Supplementary Methods

Cell-line testing

Mycoplasma testing was conducted for 15 of 19 cell-lines not purchased from the American Type Culture Collection (ATCC) within the last 6 months. Briefly, 50μL MycoAlert™ Plus reagent was added to 50uL cell culture medium supernatant and read after 5 mins using a microplate reader (CLARIOstar, BMG LABTECH) to generate Readout A. 50μL MycoAlert™ Plus substrate was added, cells were incubated on a shaker at room temperature for 10 mins, and then read using the described method to generate readout B. The ratio of readout B/A was used to determine presence of mycoplasma (cell-lines with a ratio between 0.9 and 1.2 were quarantined for 24 hours and retested); a ratio less than 0.9 was considered as negative for mycoplasma.

STR DNA profiling was conducted to authenticate cell-lines (14 of 19 cell-lines not purchased from the ATCC within the last 6 months). DNA from cell-lines was purified using QIAquick PCR Purification Kit (purchased from Qiagen, catalog# 28104). Purified DNA was then sent to the Centre for Applied Genomics at the SickKids Hospital (Toronto, ON, Canada) for testing.

Cell-lines received from ATCC were not further tested (ie: for mycoplasma or STR) as they were cultured and assayed within 6 months of purchase. The ATCC ensures authentication of human cell-lines through STR profiling and mycoplasma testing.

Immunoblotting and apoptosis assessment

The cells were treated with 0.5μM and 1μM of TAK-243 for 24 hours, washed with PBS and lysed using RIPA buffer. The cell lysate was centrifuged at 21,000*g* for 45 min at 4°C and supernatant was collected. Proteins were separated from the supernatant on 8% SDS–polyacrylamide gel using the Tricine buffer system. The proteins were then transferred onto the PVDF membrane at 20V for 16-20 hours at 4°C. After blocking with 3% BSA in TBST for 1 hour at room temperature, the membrane was incubated with anti-PARP1 (cat#: sc-8007, 1:500, Santa Cruz), anti-cleaved PARP (cat#: 9541S, 1:1000, CST) and anti-β actin (cat#: 3700S, 1:15000, CST) at 4°C overnight. After washing with TBST thrice, the membrane was incubated with IRDye donkey-anti-rabbit 800CW (LI-COR Biosciences, P/N: 926-32213) and IRDye donkey-anti-mouse 680RD (LI-COR Biosciences, P/N: 926-68072) secondary antibodies. Near-infrared imaging (LI-COR; Odyssey) was performed and bands were quantified using ImageStudio (LI-COR; version 5.2.5). After normalizing the bands with β-actin for loading control, the ratio of PARP1 to cleaved-PARP was calculated.

Cell viability assays

The drug response of 26 SCLC cell-lines was assessed using the CellTiter Glo 2.0 assay (Promega, #G9243). Briefly, cell-lines were plated in 384 well plates using the automated Thermo Scientific Multidrop Combi Reagent Dispenser (#5840300). Once cells were plated, drugs were added (TAK-243, range: 0-1 μM; Cisplatin/Etoposide 1:1 ratio, range: 0-10 μM; olaparib, range 0.013-500 μM) using the Tecan D300e Digital Dispenser. DMSO and staurosporine (10mM) were used as the vehicle (100% viability)

and kill positive (100% cell death, for background normalization) controls, respectively in all cell-viability experiments. Plates were incubated at 37°C in a humidified CO_2 incubator for 3 days. Cell viability was assessed using the Celltiter Glo 2.0 by measuring luminescence a microplate reader (CLARIOstar, BMG LABTECH).

To evaluate drug synergy between TAK-243 and C/E, cell-lines were seeded in 384 well plates and treated with 144 cell-line specific concentration combinations of TAK-243 and C/E (1:1 ratio of C/E) to result in a 12 by 12 dose-response matrix. The drug combinations of TAK-243 (0.00026 - 2μM) and C/E (0.0055 - 15μM) were chosen for each cell-line based on their specific single agent sensitivity to each drug individually, with stepwise log-fold increments between doses. Similarly, to evaluate drug synergy between TAK-243 and olaparib, cell-lines were seeded in 384 well plates and treated with 56 concentration combinations of TAK-243 (0.00026 - 1μM) and olaparib (0.013 - 250μM) in triplicates to result in an 8 by 7 dose-response matrix. For synergy experiments, cell viability was assessed using the alamarBlue resazurin conversion assay (ThermoFisher Scientific, #DAL1025) by measuring fluorescence after six days of drug exposure using a microplate reader (CLARIOstar, BMG LABTECH).

***In vivo* studies**

Patient-derived xenograft (PDX) dissociated tumor cells were passaged in accordance with previous methodologies[1,2]. Once tumors reached an average starting tumor volume between 100-200mm^3, animals were randomized into experimental or control groups. Mouse weight and tumour volumes were measured biweekly using a digital caliper and scale. Tumour volumes (TV) were calculated by the following equation:

TV = $xy^2(0.52)$. All animals were euthanized once a tumour endpoint of $1000mm^3$ (volume) or 1.5cm (length) was reached or if any other humane endpoint was observed as recommended by the Animal Resource Centre veterinarian.

Statistical Analysis

Dose response analysis

Drug dose response was modelled by the LL.4 four-parameter log-logistic function:

$$f(x\ (b,c,d,e)) = c + \frac{d-c}{1+\exp\ (b(\log\ (x) - \log(e)))}$$

using the *drc* analysis package (version 3.0-1)[3] in RStudio. *b* denotes the steepness of the dose-response curve, *c* and *d* represent the lower and upper limits of the response, and *e* is the half-maximal effective dose (EC50).

To evaluate TAK-243 single agent activity, cell survival was calculated relative to control cells (treated with DMSO and considered as 100% viable) and to kill positive cells treated with staurosporine (considered to have 0% survival). If large discrepancies between control readouts were observed due to edge-effect, wells treated with the lowest concentration of TAK-243 (0.25-0.5nM) were considered 100% viable. Outliers were excluded from analysis.

For single agent analysis, the upper limit of dose response curves was set to 100%, while no limit constraints were initially specified to generate curves for combination experiments. For combination experiments, dose response of cell-lines was reanalyzed by calculating cell survival relative to the survival of wells treated only with the single agent of the fixed drug (i.e. TAK-243 or C/E alone), to account for additive drug interactions.

The AUC of this normalized response was determined. ∆AUC was measured using the following equation:

$$\Delta \mathrm{AUC} = \frac{\left(\mathrm{AUC}_{\mathrm{Single\ agent}} - \mathrm{Normalized\ AUC}_{\mathrm{combination}}\right)}{\mathrm{AUC}_{\mathrm{Single\ agent}}} \times 100$$

Kaplan Meier survival analysis

Adjusted *p*-values using the Benjamini-Hochberg method were utilized when comparing multiple Kaplan-Meier survival curves.

References:

1. Daniel, V. C. *et al.* A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. *Cancer Res.* **69**, 3364–3373 (2009).
2. Hann, C. L. *et al.* Therapeutic efficacy of ABT-737, a selective inhibitor of BCL-2, in small cell lung cancer. *Cancer Res.* **68**, 2321–2328 (2008).
3. Ritz, C., Baty, F., Streibig, J. C. & Gerhard, D. Dose-response analysis using R. *PLoS One* **10**, (2015).