Supplementary Methods

Cell culture and spheroid generation and treatment

The human OAC cell lines OE33, OE19 and SK-GT-4 were purchased from the European Collection of Authenticated Cell Cultures, UK (ECACC). Cell lines were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium with L-glutamine (Gibco, Life Technologies, UK) supplemented with 10% (v/v) heat inactivated foetal bovine serum (FBS; Gibco, Life Technologies, UK). Cells were incubated at 37°C, with 95% humidified air and 5% CO_2. All cell lines tested negative for mycoplasma infection.

Spheroids were generated by seeding cells at a density of $2.5x10^4$ cells per well in ultra-low adherence round-bottomed 96-well plates (Corning). After aggregation, spheroids were treated according to specific experimental conditions, with media replaced every 2 days. Spheroids were imaged using the GelCount instrument (Oxford Optronix), and spheroid size was determined as previously reported [1,2]

Transfection with Pre-miR miRNA pre-cursor molecules

Ambion pre-miR miRNA precursor molecules (Thermo Fisher Scientific, UK) were used to achieve transient miR-187 overexpression. Cells were seeded into 6 cm dishes at a density of $0.8x10^6$ cells/ dish prior to reverse transfection with 5 nM Pre-miR-187 precursor molecules in combination with Lipofectamine RNAiMAX (Invitrogen, UK). Non-transfected control cells were concurrently seeded and treated using an identical protocol whereby the miRNA precursor molecules were substituted for the vehicle control (nuclease free water).

Patient treatment and histology

Following ethical approval (Joint St James's Hospital/AMNCH ethical review board, Reference ID 2011/27/01) and written informed consent, diagnostic biopsy tumour specimens were taken from patients with a diagnosis of operable OAC, prior to neoadjuvant therapy. All patients received a complete course of neoadjuvant CRT. Chemotherapy consisted of 2 courses of 5-fluorouracil (5-FU) and cisplatin, as previously described [3]. Patients received 40.05 Gy in 15 daily fractions (2.67 Gy/fraction) over 3 weeks as previously described [3]. Surgical resection was performed approximately one month following completion of the CRT regimen. All resected oesophagectomy specimens were assessed by an experienced pathologist. Tumour response to treatment was assigned 1 of 5 tumour

regression grades (TRG) as previously described [4]. Good responders were classified as patients achieving a TRG of 1 or 2, whilst poor responders were classified as patients having a TRG of 3, 4 or 5, as previously described [5]. For the purposes of this study patients with a TRG 3 were excluded.

Tissue collection

Diagnostic endoscopic biopsies were obtained by a qualified endoscopist prior to neoadjuvant therapy. Immediately adjacent tissue was taken for histologic confirmation, which was performed using routine Haematoxylin and Eosin staining. Specimens were immediately placed in RNAlater (Ambion) and refrigerated for 24 h, before removal of RNA later and storage at -80^{o}C.

Clonogenic assay

Cell seeding densities were optimised for each treatment condition to ensure that at least 100 colonies, each consisting of at least 50 cells were counted. Cells were seeded directly into 6 well plates at the optimised seeding densities (500-5000 cells/well). Once adhered, cells were treated with GDC-0941 or the DMSO 18 hours and then irradiated/ mock irradiated. Clonogenic plates were then placed in the incubator 7-10 days, fixed, and stained through the application of crystal violet staining solution (0.1% w/v crystal violet, 70% v/v methanol, 30% v/v dH_2O). Colonies were counted using a Gel Count system (Oxford Optronics, UK). Plating efficiencies were calculated as follows: average colony number / number of cells seeded. The surviving fraction (SF) for each treatment group was then determined as follows: (average colony number/plating efficiency of control) X seeding density [6].

Cell viability assay

Short term cell viability was assessed using the Cell Titer 96 AQeous One Solution MTS cell proliferation assay (Promega, UK) as previously described [2]. Cells were seeded into 96 well plates at a seeding density of 3000 cells/well and incubated over night to allow cells to adhere. Complete medium was replaced with treatment conditions and plates were returned to the incubator for a further 48 h, and MTS reagent added as per manufacturer's instructions. Media only absorbance was subtracted from mean absorbance values and cell viability for each treatment condition was calculated relative to DMSO vehicle control, normalised to 100% viability.

Western blotting

Cells were lysed in UTB (9M Urea, 75 mM Tris- HCL pH 7.5, 0.1M β- Mercaptoethanol) as previously reported [7]. Tumour samples were prepared for lysis using a BioPulverizer and Cryo-cup grinder (BioSpec, USA) and lysed in RIPA buffer (Cell Signalling Technology, USA) supplemented with protease inhibitors (Mini, EDTA-free Protease Inhibitor Cocktail, Roche, UK) and phosphatase inhibitors (PhosSTOP, Roche, UK). Western blotting was performed as previously reported [7]. Antibodies used include AKT (pan), phospho AKT Serine 473, PARP, ß-actin or GAPDH, details noted in Supplementary Table 2. Detection was carried out using the ChemiDoc XRS+ (BioRad, UK). Densitometric analysis of band intensity of blots was carried out using Image J (NIH, USA).