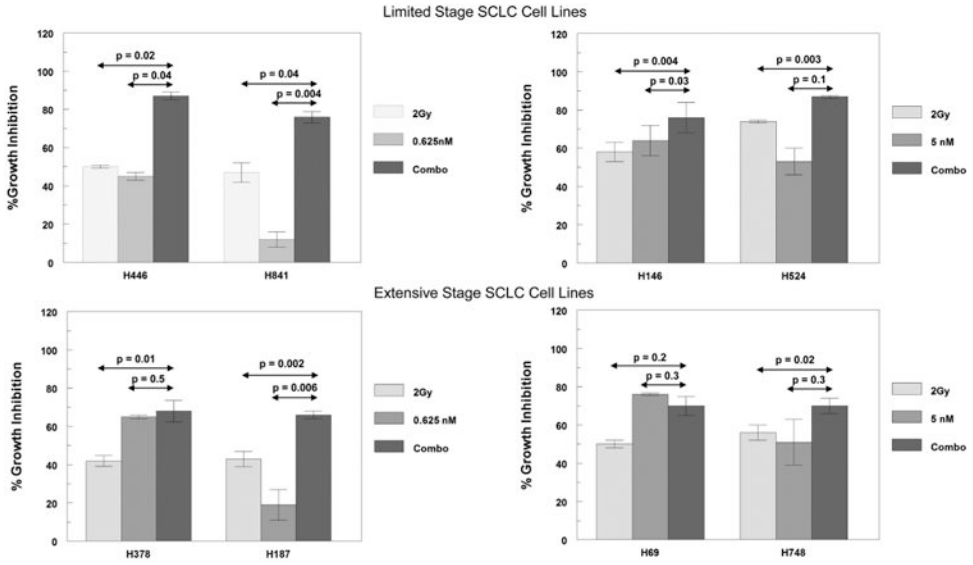

Fig. 2.

Growth inhibition by eribulin in concurrent combination with radiation in SCLC lines established from patients with LS and ES disease. A–B. LS-SCLC lines: A. The single agent eribulin IC_{50} concentration in the H446 and H841 lines were 0.55 and 1.3 nM respectively. In both lines growth inhibition by 0.625 nM eribulin + 2 Gy radiation was significantly greater then radiation alone or eribulin alone (p values < .05). B. The eribulin IC_{50} concentration in the H146 and H524 lines were 4.7 and 5 nM respectively. Growth inhibition by 5 nM eribulin plus 2 Gy radiation in the H146 and H524 lines was significantly greater than that observed with radiation alone in and eribulin alone (p values < .05). Growth inhibition by the combination of 5 nM eribulin in combination with 2 Gy radiation was significantly greater then radiation alone (p = .003) but was not significantly greater than eribulin alone (p = .1). C–D. ES-SCLC lines: C. The eribulin IC_{50} concentration in the H378 and H187 lines were 0.46 and 1 nM respectively. Growth inhibition by 2 Gy radiation + 0.625 nM eribulin was significantly greater than 2 Gy radiation alone in the H378 line and the H187 line p values < .05). Growth inhibition by the combination of radiation + eribulin was significantly greater then eribulin alone only in the H187 line (p = .0064). D. The eribulin IC_{50} concentration in the H69 and H748 lines were 3.6 and 6.2 nM respectively. Significant growth inhibition by 5 nM eribulin + 2 Gy radiation was observed in the H748 line (p < .05) but not in the H69 cell lines (p = .156). Growth inhibition by the combination of radiation + eribulin was not significantly greater then eribulin alone in either the H69 or H748 lines (p values > .05).

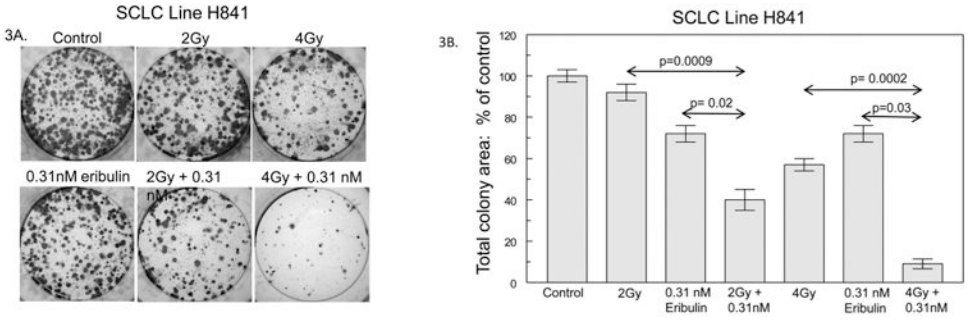

Fig. 3.

Eribulin enhanced the effect of radiation on clonogenic survival. A. The adherent H841line was treated with 2 Gy and 4 Gy radiation alone or concurrently with 0.31 nM eribulin. At 10 days post-treatment the colonies were stained with crystal violet and photographed. A representative well of the three replicates is shown. B. Mean total colony area (± SEM, n = 3) for the indicated erlotinib and radiation treatments were quantified using Metamorph Software and the data are graphically presented. Inhibition of colony formation by 0.31 nM eribulin + 2 Gy and 4 Gy was statistically greater than that observed with radiation alone and eribulin alone (p values < .05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

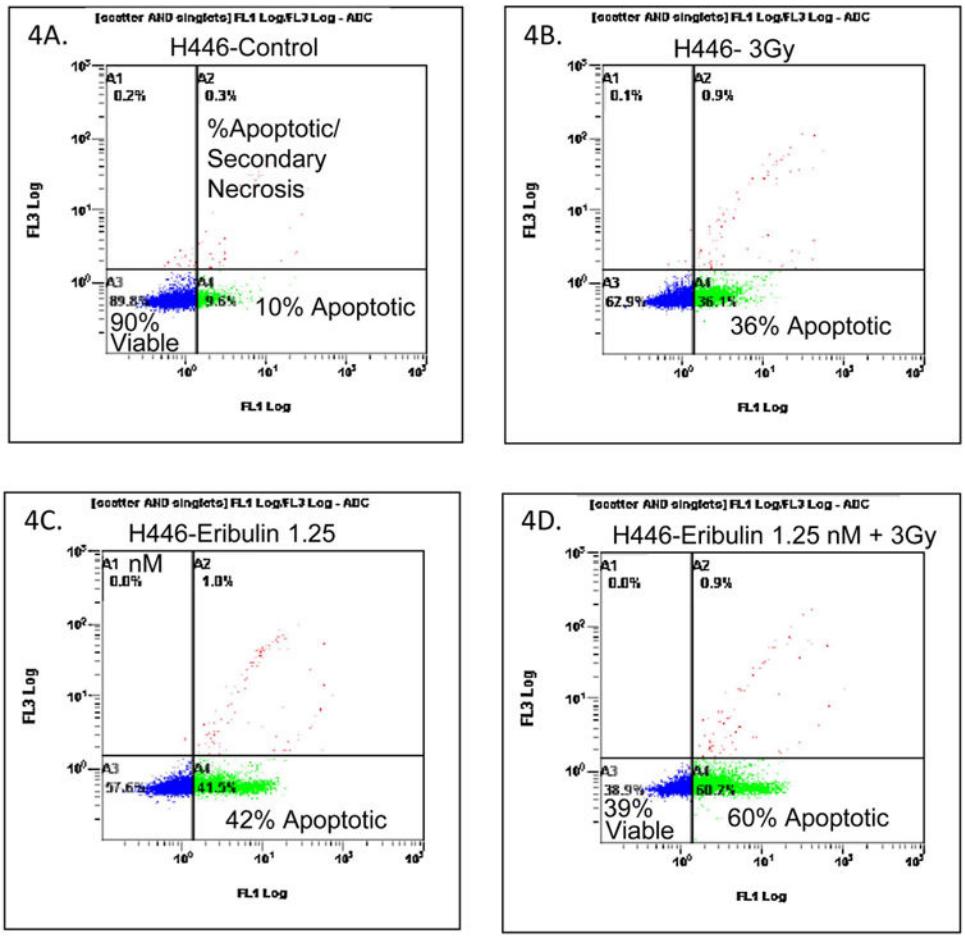

Fig. 4.

The effects of eribulin in combination with radiation on apoptosis. The H446 line was treated with 3 Gy radiation, 1.25 nM eribulin or the concurrent combination. The cells were harvested following 48 h of treatment and analyzed by flow cytometry. The %apoptotic cells was 10% in the control cells, 36% in the 3 Gy radiation alone, 42% following eribulin alone and 60% in the combination of 3 Gy radiation and 1.25 nM eribulin.

Table 1

Cell Line Characteristics

Cell Line	Stage*	Gender*	PY*	Prior Chemo*	Prior Rads*	DT+	E IC_{50} nM
H211	ES	F	35	Y	Y	56 H	0.42
H82	ES	M	UNK	Y	Y	26 H	0.43
H378	ES	F	50	Y	Y	93 H	0.46
H2171	ES	M	150	Y	Y	49 H	0.48
H446	LS	M	80	Y	N	41 H	0.55
H1963	LS	M	30	N	N	44 H	0.6
H187	ES	M	50	N	N	68 H	1
H2081	ES	F	25	Y	Y	ND	1.2
H841	LS	M	70	Y	Y	31 H	1.3
H774	ES	M	30	N	N	ND	3
H889	ES	F	30	N	N	61 H	3.2
H69	ES	M	UNK	Y	Y	51 H	3.6
DMS114	UNK	M	UNK	N	UNK	91 H	3.7
H146	LS	M	100	Y	N	53 H	4.7
H345	LS	M	60	Y	Y	65 H	4.8
H524	LS	M	30	Y	N	38 H	5
H748	ES	M	50	Y	Y	ND	6.2

*Obtained from Ref 15; ES = extensive stage; LS = limited stage; F = female; M = male; PY = pack years; UNK = unknown; Chemo = chemotherapy; Rads = radiation treatment; DT = doubling time; + Obtained from Ref 16; H = hours; E = eribulin.

Table 2

%G2-M Arrest Induced by Eribulin (E) + Radiation in SCLC Lines.

Limited Stage Lines	**%G2-M**	**Extensive Stage Lines**	**%G2-M**	
H446 E IC_{50} 0.55 nM	**24 H**	**H378 E IC_{50} 0.42 nM**	**24 H vs 48 H**	
1.25 nM Eribulin	34	1.25 nM Eribulin	50	84
3 Gy	44	3 Gy	48	41
3 Gy + 1.25 nM	70	3 Gy + 1.25 nM	53	93
H841 E IC_{50} 1.3 nM		H187 E IC_{50} 1 nM		
1.25 nM	32	1.25 nM	28	59
3 Gy	37	3 Gy	66	30
3 Gy +1.25 nM	59	3 Gy + 1.25 nM	64	78
H146 E IC_{50} 4.7 nM		H748 E IC_{50} 6.2 nM		
5 nM	48	5 nM	62	77
2 Gy	66	3 Gy	51	58
2 Gy + 5 nM	83	3 Gy + 5 nM	54	79
H524 E IC_{50} 5 nM		H69 E IC_{50} 3.6 nM		
5 nM	43	5 nM	26	31
2 Gy	48	3 Gy	59	41
2 Gy + 5 nM	68	3 Gy + 5 nM	61	45