Identification of anticancer drugs to radiosensitise BRAF-wild-type and mutant colorectal cancer

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ABSTRACT
Objective: Patients with BRAF-mutant colorectal cancer (CRC) have a poor prognosis. Molecular status is not currently used to select which drug to use in combination with radiotherapy. Our aim was to identify drugs that radiosensitise CRC cells with known BRAF status.

Methods: We screened 298 oncological drugs with and without ionising radiation in colorectal cancer cells isogenic for BRAF. Hits from rank product analysis were validated in a 16-cell line panel of human CRC cell lines, using clonogenic survival assays and xenograft models in vivo.

Results: Most consistently identified hits were drugs targeting cell growth/proliferation or DNA damage repair. The most effective class of drugs that radiosensitised wild-type and mutant cell lines was PARP inhibitors. In clonogenic survival assays, talazoparib produced a radiation enhancement ratio of 1.9 in DLD1 (BRAF-wildtype) cells and 1.8 in RKO (BRAF V600E) cells. In DLD1 xenografts, talazoparib significantly increased the inhibitory effect of radiation on tumour growth (P ≤ 0.01).

Conclusions: Our method for screening large drug libraries for radiosensitisation has identified PARP inhibitors as promising radiosensitisers of colorectal cancer cells with wild-type and mutant BRAF backgrounds.

KEYWORDS
Radiosensitizer; colorectal cancer; PARP inhibitor; radiotherapy

Introduction

Colorectal cancer (CRC) is one of the most common forms of cancer, accounting for approximately 1 in 10 new cancer diagnoses worldwide in 20121. Radiotherapy is commonly used to treat rectal cancers prior to surgery or to treat inoperable colorectal metastases, in the form of stereotactic body radiotherapy or selective internal radiotherapy2-4. International standard combination therapy for rectal cancer, radiotherapy delivered with 5-fluorouracil (5FU) as a radiosensitiser, is given either as an infusion or as an oral prodrug (capecitabine). There is currently no molecular basis for the selection of patients for radiotherapy, nor for the selection of any alternative drug to use as a radiosensitiser. With the current standard, sufficient downsizing by chemoradiotherapy is obtained by approximately half of patients treated5. There is scope for improving the radiotherapy approaches currently offered to patients. Clinical trials have added additional drugs to 5FU as a combination radiosensitising approach6,7 without molecular selection, but these trials have not changed the international standard.

Colorectal tumours have a heterogeneous molecular
background. Commonly occurring CRC mutations that may be prognostic or can affect treatment decisions include KRAS, BRAF and PIK3CA mutations, which are found in 42%, 9% and 13% of CRC patients respectively. KRAS, BRAF and PIK3CA are vital components of two main cellular signalling pathways; RAS/MEK/ERK and PI3K/akt/mTOR; strongly inter-connected pathways that play central roles in tumorigenesis by regulating cell survival, proliferation, metabolism, and motility. The KRAS gene is a member of the oncogenic RAS gene family and binds to effector kinases including BRAF and phosphatidylinositol 3-kinase (PI3K). The PIK3CA gene encodes the PI3K p110α subunit, which interacts with RAS proteins.

The commonest BRAF mutation in colorectal cancer, the V600E substitution, results in elevated kinase activity and constitutive downstream MEK and ERK phosphorylation. The presence of BRAF V600E in advanced CRC correlates with poor prognosis with markedly worse progression after chemotherapy. BRAF mutation is predictive of poor response to cetuximab in metastatic CRC, also observed for KRAS and PIK3CA mutations. Although patients with BRAF-mutant cancers do less well with chemotherapy, anti-EGFR therapies and surgery, there is currently no suggestion that they benefit less from radiotherapy. Although BRAF mutation is relatively rare in rectal cancer, radiotherapy can also be used to treat inoperable liver metastases from CRC. It has been suggested that CRC liver metastases respond less well to radiotherapy than liver metastases from other primary malignancies, hence the addition of a radiosensitising drug may be of value to improve the therapeutic index during radiotherapy.

Our aim was to develop a radiosensitiser drug discovery assay enabling identification of drugs that will enhance radiotherapy more effectively than the current standard, 5FU, and demonstrate activity in defined molecular backgrounds. Firstly, we developed a high throughput screen (HTS), in CRC cell lines, to identify drugs that could be effective radiosensitisers in the context of BRAF V600E activating mutations. The drugs identified during the screen were validated across an extensive panel of human CRC cell lines, selected to represent aspects of the molecular landscape of CRC; including BRAF V600E in both MSI and MSS backgrounds, and a spectrum of KRAS, PIK3CA and p53 mutations. Such cell line panels recapitulate the different subtypes found in CRC, are representative of genetic alterations found in primary cancers and are good predictors of clinical efficacy during drug development programmes. Here, we use this model to test new drug-radiotherapy combinations for the first time, identifying PARP inhibitors as the most strongly radiosensitising class of agent before validating by clonogenic survival assays and in vivo xenograft studies.

Materials and methods

Cell lines, drug library and irradiations

The parental CRC cell lines RKO (BRAF V600E/V600E/WT) and VACO432 (V600E/WT) and their isogenic pairs RKO-T29 (BRAF WT/WT) and VACO432-VT1 (BRAF WT/) were a gift from Sandra Van Schaeybroeck, Queens University, Belfast, UK (mutation status confirmed by sequencing). The panel of colorectal cancer cell lines utilised for cell proliferation assays was obtained from Prof. Walter Bodmer, University of Oxford, UK. The cell line panel is listed in Supplementary Table S1, and has been previously described. Non-malignant cell lines were obtained from Prof. Gillies McKenna, University of Oxford, UK. All cell lines were used within 12 passages, or where necessary, replenished using frozen aliquots of the initial passage. Isogenic cell lines were grown in McCoy’s 5A (Modified) Medium, and other cell lines in DMEM; both supplemented with 10% Fetal Bovine Serum and 1 × penicillin/streptomycin (Thermofisher Scientific Inc., MA, USA), in a 37°C, 5% CO₂, humidified incubator. The small compound anti-cancer drug library was provided in 384-well plate format (Target Discovery Institute, University of Oxford), and contained 222 drugs from the TDI Extended Oncology Drugs Library (ODL) and 76 from the NCI Developmental Therapeutics Program (DTP) Approved Oncology Drug set (Supplementary Table S2).

A GSR D1 irradiator (Gamma-Service Medical GmbH, Leipzig, Germany) was used for cell irradiations. For xenografts, a RS320 X-ray irradiator (Gulmay Limited, Byfleet, UK) was used (1.6 Gy/min), with lead shielding to localise dose to tumor. Dosimetry was calculated from optical density of scanned Gafchromic EBT3 film (Ashland, NJ, USA), corrected and calibrated to the National Physical Laboratory (Teddington, UK) primary standard.

High-throughput drug screen with ionising radiation

Methodology and data analysis followed internationally recognised high-throughput screening guidelines. BRAF V600E isogenic RKO and VACO432 cells were seeded in 52 μL/well by Flexdrop (PerkinElmer, MA, USA). Seeding
density in 384-well plates was 300 cells/well (RKO) and 1,000 cells/well (VACO432). Eighteen hours after seeding, cells were screened with 298 oncological drugs, in 5-fold dilutions from 10 μM–16 nM. Janus workstations (PerkinElmer, MA, USA) were used to transfer 13 μL of compound from library plate to cell culture plates. Positive controls were PI103 and vorinostat, negative controls were vehicle (DMSO) alone. After 6 h, plates were either mock-irradiated, or irradiated with 4 Gy. Media was replaced 24 h following treatment, and surviving cells allowed to proliferate for five doubling times as optimised in preliminary screens. Cell viability was measured by resazurin (10 μg/mL) in phenol red-free DMEM. Metabolically viable cells reduce resazurin to fluorescent resorufin, which was quantified by PerkinElmer Envision microplate reader (540 nm excitation/590 nm emission). Control wells reached 90%–100% confluency at the time of assay performance, control irradiated wells were around 60% confluent. Raw data were normalized by rescaling to plate mean intensity and to negative controls. Quality plots were contrasted to assess artifacts and reproducibility. Normalized data Z are presented, as the applied rescaling by plate mean is effectively a z-score standardization. Selection of candidate hits was based on rank product analysis, adapting a published method. Specifically, for each pair of conditions (i.e. with/without irradiation), the differences between normalised screen intensities were calculated for each well, hence each drug. These differences are presented as Delta-Z (ΔZ) scores. Rank product applied to these differences identified compounds producing large and consistent changes. Probability of false discovery was computed by permutation, with n = 100. Analyses were implemented in R version 2.1 (https://cran.r-project.org/); heatmaps were generated by modifying D3.js libraries (https://d3js.org/).

Cell proliferation and colony formation assays

Our method for comparison of IC_{50} in the presence or absence of radiation has been described previously. Clonogenic survival was measured following a standard method, with plating efficiency and surviving fractions calculated as described. Briefly, cells were seeded into 10 cm culture dishes, normally 500 cells/plate (for 0 Gy plates), increasing by 10-fold for each 4 Gy administered, to 500,000 cells/plate (12 Gy). After attachment (overnight), cells were drug-treated, and six hours later exposed to 0, 4, 8 or 12 Gy radiation. Culture medium was replaced 24 hours post-irradiation, plates were incubated to form visible colonies > 50 cells (10 – 15 days) and fixed with 0.4% methylene blue in methanol. Survival curves were fitted using Graphpad Prism v7.0A. Radiation enhancement ratio (RER) was obtained from the ratio of radiation dose at 1% survival of vehicle compared with drug treated cells.

Xenograft studies

Animal experiments were performed following local ethical review under licence from the UK Home Office (ASPA 1986, revised January 2013). Female Balb/c nude mice (6–8 weeks old) were anaesthetised with 2% isoflurane and subcutaneously injected with 50% matrigel containing 5x10^6 DLD1 cells or 5 x 10^6 RKO/mouse (n = 24) into the back. When tumor volume reached 100 mm^3, mice were randomly placed into 4 groups (n = 6/group). Oral treatments were by gavage, in two doses on the first and fourth days of treatment. Group (1) received vehicle only, 10% dimethylacetamide/6% solutol HS/PBS (0.1mL/10 g body weight). Group (2) received talazoparib; 0.1 mg/kg in vehicle. Radiation treatments comprised 2 × 5 Gy, localised to the tumor, also on the first and fourth days of treatment. Group (3) received radiation only, 5 Gy one hour after each vehicle treatment. Group (4) received combination treatment, 5 Gy one hour after each talazoparib treatment. Tumor size was measured by caliper 3 × per week. Mice were sacrificed when tumours reached 400 mm^3 or 42 days following the first treatment. Tumours were formalin fixed and stained for the hypoxia marker CA9 as previously described.

Results

Development of a high throughput screen with ionising radiation

In order to identify drugs that radiosensitise CRC cells mutated for BRAF V600E, isogenic cell lines containing either BRAF V600E or BRAF WT variants were screened against a 298-compound library of approved anticancer drugs. Mutation status for KRAS, PIK3CA and p53 for these cell lines is shown in Figure 1A, with the screen protocol outlined in Figure 1B.

A prerequisite for high-throughput detection of radiosensitisers is an assay that is predictive of the effects of drug/ radiation combinations on clonogenic cell survival. Extended incubation following irradiation improves correlation with radiosensitisation, and we incorporated 5 days incubation following radiation treatment; improving correlation to clonogenic survival, but avoiding compromises to cell metabolism and thus assay performance. Serial
dilution of cells in the presence of resazurin showed equivalent fluorescence, linear in relation to cell number, for both non-irradiated cells, and cells 5 days post-irradiation (data not shown). This indicates that the metabolic assay was a good surrogate for cell number at this timepoint.

Screens were carried out in duplicate and quality plots demonstrated good reproducibility (Figure 1C), with mean Pearson correlation between pairs of replicates of 0.88 and average Z factor of 0.58 for irradiated and 0.53 for non-irradiated plates. Cell viability was compared between normalized irradiated and non-irradiated plates, generating heatmaps of the difference, ΔZ, for each compound. Hit selection (Figure 1D) was based on rank product analysis, with the probability of false discovery computed by permutations (see Materials and methods). Potential hits were drugs that sensitised the BRAF-mutant isogenic variant, at one or more concentrations, with probability of false positive (PFP) ≤ 0.05. Some plates showed a pronounced ‘edge effect’, and for this reason, analysis was repeated considering the edge wells as a separate population (Figure 1E). Hits with significant ΔZ score between irradiated and non-irradiated samples, with radiosensitisation factor < 1 (normalised against control plates) and P-value ≤ 0.05 were selected as significant. Positive controls were consistently identified as hits, with ΔZ scores ≤ 2, comparable to results obtained in manual assays.

**Figure 1** High-throughput screening of FDA approved cancer drugs to identify which drugs should be used for radiosensitisation in the context of single gene mutations in colorectal cancer. (A, B) CRC cells isogenic for BRAF V600E and with defined KRAS, PIK3CA and p53 status were screened with the DTP approved oncology drug library +/- irradiation and allowed to grow for five doubling times. Cell viability was compared between irradiated and non-irradiated plates. (C) Raw data were normalized by rescaling both to the plate mean and negative controls, and quality plots contrasted. (D) Heatmaps were generated for each individual plate. ΔZ scores were calculated between irradiated and non-irradiated plates. Selection of candidate hits was based on a rank product method (see methods). Probability of false discovery was computed by permutation, with 100 permutations. (E) Example heatmap generated for one of the HTS plates. Hits were identified as drugs with a ΔZ score significantly higher than expected by chance when irradiated and non-irradiated samples were compared.
BRAF V600E screen in isogenic cell lines following irradiation

Drugs were ranked according to radiosensitisation against BRAF-mutated cells. The fifteen drugs with the highest significance against BRAF-mutated cells are shown in Table 1. Seven hits have previously been identified as radiosensitisers in the published literature, helping to validate our methodology. Five hits were inhibitors of RAS/RAF/MEK/ERK pathway (trametinib, TAK-733, pimasertib, doramapimod and dactolisib), predominantly acting in BRAF WT and V600E. Eight drugs reached significance in the BRAF-mutant cell line but not in BRAF WT, including the CHK1 inhibitor, PF477736. Another CHK1 inhibitor, AZD7762, radiosensitised both BRAF variants.

The poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib, significantly increased sensitivity to irradiation in BRAF V600E RKO cells. In a separate screen of BRAF isogenic Vaco432 cells, olaparib also radiosensitised BRAF V600E Vaco432 cells at 16 nM and 80 nM (P ≤ 0.05, data not shown). Based on these data, radiosensitisation by PARP inhibitors (PARPi) in RKO isogenic for V600E and WT, was validated by long-term proliferation assay at a broad concentration range and by clonogenic cell survival assay (Figure 2). Olaparib as a single agent had little effect on survival, but combination treatment caused a significant increase in radiation sensitivity, albeit with similar effect in both BRAF WT and V600E variants.

Radiosensitisation in an extended CRC cell line panel

To validate the screen, we used a cell line panel inclusive of the different molecular subtypes of CRC. We specifically prioritised the drug hits with the most immediate scope for translation to clinical trials in combination with radiotherapy. The cell line panel was selected so that several cell lines exhibited each gene mutation of interest. Fifteen cell lines with defined BRAF, p53, KRAS, PIK3CA and mismatch repair status were used. The compounds chosen for further testing are shown in Table 2, along with p-values indicating whether significant IC50 shift was observed following normalisation for radiation effect. The complete IC50 results determined by these assays are shown in Supplementary Table S3.

From these assays, olaparib and rucaparib displayed potent

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**Table 1** Fifteen radiosensitisers identified for BRAF-mutant cells

| Compound | Effective concentration in RKO (BRAF mut) (μM) | Effective concentration in RKO (BRAF WT) (μM) | Mechanism of action |
|----------|-----------------------------------------------|-----------------------------------------------|---------------------|
| Dactolisib | 0.016, 0.4 | 0.016 | Dual PI3K/mTOR inhibitor |
| Panobinastat | 0.016 | ns | HDAC inhibitor |
| Trametinib | 0.016 | 0.016 | MEK inhibitor |
| ABT-199 | 0.08 | 0.08 | Bcl-2 inhibitor |
| Olaparib | 0.08 | ns | PARP inhibitor |
| Tosedostat | 0.08 | ns | Peptidase inhibitor |
| AZD 7762 | 0.08 | 0.08 | Chk inhibitor |
| Pimasertib | 0.4, 0.08 | 0.08 | MEK inhibitor |
| PF477736 | 0.08 | ns | Chk1 inhibitor |
| 17-AAG | 0.08 | ns | Hsp90 inhibitor |
| Doramapimod | 0.08 | ns | p38 MAPK inhibitor |
| Danusertib | 0.08 | ns | aurora kinase inhibitor |
| Serdametan | 0.4 | 0.4 | MDM2 inhibitor |
| Tak-733 | 0.4 | 0.4 | MEK inhibitor |
| Auranofin | 0.4 | ns | Gold complex |

RKO colorectal cancer cells BRAF V600E or WT were screened with 298 approved oncology drugs alone or in combination with irradiation. Radiosensitisation factors were calculated from the ratio of fluorescence of irradiated versus non-irradiated plates. The most significant hits for BRAF-mutant variant RKO cells are shown; each hit has radiosensitisation factor < 1, PFP ≤0.05 and P-values ≤ 0.05; ‘ns’ indicates that significance was not reached in the BRAF WT cell line for the drug tested.
radiosensitising ability across multiple cell lines. IC\textsubscript{50} curves (normalised for radiation effect) were significantly different ($P \leq 0.01$) for all except three cell lines; namely, C10, CW2 and Colo678 (Table 2).

Both Chk1 inhibitors, and trametinib, were also effective radiosensitisers in the majority of cell lines tested. Vemurafenib was ineffective in BRAF WT (IC\textsubscript{50} frequently not reached), but showed some efficacy in BRAF mutated cell lines, (not significant for radiosensitisation). This limited effect may arise from feedback activation of EGFR, PI3K or alternative signaling pathways, reducing vemurafenib efficacy in CRC when compared to melanoma\textsuperscript{37}.

Validation of radiosensitisation by PARP inhibitors with clonogenic survival assays

As PARPi were the most effective radiosensitisers of the CRC cell line panel, clonogenic survival assays were used to measure radiation enhancement ratios (RERs) in 3 cell lines that were strongly radiosensitised (> 10-fold IC\textsubscript{50} shift) and 3 cell lines with IC\textsubscript{50} shift < 10-fold. To potentially improve PARPi radiosensitisation of these resistant cell lines, a more trapping PARPi, talazoparib, was included in these assays. Survival curves (Figure 3), and RERs (Table 3) reflected the proliferation assay results: Olaparib and rucaparib significantly radiosensitised RKO, DLD1, and HT29 compared to vehicle-treated cells, while radiosensitisation of HT55, Colo678, and C10 was limited – although significant for HT55 cells treated with rucaparib. Talazoparib significantly radiosensitised all cell lines tested, and was overall the most effective radiosensitiser (average RERs 1.21–1.92), followed by rucaparib (average RERs 1.15–1.41) and finally olaparib (average RERs 1.12–1.4).

To indicate potential normal tissue toxicity, PARPi experiments were repeated in three non-malignant cell lines, HFLA, MRC5 and RPE. In clonogenic assays (Table 3), these non-malignant cells were significantly radiosensitised by talazoparib. Radiosensitisation by rucaparib was significant for HFLA and MRC5, and radiosensitisation by olaparib was significant only for MRC5 cells ($P \leq 0.05$).
Table 2  

| Cell line | LS411  | Vaco5 | RKO | HT29 | OXCO4 | CCKX1 | HCA7 | DLD1 | CW2 | C10 | HTSS | C99 | Colo678 | SW403 | SW122 |
|-----------|--------|-------|-----|------|-------|-------|------|------|-----|-----|------|----|---------|-------|-------|
| BRAF      | BRAF   | BRAF  | BRAF | BRAF | BRAF  | BRAFWT| BRAFWT| BRAFWT| BRAFWT| BRAFWT| BRAFWT| BRAFWT| BRAFWT | BRAFWT| BRAFWT|
| MSI status| MSI   | MSI   | MSI  | MSI  | MSI   | MSI   | MSI   | MSI   | MSI  | MSI  | MSI   | MSI | MSI     | MSI   | MSI   |
| KRAS      | KRAS   | KRAS  | KRAS | KRAS | KRAS  | KRAS  | KRAS  | KRAS  | KRAS | KRAS | KRAS  | KRAS | KRAS    | KRAS  | KRAS  |
| EGFR      | EGFR   | EGFR  | EGFR | EGFR | Not known | EGFR | EGFR | EGFR | EGFR | EGFR | EGFR  | EGFR | EGFR    | EGFR  | EGFR  |

| Compound | Target | Radiosensitisation response (+ indicates significant radiosensitisation, with P value given below) |
|----------|--------|------------------------------------------------------|
| 5-fluorouracil | Thymidylate synthase | ns ns ns ns ns ns ns +≤ 0.01 ns ns ns ns ns ns ns |
| SAHA     | HDAC   | ns ns +≤ 0.05 ns +≤ 0.01 ns ns ns ns ns +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.05 |
| PI-103   | PI3K/ DNAPK/ mTOR | +≤ 0.05 ns +≤ 0.01 ns +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.01 ns +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.05 |
| Olaparib | PARP   | +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 ns ns +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 |
| Rucaparib| PARP   | +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.01 ns +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.01 |
| AZD-7762 | CHK1 and 2 | ns ns +≤ 0.05 ns +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.05 ns +≤ 0.01 +≤ 0.05 |
| PF477736 | CHK1 and 2 | +≤ 0.05 +≤ 0.05 +≤ 0.05 +≤ 0.05 +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.01 +≤ 0.05 ns |
| AZD-6244 | MEK1 and 2 | ns +≤ 0.05 +≤ 0.01 ns +≤ 0.01 ns +≤ 0.01 ns +≤ 0.01 +≤ 0.01 +≤ 0.05 +≤ 0.05 ns +≤ 0.01 +≤ 0.01 +≤ 0.01 |
| Trametinib | MEK1 and 2 | +≤ 0.05 ns +≤ 0.01 ns ns +≤ 0.05 +≤ 0.01 +≤ 0.01 ns +≤ 0.05 ns +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.05 |
| Mitoxantrone | TOPO II | ns ns ns +≤ 0.05 ns +≤ 0.05 +≤ 0.01 +≤ 0.01 +≤ 0.01 ns +≤ 0.05 ns +≤ 0.01 +≤ 0.01 |
| Vemurafenib | BRAF V600E | ns ns ns ns ns ns ns ns ns ns ns ns ns ns ns |

A panel of fifteen colorectal cell lines, selected for BRAF status in a heterogeneous mutational background, were treated with 11 drugs with or without 4 Gy radiation. Radiosensitisation shown is for the clinical radiosensitisers, 5-fluorouracil; two positive control drugs, SAHA and PI103; and compounds selected on the basis of primary screen P-values and potential clinical utility. Significance was determined by paired t-test on IC_{50} curve values following normalisation for radiation; ‘+’ indicates significant radiosensitisation, with the P-value indicated.
Figure 3  Clonogenic assays to confirm radiosensitisation of multiple cell lines by PARP inhibitors. (A) Colorectal cancer cell lines were plated, rested overnight, drugged and 6 hours later, the cells were either mock irradiated, or irradiated at 4, 8, or 12 Gy. Separation between the control (DMSO) and treated curves indicates radiosensitivity induced by the compound. (B) Human lung fibroblast (HFLA and MRC5) and retinal epithelial (RPE) non-malignant cell lines were drugged in an identical manner and irradiated with 0, 4 or 8 Gy to determine non-cancer cell survival following similar treatment. Data show mean of n=3 experiments±SEM.

Validation of PARP inhibitors as radiosensitisers in xenograft studies

The PARP inhibitor talazoparib was the most effective radiosensitiser and had not previously been tested with radiotherapy in animal CRC models. To confirm the in vitro radiosensitisation by PARPi in an in vivo model, talazoparib was tested against two cell lines that were effectively radiosensitised by the drug in 2D assays. Mice were inoculated with subcutaneous tumors consisting of RKO or DLD1 cells, and treated with talazoparib or vehicle, either alone or one hour before each of 2 × 5 Gy radiation treatments. In DLD1 cells (Figure 4A), single treatment with talazoparib or radiation alone did not inhibit tumour growth. Combined talazoparib/radiation treatment was tolerated by the mice, and significantly reduced tumour growth compared with radiation alone (P ≤ 0.01). For the RKO cell xenograft model, there was no significant difference between the effect of radiation alone, and the radiation/talazoparib combination. Tumour histology, levels of perinecrotic hypoxia (CA9 staining) and necrosis were similar for both cell types (Figure 4B).

Discussion

The aim of this study was to identify treatment options to radiosensitise colorectal cancer cells in the context of key mutations that characterise the disease. Biopsies from CRC patients are routinely screened for BRAF, KRAS and PIK3CA mutations, but this information is not currently used in treatment decisions regarding radiotherapy. There is preclinical evidence that single gene alterations in cancer can determine the extent of radiosensitisation exerted by different drugs. Examples include mammalian AMP-activated protein kinase dependence of pancreatic cancer cells to radiosensitisation by metformin38, the role of mismatch repair deficiency in radiosensitisation of CRC cell lines by gemcitabine39-40 and p53-dependent radiosensitisation by valproic acid41. Radiosensitisation drug discovery across different genetic backgrounds may enable a
change from a “one size fits all” chemo-radiotherapy to the identification of the most appropriate drugs for radiotherapy based on the genetic profile of the cancer.

To address our primary aim, we developed a novel high-throughput screen to test drug library/radiotherapy combination against cell lines. For drug repurposing, which allows more rapid translation into the clinic, we used a library of drugs already in clinical use or in clinical trials. Previous investigators using more focused library screens have successfully identified radiosensitisers of CRC\(^4\) and our study identified the same drugs with radiosensitising potential, the CHK inhibitor, AZD-7762, and the dual mTOR/P13K inhibitor, dactosilib. We initially used isogenic cell lines to identify radiosensitisers active in a BRAFT V600E background. Reassuringly, our results confirmed radiosensitisation by agents from drug classes previously shown to have radiosensitising activity in other published papers, such as inhibitors of the RAS/MEK/ERK, and PI3K/MTOR pathways. In addition, we identified compounds not previously known to be radiosensitisers (Table 1). Of the drugs targeting mutated BRAFT ( vemurafenib, dabrafenib, RAF265), only vemurafenib reached the threshold for hit-detection in the screen, possibly because vemurafenib is a more potent radiosensitizer, at least compared with dabrafenib\(^4\).

Cell lines manipulated by gene mutation might not be entirely representative of the molecular landscape of cancer in patients. We therefore validated results from isogenic cell lines in a panel of human colorectal cancer cell lines, inclusive of common CRC mutations and previously shown to be a useful model for drug development\(^22,44\). This approach was also novel since this cell line panel has not previously been used to test new drug-radiotherapy combinations. The results (shown in Table 2), confirmed PARPi as significant radiosensitisers, notably across a much broader range of cell lines than 5FU, the current clinical standard, suggesting that 5FU may not be the optimal treatment for all CRC patients compared to newer and more targeted drugs. This reflects data in other studies in CRC, which show that radiosensitisation by 5FU varies depending on the cell line used\(^45,46\). Additionally, the timing of 5FU exposure may influence the degree of radiosensitisation\(^47\).

In future, immunotherapy is likely to be of increasing importance in CRC treatment, although at present it is only used to treat the more immunogenic MSI-high tumours\(^48\). Despite this, radiotherapy is likely to remain an important treatment for rectal cancer and metastatic disease, particularly when the cost effectiveness of treatment is considered. The broad range of cell lines for which PARPi have successfully been identified radiosensitisers, notably across a much broader range of cell lines than 5FU, the current clinical standard, suggesting that 5FU may not be the optimal treatment for all CRC patients compared to newer and more targeted drugs. This reflects data in other studies in CRC, which show that radiosensitisation by 5FU varies depending on the cell line used\(^45,46\). Additionally, the timing of 5FU exposure may influence the degree of radiosensitisation\(^47\).

Three PARPi, olaparib, rucaparib, and niraparib, have been approved by the US FDA for the treatment of ovarian cancer, including BRCA-deficient tumours that have deficient homologous recombination repair. PARPi function

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### Table 3 Radiation enhancement ratios of PARP inhibitors for colorectal cancer and non-malignant cell lines

| Gene mutation status | Radiation enhancement ratio \( (P\text{-value}) \) |
|----------------------|-----------------------------------------------|
|                      | Olaparib  | Rucaparib  | Talazoparib |
| **CRC cell lines**    |          |            |            |
| RKO p.V600E WT p.H1047R WT | 1.48 \( (P \leq 0.05) \) | 1.41 \( (P \leq 0.05) \) | 1.71 \( (P \leq 0.001) \) |
| HT29 p.V600E WT WT R273H | 1.44 \( (P \leq 0.01) \) | 1.28 \( (P \leq 0.001) \) | 1.82 \( (P \leq 0.001) \) |
| DLD1 WT G13D p.E545K S241F | 1.18 \( (P \leq 0.01) \) | 1.21 \( (P \leq 0.01) \) | 1.92 \( (P \leq 0.001) \) |
| HT55 WT WT WT R213L | 1.12 (ns) | 1.18 (ns) | 1.48 \( (P \leq 0.001) \) |
| C10 WT WT WT G245S | 1.12 (ns) | 1.15 (ns) | 1.21 \( (P \leq 0.001) \) |
| Colo678 WT G12D WT WT | 1.09 (ns) | 1.3 \( (P \leq 0.05) \) | 1.29 (ns) |
| **Non-malignant cell lines** |          |            |            |
| HFLA n/a n/a n/a n/a | 1.35 \( (P \leq 0.05) \) | 1.34 \( (P \leq 0.05) \) | 1.52 \( (P \leq 0.01) \) |
| MRC5 n/a n/a n/a n/a | 1.1 (ns) | 1.07 (ns) | 1.24 \( (P \leq 0.01) \) |
| RPE n/a n/a n/a n/a | 1.12 (ns) | 1.3 \( (P \leq 0.05) \) | 1.71 \( (P \leq 0.001) \) |

Radiation enhancement ratios were calculated from clonogenic survival assays (normalised, by plating efficiency, for effect of drug alone) and comprise the ratio of radiation dose leading to 1% cell survival to the radiation dose producing 1% survival in the combined treatment. Significance \((P \leq 0.05)\), displayed by in bold, was calculated by one-way ANOVA, with multiple comparisons of each drug against the DMSO control.
by inhibiting the binding, or enzymatic activity, of PARP to single strand breaks in DNA. The absence of SSB repair leads to double strand break (DSB) formation at the approaching replication fork, and cell death. It has been shown that PARPi have an increased radiosensitising effect on DSB- repair deficient tumour cells compared with DSB- repair proficient lines. Compared to olaparib and rucaparib, we found that talazoparib treatment led to higher RERs. PARPi affect cell proliferation by two main actions: inhibiting PARP enzymatic function, and by binding (‘trapping’) PARP to DNA. Olaparib and rucaparib function primarily through inhibiting enzymatic function, whereas talazoparib ‘traps’ PARP at DNA damage sites, with increased anti-proliferative effect, potentially contributing to more effective radiosensitisation.

We proceeded to show that the PARP inhibitor, talazoparib, radiosensitised DLD1 xenografts in vivo. The combined treatment caused a prolonged tumour growth delay, in excess of the effects demonstrated elsewhere for combined 5FU/radiation treatment for HCT116 and WiDr CRC xenografts. It is unclear why talazoparib did not significantly radiosensitise BRAF mutated RKO xenografts in vivo. It has been shown that BRAF-mutant early neoplastic lesions have upregulation of gene sets involved in aberrant DNA methylation and that BRAF-mutant cancers can have distinct tumour-associated-stroma and components of the extracellular matrix that are different from wild-type cancers. These complexities may explain the discrepancy between the highly significant results we obtained in 2D culture and the non-significant results we obtained in vivo using the same cell line. Future studies should consider the use of other models, such as patient-derived xenografts or immunocompetent mouse models, to explore this discrepancy further.

Some investigators advocate preclinical comparison of non-malignant with malignant cell lines to identify cancer-
specific drugs. In our study, olaparib did not cause significant radiosensitisation of two non-malignant cell lines, HLA and RPE. An in vivo study of intestinal crypt damage, in which fractionated radiotherapy was combined with olaparib, did not appear to cause additional gut toxicity compared to radiotherapy without drug. Contrastingly, clinical studies of PARPi have documented bowel toxicities as side effects of treatment and total body irradiation of a p21-reporter mouse has shown that olaparib can exacerbate DNA damage in normal tissues when combined with radiation. It should be noted that, in our study, rucaparib and talazoparib caused significant radiosensitisation of 2 non-malignant cells tested by clonogenic survival assays. Although talazoparib has already completed phase I development as a single agent, we recommend that the normal tissue toxicity from the combination of PARPi with radiotherapy should be assessed further in preclinical normal tissue toxicity models and monitored closely in early-phase clinical trials.

In conclusion, our novel approach to radiosensitisation drug discovery in cells isogenic for the BRAF V600E mutation, has led to the identification of PARPi as radiosensitisers for CRC. Validation in a broad panel of human CRC cell lines, and an in vivo xenograft model, has shown potentially broader radiosensitising activity than the current clinical standard of care, 5FU. Following toxicity evaluation of the combination of PARPi with radiotherapy in other preclinical models, we propose that PARP inhibition should be tested in combination with radiotherapy for rectal cancer or metastatic CRC treatment, with careful monitoring of potential toxicities.

Acknowledgements

This work was supported by Bowel Disease Research Foundation, Oxford Cancer Research Centre, the National Institute for Health Research University College London Hospitals Biomedical Research Centre, the Cancer Research UK University College London Experimental Cancer Medicine Centre, CRUK-UCL Centre Award (Grant No. C416/A25145), the Cancer Research UK Centers Network Accelerator Award Grant (Grant No. A21993) to the ART-NET Consortium, and the NIHR Oxford Biomedical Research Centre.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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Cite this article as: Carter R, Cheraghchi-Bashi A, Westhorpe A, Yu S, Shanneik Y, Seraia E, et al. Identification of anticancer drugs to radiosensitize BRAF-wild-type and mutant colorectal cancer. Cancer Biol Med. 2019; 16: 234-46. doi: 10.20892/j.issn.2095-3941.2018.0284
Supplementary materials

Table S1  Details of the cell lines

| Cell line | BRAF | KRAS | PIK3CA | P53 | MSI/MSS | CIMP |
|-----------|------|------|--------|-----|---------|------|
| C10       | WT   | WT   | WT     | WT  | MSS     | CIMP-|
| C99       | WT   | WT   | WT     | WT  | MSS     | CIMP-|
| CCK81     | WT   | WT   | C420R, C472Y | P278H | MSI     | CIMP-|
| COLO678   | WT   | G12D | WT     | WT  | MSS     | CIMP+|
| CW2 †     | WT   | P140H| P283S  | WT  | MSI     | na.  |
| DLD1      | WT   | G13D | E545K  | S241F  | MSI     | CIMP+|
| HCA7      | WT   | WT   | WT     | P301fs*44 | MSI     | CIMP+|
| HT29      | V600E| WT   | WT     | R273H | MSS     | CIMP+|
| HT55      | V600E| WT   | WT     | .R213L | MSS     | CIMP-|
| LS411     | V600E| WT   | WT     | Y126* | MSI     | CIMP+|
| OXCO4 †   | V600E| WT   | WT     | mutant | MSS     | na.  |
| RKO       | V600E| WT   | H1047R | WT  | MSI     | CIMP+|
| SW1222    | WT   | A146V| WT     | WT  | MSS     | CIMP-|
| SW403     | WT   | G12V | Q546K  | E51*  | MSS     | CIMP-|
| VACOS †   | WT   | WT   | H1047R | mutant | MSI     | na.  |

Table of cell lines comprising the panel for screen validation: Data is from Mouradov et al., Cancer Res. 2014; 74: 3238-47, except where indicated. † Indicates data from Prof. Walter Bodmer, personal communication. na. Indicates information not available.

Table S2  Anticancer drugs comprising the small compound library for the screen

| Drug Name | Details |
|-----------|---------|
| (5Z)-7-Oxozeaenol | Bleomycin |
| (R)-Flurbiprofen (Tarenflurbil) | BMS-754807 |
| 1-methyl-D-tryptophan, 95% | BMS-911543 |
| 17-AAG (Tanespimycin, Geldanamycin) | Bortezomib |
| 17-DMAG (Alvespimycin) | Bosutinib |
| 2-methoxyestradiol (Panzem) | Brivanib |
| 4-hydroxytamoxifen | Busulfan |
| Abitretaxte/Methotrexate | Cabazitaxel |
| ABT-199 | CAL-101 |
| ABT-263 (Navitoclax) | Camptothecin |
| ABT-751 | Canertinib |
| ABT-869_Linifanib | Capcitabine |
| ABT-888 (Veliparib) | Carboplatin |
| AC220_Quizartinib | Carfilzomib |
| Acrichine | Carmustine |

Table of compounds tested from the combined TDI Extended Oncology Drugs Library (ODL) and the NCI Developmental Therapeutics Program (DTP) Approved Oncology Drug Library.
| Drug Name                | Compound Name                          | Formula      | Chemical Structure | Toxicity                  |
|-------------------------|----------------------------------------|--------------|--------------------|---------------------------|
| AG-014699_Rucaparib     | Celecoxib                              | GSK 650394   |                    | NVP-AUY922, Sunitinib     |
| Allopurinol             | CHIR-258 (Dovitinib)                   | GSK1120212_Trametinib |                | NVP-BEZ235_Dactolisib, TAK-733 |
| Altretamine             | Chlorambucil                           | GSK2126458   |                    | NVP-BGJ398, TAK-901       |
| Amifostine              | Chloroquine diphosphate                | GSK2636771   | (Diphosphate salt) | Tamoxifen citrate         |
| Aminoglutethimide       | CHR 2797_Tosedostat                    | HA-1077 (Fasudil) |                | Obatoclax Mesylate (GX15-070), Tandutinib |
| Aminolevulinic acid     | CI-994_Tacedinaline                    | Homoharringtonine |                | Olaparib, Tasocitinib_Tofacitinib |
| Amonafide               | Cisplatin aq                           | Hydroxyurea  |                    | OSI-027, Temozolomide     |
| Anagrelide              | Cladribine                             | J-BET151 (GSK1210151A) |                | OSI-906_Linsitinib, Teniposide |
| Anastrozole             | Clafen (Cyclophosphamide, Endoxan)     | Idarubicin HCl |                    | Oxaliplatin, Tetramisole HCl |
| AP24534 (Ponatinib)     | Clofarabine                            | Ifosfamide   |                    | PAC-1, TGX-221            |
| ARQ 179_Tivantinib      | Clomifene citrate                      | Imatinib     |                    | Paclitaxel, Thalidomide   |
| AYY-162_MEK-162         | CPI-613                                | Imiquimod    |                    | Panobinostat, Thio-TEPA   |
| Arsenic(III) oxide      | Crenolanib                             | INCB018424 (free base, Ruxolitinib) |                | Pazopanib, Thioguanine    |
| AS703026_Pimasertib     | Crizotinib                             | Indibulin    |                    | PCI-32765_1brotinib, Thiopeta |
| Aspirin (Acetylsalicylic Acid) | CUDC-101                              | Iniparib (BSI-201, IND-71677) |                | PD-0332991, Tipifarnib (Zarnestra) |
| AT 101                  | Cyclophosphamide                       | INK128       |                    | Pemetrexed, Topotecan HCl |
| AT-406                  | CYT-387_Momelotinib                    | Irinotecan   |                    | Pentostatin, Toremifene citrate |
| AT9283                  | Cytarabine HCl                         | Ixabepilone  |                    | Perifosine aq/PBS, Tretinoine |
| Atorvastatin Ca         | Dabrafenib Mesylate                    | JNU 26854165 (Serdemetan) |                | PF 431396, Triethylene melamine |
| Auranofin               | Dacarbazine                            | JNU_26481585_Quisinostat |                | PF 477736, Tubacin         |
| AV-951 (Tivozanib)      | Dacominib (monohydrate) (PF-00299804)  | KX2-391      |                    | PF-04691502, Tubastatin A HCl |
| AVN944                  | Dactinomycin                           | Lapatinib, di-p-toluenesulfonate salt |                | PF-04708671, UCN-01       |
| Axitinib                | Dasatinib                              | Lasosifene   |                    | PF-2341066 (Crizotinib), Uracil mustard |
| AZ 3146                 | Daunorubicin HCl                       | Lenalidomide |                    | PF-3845, Valproic acid    |
| Azacitidine             | DCC-2036_Rebaminib                     | Lestaquinib  |                    | PF4800567 hydrochloride, Valrubicin |
| AZD 7762 hydrochloride  | Decitabine                             | Letrozole    |                    | PF670462, Vandetanib      |
| AZD1152-HQPA            | Decitabine (Dacogen)                   | Lomeguatrib |                    | PHA-739358 (Danusertib), Varespladib |
| AZD1480                 | Deferoxamine mesylate                  | Lomustine, CCNU |                | PIK-75 HCl, Vatalanib     |
| AZD2014                 | Dexamethasone (Decadron)               | LY 333531 mesylate-Ruboxistaurin |                | Pilocarpine, Vemurafenib |
| AZD4547                 | Dexrazoxone                            | LY2157299   |                    | Pipobroman, VER 155008    |

Continued
Continued

AZD6244 (Selumetinib) | Dinaciclib (SCH727965) | LY2228820 (CP868569) | PKC412_Midostaurin | Vinblastine sulfate
---|---|---|---|---
AZD8055 | Doxorubicin HCl | LY2603618_Rabusertib | Plerixafor | Vincristine Sulfate (Oncovin)
BAY 73-4506_Regorafenib | Doxorubicin | LY2784544_Gandotinib | Plicamycin | Vinorelbine tartrate
Belinostat (PXD101) | Doxorubicin | Masitinib | PLX4032_Vemurafenib | Vismodegib
Bendamustine HCl | EMD1214063 | MDV3100_Enzaludamide | Pralatrexate | Vorinostat
Bexarotene | Entinostat | Megestrol acetate | Pravastatin | VX-11e
BI 2536 | Enzastaurin | Melphalan | Prednisolone | XAV-939
BI 6727_Volasertib | Epothilone B (Patupilone) | Mercaptopurine | Prednison | XL-147
BIBF 1120_Nintedanib | Erlotinib HCl | Metformin hydrochloride aq | Prima-1 Met | XL184_Cabozantinib
BBW2992 (Tovok)_Afatining | Estramustine sodium phosphate | Methotrexate | Procarbazine | XL880 (Foretinib)
Bicalutamide | Etoposide | Methoxsalen | PX-866_Sonolisib | YM155
BIIB021 | Everolimus | Methylprednisolone | Quinacrine HCl | Zoledronic acid
Bimatoprost | Entinostat | Mithramycin A | Raloxifene HCl | ZSTK474
BIRB 796 (Doramapimod) | FG-4592 | MGCD0103_Mocetinostat | Rafinpx0 | ZSTK474
BKM-120_Buparlisib | Finasteride | Mithramycin A | Raloxifene HCl | ZSTK474

Table S3 IC\textsubscript{50} (μM) for each drug at 0 and 4 Gy in a panel of colorectal cancer cell lines

| Cell line | 5-FU IC\textsubscript{50} | Vorinostat IC\textsubscript{50} | PI-103 IC\textsubscript{50} | Olaparib IC\textsubscript{50} | Rucaparib IC\textsubscript{50} | Mitoxantrone IC\textsubscript{50} |
|-----------|------------------|------------------|------------------|------------------|------------------|------------------|
| LS411 0 Gy | 24.67 (17.58 to 35.49) | 6.79 (3.99 to 11.91) | 5.23 (3.47 to 8.12) | 24.46 (15.71 to 38.89) | *62.18 (12.32 to 22.98) | 16.75 (12.32 to 22.98) |
| LS411 4 Gy | 21.95 (12.37 to 40.8) | 14.55 (9.69 to 22.27) | 2.76 (1.57 to 4.96) | 2.11 (1.09 to 4.18) | 1.47 (0.49 to 8.54) | 7.8 (5.63 to 10.89) |
| VACOS 0 Gy | 3.54 (1.95 to 3.35) | 3.45 (2.47 to 4.9) | 1.91 (1.05 to 3.58) | 10.5 (3.83 to 29.59) | 34.03 (21.2 to 58.03) | 3.49 (0.87 to 14.64) |
| VACOS 4 Gy | 0.99 (0.83 to 1.19) | 3.37 (2.85 to 4) | 0.48 (0.34 to 0.71) | 0.75 (0.43 to 1.3) | 3.07 (0.61 to 11.91) | 1.24 (0.56 to 2.99) |
| RKO 0 Gy | 2.51 (1.93 to 3.29) | 6.51 (3.89 to 11.26) | 1.55 (0.95 to 2.54) | 6.51 (4.32 to 17.22) | 61.23 (30.07 to 167.4) | 9.75 (6.21 to 15.44) |
| RKO 4 Gy | 1.15 (1.73 to 1) | 2.14 (1.2 to 4.15) | 0.33 (0.2 to 0.57) | 0.35 (0.15 to 0.78) | 0.3 (0.03 to 1.59) | 2.9 (1.59 to 5.46) |
| HT29 0 Gy | 9.12 (6.67 to 12.66) | 3.47 (2.42 to 5.03) | * >20 | 17.93 (4.14 to 75.29) | 51.82 (33.61 to 86.34) | 6.58 (1.24 to 78.99) |
| HT29 4 Gy | 6.6 (5.24 to 8.36) | 4.18 (2.6 to 6.94) | * 12.94 | 2.21 (1.25 to 3.55) | 5.48 (2.49 to 11.75) | 3.06 (0.88 to 12) |
| OXCO4 0 Gy | 16.71 (14.13 to 19.85) | 6.09 (3.69 to 10.41) | 2.42 (1.82 to 3.24) | 26.88 (16.82 to 43.79) | 13.11 (10.42 to 16.58) | 0.89 (0.61 to 1.33) |
| OXCO4 4 Gy | 9.45 (7.92 to 11.32) | 3.82 (2.69 to 5.49) | 1.13 (0.87 to 1.47) | 6.07 (4.73 to 7.82) | 2.5 (1.74 to 3.61) | 0.59 (0.44 to 0.78) |

IC\textsubscript{50} was calculated using Graphpad Prism following normalisation for radiation effect, and is shown in μM, with 95% confidence limits in parenthesis. * Where the curve shape did not allow calculation of IC\textsubscript{50} in Graphpad, IC\textsubscript{50} was calculated manually by interpolation. * > indicates the highest concentration tested in cell lines where the IC\textsubscript{50} was not reached.

Continued
| Cell line   | 5-FU IC_{50} (Gy) | Vorinostat IC_{50} (Gy) | PI-103 IC_{50} (Gy) | Olaparib IC_{50} (Gy) | Rucaparib IC_{50} (Gy) | Mitoxantrone IC_{50} (Gy) |
|------------|-------------------|-------------------------|---------------------|-----------------------|------------------------|--------------------------|
| CW2 0 Gy   | 20.19 (15.17 to 27.24) | 4.49 (2.9 to 7.08) | 4.97 (3.14 to 8.17) | 17.05 (6.48 to 44.79) | 36.91 (30.48 to 45.16) | 19.81 (11.2 to 35.76) |
| CW2 4 Gy   | *20.1             | 5.33 (3.59 to 7.99) | 4.21 (1.49 to 14.87) | * >20                  | * >30                  | 21.02 (12.56 to 186) |
| DLD1 0 Gy  | 8.6 (6.77 to 10.99) | 6.26 (3.07 to 13.72) | 1.69 (0.94 to 3.08) | * >100                 | *30.41                 | 4.08 (1.49 to 12.46) |
| DLD1 4 Gy  | 7.78 (5.26 to 11.81) | 3.25 (2.02 to 5.42) | 0.52 (0.29 to 0.95) | 1.74 (0.89 to 3.5) | 0.44 (0.15 to 2.1) | 1.9 (1.33 to 2.72) |
| CCK81 0 Gy | 29.85 (23.48 to 38) | 10.4 (5.37 to 21.34) | 1.27 (0.92 to 1.76) | >100                   | *48.51                 | *16.51                   |
| CCK81 4 Gy | 20.77 (16.64 to 26.01) | 7.84 (4.13 to 15.86) | 1.07 (0.81 to 1.44) | 13.05 (7.62 to 22.53) | 45.05 (11.83 to 105.1) | 22.6 (11.62 to 60.7) |
| C10 0 Gy   | 43.38 (31.27 to 60.93) | 2.05 (0.82 to 6.16) | 0.98 (0.45 to 2.19) | * >100                 | 23.7 (20.64 to 222.2) | 3.92 (1.84 to 8.96) |
| C10 4 Gy   | 39.86 (17.15 to 101.3) | 10.21 (1.3 to 74.5) | 0.53 (0.27 to 1.17) | * >100                 | 22.4 (6.87 to 130) | 2.18 (1.14 to 4.29) |
| SW403 0 Gy | 1.31 (0.86 to 2.02) | 17.71 (7.57 to 49.28) | * >20 | 6.18 (1.46 to 26.89) | 40.51 (27.91 to 61.56) | 2.17 (0.94 to 5.27) |
| SW403 4 Gy | 0.73 (0.49 to 1.09) | 7.28 (4.53 to 12.05) | 10.81 (5.35 to 27.22) | 0.85 (0.28 to 2.46) | 12.39 (5.71 to 26.75) | 1.46 (0.66 to 3.41) |
| COL0678 0 Gy | 85 (37.7 to 197.4) | 8.68 (1.84 to 64.49) | 2.3 (1.08 to 5.15) | * >200 | *48.67 | 5.96 (2.98 to 12.18) |
| COL0678 4 Gy | 81.5 (70.58 to 129.3) | *5.5 | 2.24 (1.09 to 4.85) | * >200 | *45.79 | 20.31 (12.29 to 34.44) |
| SW1222 0 Gy | 10.58 (5.80 to 20.42) | 41.93 (16.03 to 134) | * >20 | 16.72 (9.97 to 28.91) | 9.78 (4.56 to 22.31) | 2.76 (1.63 to 4.8) |
| SW1222 4 Gy | 3.23 (2.19 to 4.75) | 4.07 (2.98 to 5.63) | 0.75 (0.63 to 0.90) | 0.42 (0.32 to 0.56) | *0.32 | 0.73 (0.32 to 1.83) |
| HCA7 0 Gy | 27.64 (22.63 to 33.87) | 1.29 (0.93 to 1.80) | 2.95 (1.71 to 5.26) | 3.99 (3.16 to 5.05) | 48.51 (35.36 to 68.98) | 1.93 (0.57 to 6.89) |
| HCA7 4 Gy | 19.82 (16.49 to 23.89) | 0.82 (0.70 to 0.97) | 1.14 (0.79 to 1.66) | 0.24 (0.18 to 0.32) | 0.36 (0.19 to 0.66) | 0.75 (0.24 to 2.53) |
| HT55 0 Gy | 10.53 (7.91 to 14.17) | 2.11 (1.51 to 3) | 2.87 (0.95 to 9.99) | 41.07 (4.96 to 28.01) | 12.2 (8.31 to 18.19) | 1.26 (0.86 to 1.85) |
| HT55 4 Gy | 12.03 (8.91 to 16.48) | 3.14 (1.99 to 5.10) | 2.58 (1.85 to 3.63) | 7.88 (0.88 to 3.66) | 1.47 (0.4 to 5.18) | 1.27 (0.79 to 2.08) |
| C99 0 Gy | 3.34 (2.11 to 5.72) | 3.53 (1.8 to 7.46) | 23.77 (8.94 to 31.98) | 14.01 (4.11 to 53.54) | 39.28 (21.29 to 81.87) | 1.15 (0.58 to 2.31) |
| C99 4 Gy | 4.44 (2.37 to 8.86) | 3.0 (1.66 to 5.92) | 0.97 (0.30 to 3.48) | 0.44 (0.22 to 0.87) | 14.2 (0.16 to 18) | 0.49 (0.27 to 0.92) |
| Cell line | AZD-7762 IC_{50} (Gy) | PF4777 IC_{50} (Gy) | AZD-6244 IC_{50} (Gy) | Trametinib IC_{50} (Gy) | Vemurafenib IC_{50} (Gy) |
| LS411 0 Gy | 2.69 (1.5 to 6.50) | 3.84 (2.91 to 5.08) | 11.92 (5.13 to 39.4) | 2.03 (0.08 to 25.26) | 58.81 (30.19 to 144.8) |
| LS411 4 Gy | 0.41 (0.25 to 0.69) | 1.49 (1.06 to 1.83) | 6.94 (2.62 to 24.83) | 1.81 (0.006 to 48.6) | 20.39 (4.63 to 210.7) |

Continued
| Cell line | 5-FU IC50 (95% CI) | Vorinostat IC50 (95% CI) | Pi-103 IC50 (95% CI) | Olaparib IC50 (95% CI) | Rucaparib IC50 (95% CI) | Mitoxantrone IC50 (95% CI) |
|-----------|-------------------|-------------------------|---------------------|-----------------------|-------------------------|--------------------------|
| RKO 0 Gy  | 0.02 (0.015 to 0.03) | 0.47 (0.31 to 0.71)    | *148.75             | 0.09 (0.03 to 0.3)    | 15.14 (4.37 to 57.2)   |
| RKO 4 Gy  | 0.005 (0.004 to 0.008) | 0.19 (0.15 to 0.25)    | 4.62 (0.74 to 46.47) | 0.03 (0.01 to 0.07)   | 4.57 (0.99 to 29.93)   |
| VACOS 0 Gy| 0.05 (0.03 to 0.11)  | 1.5 (1.06 to 2.16)     | 14.81 (7.95 to 29.18)| 0.01 (0.007 to 0.017) | 9.37 (6.57 to 13.45)   |
| VACOS 4 Gy| 0.01 (0.004 to 0.02) | 0.28 (0.23 to 0.34)    | 7.08 (4.28 to 11.86) | 0.003 (0.003 to 0.004)| 3.86 (2.6 to 5.84)     |
| HT29 0 Gy | 0.03 (0.02 to 0.06)  | 4.08 (2.58 to 6.89)    | 2.34 (1.02 to 5.62)  | 0.02 (0.01 to 0.04)   | 13.1 (5.49 to 32.24)   |
| HT29 4 Gy | 0.01 (0.003 to 0.03) | 1.57 (1.03 to 2.45)    | 1.87 (0.62 to 6.39)  | 0.01 (0.007 to 0.02)  | 11.76 (6.68 to 20.96)  |
| OXCO4 0 Gy| 2.14 (1.39 to 3.73)  | 1.54 (1.08 to 2.22)    | 3.04 (1.76 to 5.35)  | *0.15                 | 14.57 (10.14 to 21.19) |
| OXCO4 4 Gy| 0.17 (0.13 to 0.22)  | 0.76 (0.54 to 1.09)    | 0.82 (0.33 to 2.4)   | *0.06                 | 10.6 (4.09 to 28.44)   |
| CW2 0 Gy  | 2.16 (1.17 to 5.32)  | 26.75 (21.9 to 32.78)  | 1.72 (0.47 to 9.39)  | 0.46 (0.2 to 1.24)    | *53.05                  |
| CW2 4 Gy  | * >2                | 20.75 (6.06 to 71.96)  | * >10               | 0.18 (0.076 to 0.44)  | *49.07                  |
| DLD1 0 Gy | 0.14 (0.1 to 0.21)   | * 15.02                | * >20               | * >1                  | *66.41                  |
| DLD1 4 Gy | 0.02 (0.01 to 0.05)  | 5.46 (2.96 to 12.91)   | * >20               | 0.08 (0.02 to 0.59)   | 33.24 (14.01 to 84.84)  |
| CCK81 0 Gy| 0.75 (0.43 to 1.42)  | * >10                  | * >20               | * >1                  | * >160                  |
| CCK81 4 Gy| 0.11 (0.08 to 0.17)  | * >10                  | * >20               | * >1                  | * >160                  |
| C10 0 Gy  | 0.12 (0.1 to 0.15)   | *10.08                 | *25.69              | 0.68 (0.26 to 3.31)   | 53.16 (30.73 to 99.45)  |
| C10 4 Gy  | 0.12 (0.08 to 0.2)   | * >10                  | * >20               | 0.29 (0.13 to 0.74)   | 48.79 (23.05 to 119.8)  |
| SW403 0 Gy| 0.26 (0.18 to 0.37)  | 0.79 (0.44 to 1.41)    | 6.45 (3.02 to 15.7)  | *2.03                 | * >80                   |
| SW403 4 Gy| 0.13 (0.1 to 0.16)   | 0.37 (0.22 to 0.62)    | 1.71 (1.08 to 2.76)  | *1.68                 | * >80                   |
| COLO678 0 Gy| * >2                | *25.14                 | 1.12 (0.72 to 1.78)  | 0.006 (0.005 to 0.008)| * >80                   |
| COLO678 4 Gy| * >2                | *25.39                 | 1.47 (1.07 to 2.04)  | 0.005 (0.002 to 0.01) | * >80                   |
| SW1222 0 Gy| 0.07 (0.05 to 0.1)   | 6.26 (4.02 to 10.64)   | 3.75 (0.64 to 45.5)  | 0.23 (0.09 to 0.69)   | *160                    |
| SW1222 4 Gy| 0.02 (0.02 to 0.02)  | 1.19 (0.76 to 1.91)    | 0.61 (0.43 to 0.87)  | 0.04 (0.02 to 0.07)   | 39.5 (27.01-57.36) }

Continued
| Cell line | S-FU IC₅₀  | Vorinostat IC₅₀ | PI-103 IC₅₀ | Olaparib IC₅₀ | Rucaparib IC₅₀ | Mitoxantrone IC₅₀ |
|-----------|-----------|-----------------|-------------|--------------|----------------|-----------------|
| HCA7 0 Gy | 0.06 (0.01 to 0.07) | 2.37 (1.76 to 3.26) | * >20 | 0.41 (0.21 to 0.89) | 196.8 (178.93 to 231) |
| HCA7 4 Gy | 0.01 (0.00 to 0.14) | 0.42 (0.36 to 0.49) | *18.77 | 0.15 (0.09 to 0.24) | 116.7 (64.29 to 256.3) |
| HT55 0 Gy | 0.06 (0.05 to 0.09) | 0.97 (0.73 to 1.28) | 1.55 (0.28 to 5.02) | 0.08 (0.03 to 0.29) | 49.39 (15.7 to 169.4) |
| HT55 4 Gy | 0.02 (0.01 to 0.02) | 0.37 (0.3 to 0.47) | 1.55 (0.41 to 3.33) | 0.05 (0.03 to 0.09) | 50.77 (22.77 to 132) |
| C99 0 Gy  | 0.12 (0.07 to 0.22) | 3.75 (1.92 to 8.38) | 1.27 (0.32 to 5.5) | 0.01 (0.007 to 0.023) | * >160 |
| C99 4 Gy  | 0.34 (0.07 to 2.32) | 1.43 (0.32 to 7.72) | 0.38 (0.16 to 1.08) | 0.004 (0.002 to 0.007) | * >160 |