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ORIGINAL ARTICLE

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 ($P \leq 0.01$).

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 2012[1]. 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 radiotherapy[2-4].

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 treated[5]. There is scope for improving the radiotherapy approaches currently offered to patients. Clinical trials have added additional drugs to 5FU as a combination radiosensitising approach[6,7] without molecular selection, but these trials have not changed the international standard.

Colorectal tumours have a heterogeneous molecular

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background[8]. 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[9]. 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[10].

The commonest *BRAF* mutation in colorectal cancer, the V600E substitution, results in elevated kinase activity and constitutive downstream MEK and ERK phosphorylation[11,12]. The presence of *BRAF* V600E in advanced CRC correlates with poor prognosis with markedly worse progression after chemotherapy[13-15]. *BRAF* mutation is predictive of poor response to cetuximab in metastatic CRC, also observed for *KRAS* and *PIK3CA* mutations[16-18]. Although patients with *BRAF*-mutant cancers do less well with chemotherapy, anti-EGFR therapies and surgery[19], 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[20], hence the addition of a radiosensitising drug may be of value to improve the therapeutic index during radiotherapy[21].

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[22]. 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/-/-*) 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[22]. 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% CO_2, 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) a Cs-137 source, (dose rate 1.5 Gy/min) 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[23]. *BRAF V600E* isogenic RKO and VACO432 cells were seeded in 52 μ L/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 μ g/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[24]. 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[25]. Clonogenic survival was measured following a standard method, with plating efficiency and surviving fractions calculated as described[26]. 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 5×10^6 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[27].

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[28], and we incorporated 5 days incubation following radiation treatment; improving correlation to clonogenic survival, but avoiding compromises to cell metabolism and thus assay performance[29]. 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.

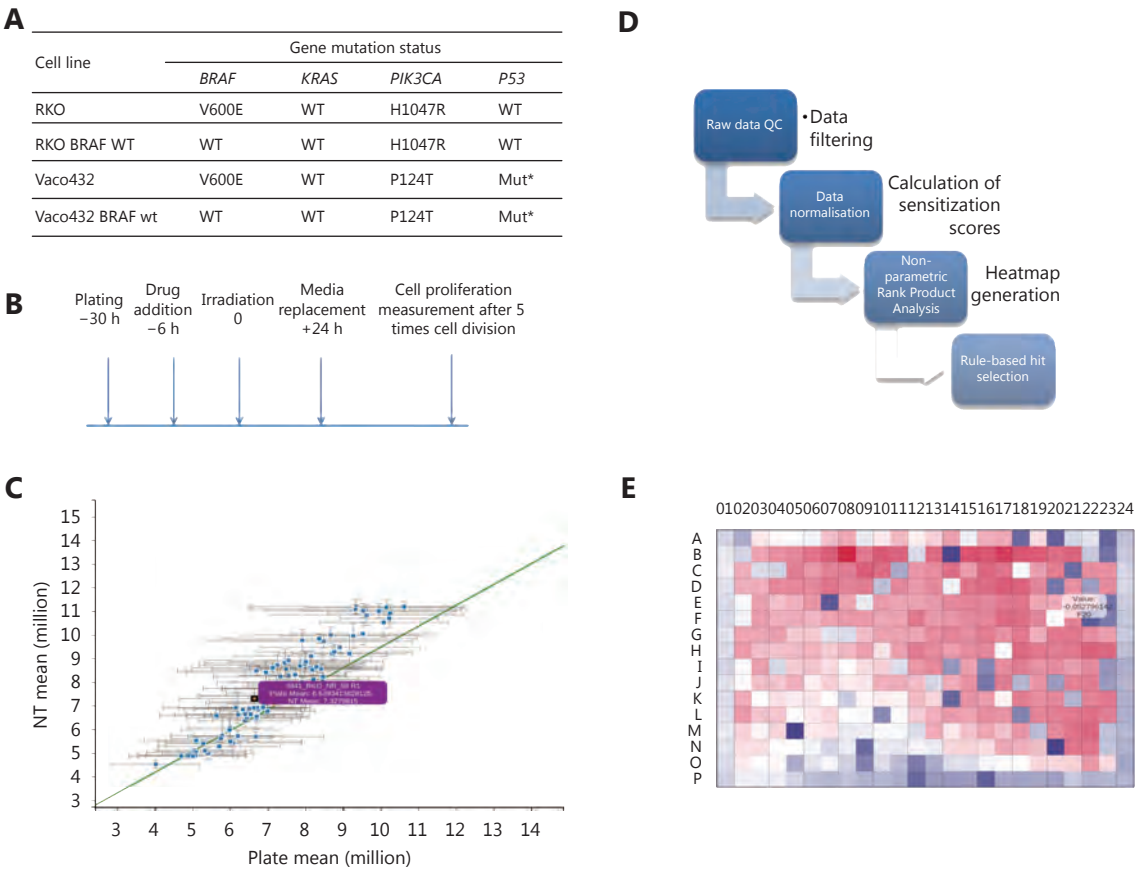

Figure 1 High-throughput screening of FDA approved cancer drugs to identify which drugs should be used for radiosensitisation in the context of single gene mutations in colorectal cancer. (A, B) CRC cells isogenic for *BRAF V600E* and with defined *KRAS, PIK3CA and p53* status were screened with the 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[30-36], helping to validate our methodology. Five hits were inhibitors of RAS/RAF/MEK/ERK pathway (trametinib, TAK-733, pimasertib, doramapoimod 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 ($P \leq 0.05$, 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 IC_{50} shift was observed following normalisation for radiation effect. The complete IC_{50} results determined by these assays are shown in **Supplementary Table S3**.

From these assays, olaparib and rucaparib displayed potent

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.

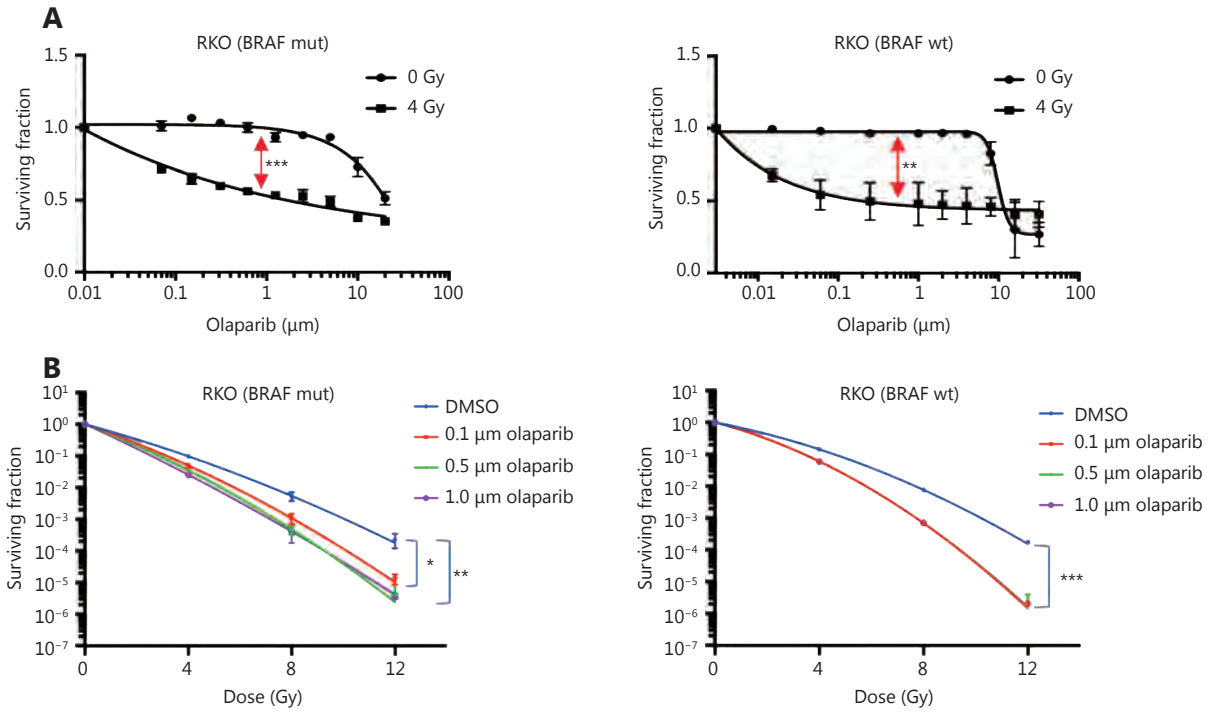

Figure 2 Validation of radiosensitisation effects of olaparib in BRAF-mutant and BRAF-WT isogenic CRC cells. Confirmation of radiosensitisation by olaparib in BRAF mutant and WT RKO cells by: (A) Long-term cell proliferation assays, showing separation (red arrows) between IC_{50} curves normalised for radiation effect, indicating significant radiosensitisation (BRAF-mutant: $P \leq 0.001$; BRAF-WT: $P \leq 0.01$, calculated by paired *t*-test). (B) Clonogenic survival assays, showing significant radiation enhancement by 0.1–1 μM olaparib at 1% cell survival (BRAF-mutant: $P \leq 0.05$; BRAF-WT: $P \leq 0.001$), calculated by one-way ANOVA in multiple comparison tests). Data show the mean of $n = 3$ experiments ± SEM (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

radiosensitising ability across multiple cell lines. IC_{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_{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[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_{50} shift) and 3 cell lines with IC_{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 *P*- values for radiosensitisation by 11 drugs across a panel of 15 CRC cell lines

Cell line		LS411	Vaco5	RKO	HT29	OXCO4	CCK81	HCA7	DLD1	CW2	C10	HT55	C99	Colo678	SW403	SW1222
BRAF		BRAF V600E	BRAF V600E	BRAF V600E	BRAF V600E	BRAFV600E	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT	BRAFWT
MSI status		MSI	MSI	MSI	MSS	MSS	MSI	MSI	MSI	MSI	MSS	MSS	MSS	MSS	MSS	MSS
KRAS		KRAS WT	KRAS WT	KRAS WT	KRAS WT	KRAS WT	KRAS WT	KRAS WT	KRAS G13D	KRAS P140H	KRAS WT	KRAS WT	KRAS WT	KRAS G12D	KRAS G12V	KRAS A146V
EGFR		EGFR MUT	EGFR WT	EGFR WT	EGFR WT	Not known	EGFR Y1069C	EGFR WT	EGFR WT	EGFR G544*FS	EGFR WT	EGFR WT	EGFR WT	EGFR WT	EGFR WT	EGFR WT
Compound	Target	Radiosensitisation response (+ indicates significant radiosensitisation, with *P* value given below)														
5-fluoro-uracil	Thymidylate synthase	ns	ns	ns	ns	ns	ns	ns	ns	$+p \leq 0.01$	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	ns	+≤ 0.01	+≤ 0.01	+≤ 0.05	+≤ 0.01
Olaparib	PARP	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	ns	ns	+≤ 0.01	+≤ 0.01	ns	+≤ 0.01	+≤ 0.01
Rucaparib	PARP	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.01	+≤ 0.05	+≤ 0.01	+≤ 0.01	+≤ 0.01	ns	ns	+≤ 0.05	+≤ 0.05	ns	+≤ 0.01	+≤ 0.01
AZD-7762	CHK1 and 2	ns	ns	+≤ 0.05	ns	+≤ 0.01	+≤ 0.01	+≤ 0.05	+≤ 0.05	+≤ 0.01	+≤ 0.01	ns	+≤ 0.01	+≤ 0.01	+≤ 0.05	ns
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	ns	ns	+≤ 0.01	+≤ 0.05
AZD-6244	MEK1 and 2	ns	+≤ 0.05	+≤ 0.01	ns	+≤ 0.01	ns	+≤ 0.01	ns	+≤ 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	ns	+≤ 0.01
Mitoxantrone	TOPO II	ns	ns	ns	+≤ 0.05	ns	+≤ 0.05	+≤ 0.05	+≤ 0.01	+≤ 0.01	ns	+≤ 0.05	ns	ns	+≤ 0.01	ns
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 radiosensitiser, 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.

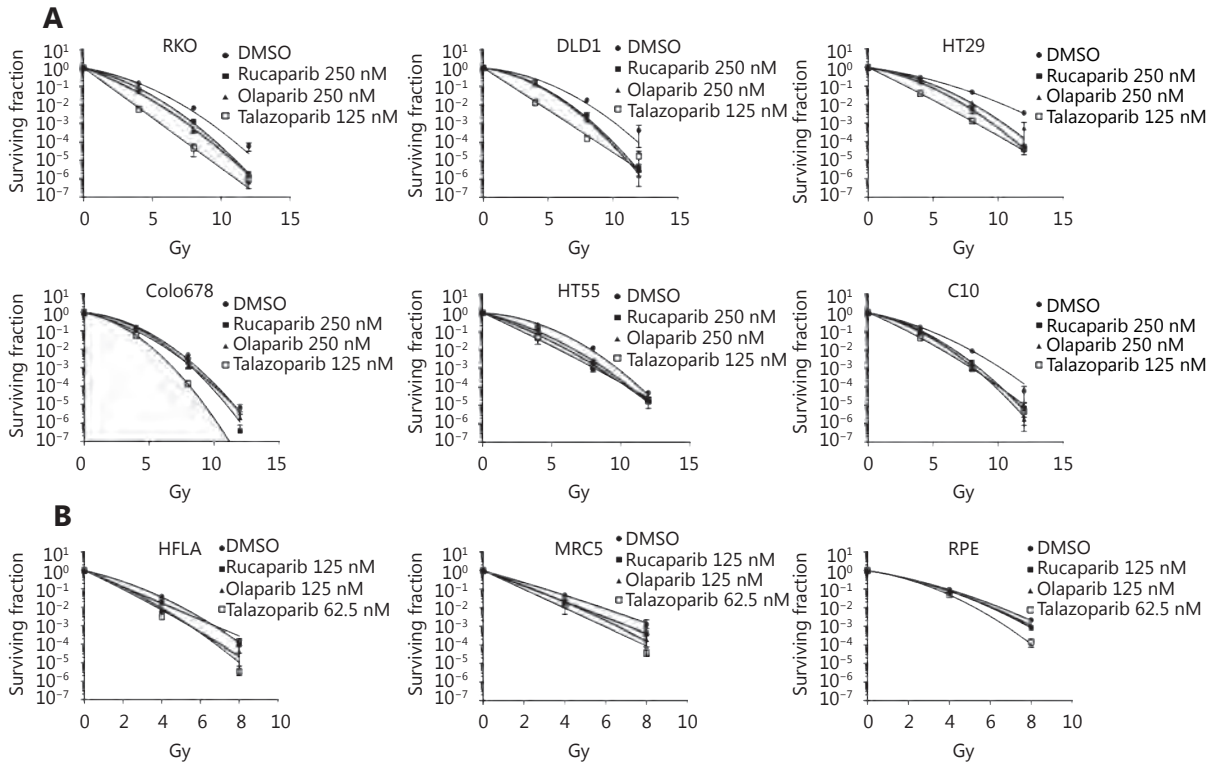

Figure 3 Clonogenic assays to confirm radiosensitisation of multiple cell lines by PARP inhibitors. (A) Colorectal cancer cell lines were plated, rested overnight, drugged and 6 hours later, the cells were either mock irradiated, or irradiated at 4, 8, or 12 Gy. Separation between the control (DMSO) 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 ($P \leq 0.01$). 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 metformin[38], the role of mismatch repair deficiency in radiosensitisation of CRC cell lines by gemcitabine[39-40] and p53-dependent radiosensitisation by valproic acid[41]. Radiosensitisation drug discovery across different genetic backgrounds may enable a

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

	Gene mutation status				Radiation enhancement ratio (*P*-value)		
	BRAF	KRAS	PIK3CA	p53	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	1.21 (ns)	**1.31 ($P \leq 0.01$)**	**1.39 ($P \leq 0.01$)**
C10	WT	WT	WT	G245S	1.12 (ns)	1.18 (ns)	**1.48 ($P \leq 0.001$)**
Colo678	WT	G12D	WT	WT	1.12 (ns)	1.15 (ns)	**1.21 ($P \leq 0.001$)**
Non-malignant cell lines							
HFLA	n/a	n/a	n/a	n/a	1.09 (ns)	**1.3 ($P \leq 0.05$)**	1.29 (ns)
MRC5	n/a	n/a	n/a	n/a	**1.35 ($P \leq 0.05$)**	**1.34 ($P \leq 0.05$)**	**1.52 ($P \leq 0.01$)**
RPE	n/a	n/a	n/a	n/a	1.1 (ns)	1.07 (ns)	**1.24 ($P \leq 0.01$)**

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.

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 in to 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[42] and our study identified the same drugs with radiosensitising potential, the CHK inhibitor, AZD-7762, and the dual mTOR/PI3K inhibitor, dactosilib. We initially used isogenic cell lines to identify radiosensitisers active in a *BRAF* 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 BRAF (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[43].

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 appear to be suitable radiosensitisers in this study may predict its potential future utility in a wide patient population.

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

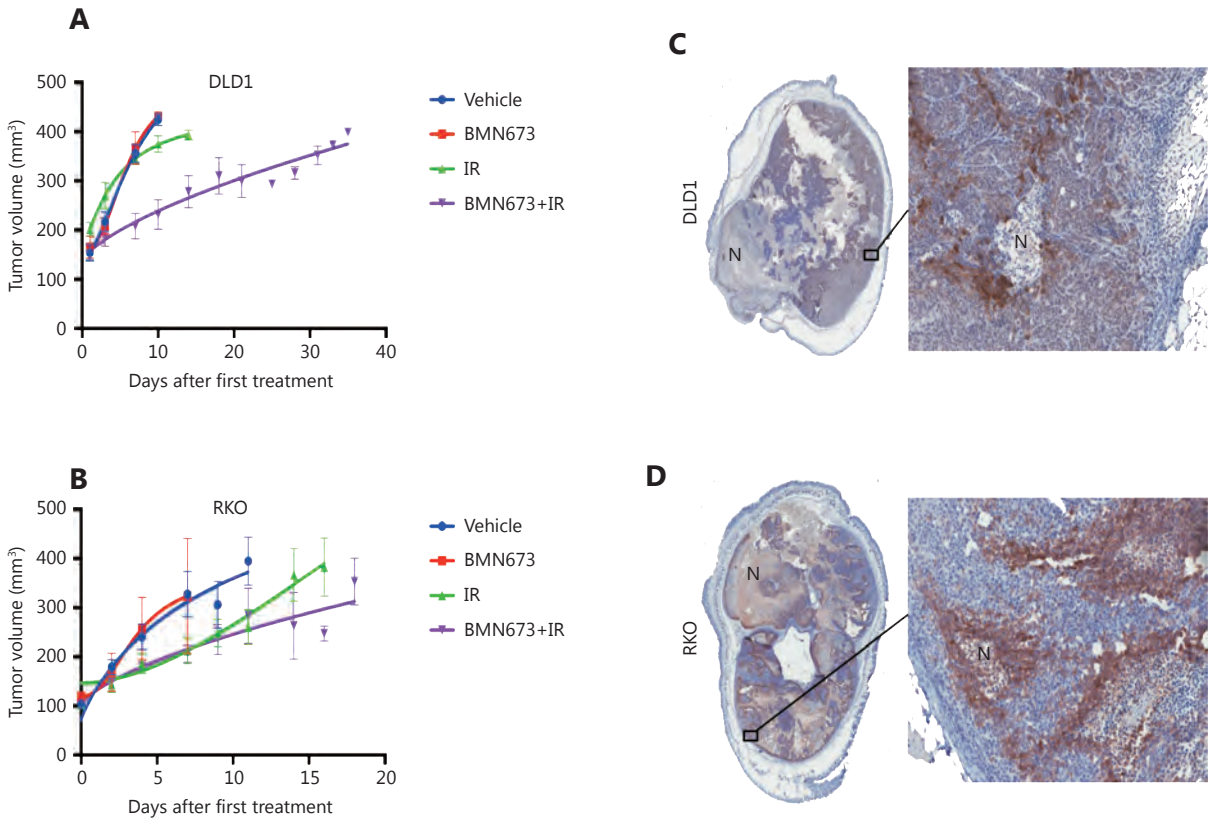

Figure 4 Talazoparib significantly enhances the response of colorectal cancer cells grown *in vivo* to ionizing radiation. (A, B) Growth of tumour cells injected subcutaneously into the back of BALB/c nude mice, treated as indicated with either; vehicle; 0.1 mg/kg talazoparib; radiation (2 x 5 Gy); or 0.1 mg/kg talazoparib 1 hour prior to each of 2 x 5 Gy radiation doses. Treatment with talazoparib+radiation significantly slowed tumor growth for (A) DLD1 cells but not (B) RKO cells. (C, D) Representative images of tumours harvested from the vehicle treated group (at 400 mm^3) show similar histology for both (C) DLD1 and (D) RKO xenografts including perinecrotic hypoxia (CA9 staining, brown) and tumor necrosis (N).

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[49]. 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[50]. 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[51,52].

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[45] and WiDr[53] 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[54] and that *BRAF*-mutant cancers can have distinct tumour-associated-stroma and components of the extracellular matrix that are different from wild-type cancers[55]. 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[56,57]. 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[58]. Contrastingly, clinical studies of PARPi have documented bowel toxicities as side effects of treatment[59] and total body irradiation of a p21-reporter mouse has shown that olaparib can exacerbate DNA damage in normal tissues when combined with radiation[60]. 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[61], 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.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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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-
VACO5 †	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

(5Z)-7-Oxozeaenol	Bleomycin	FK 506_Tacrolimus	Mitomycin C	Rapamycin (sirolimus)
(R)-Flurbiprofen (Tarenflurbil)	BMS-754807	FK-866 HCl_Daporinad	Mitotane	RD162
1-methyl-D-tryptophan, 95%	BMS-911543	Floxuridine	Mitoxantrone	RDEA119_Refametinib
17-AAG (Tanespimycin, Geldanamycin)	Bortezomib	Fludarabine	MK-0752	Ridaforolimus
17-DMAG (Alvespimycin)	Bosutinib	Fluorouracil	MK-2206	Rofecoxib (Vioxx)
2-methoxyestradiol (Panzem)	Brivanib	Flutamide	MK-4827, HCl salt	Romidepsin
4-hydroxytamoxifen	Busulfan	Fulvestrant	MK1775	Roscovitine_Selicilib
Abitrexate/Methotrexate	Cabazitaxel	Galiellalactone	MLN4924	S-trityl-L-cysteine, 40 mM
ABT-199	CAL-101	GDC-0068	MLN8237_Alisertib	SB 743921
ABT-263 (Navitoclax)	Camptothecin	GDC-0941_Pictilisib	Motesanib Di phosphate (AMG-706)	Simvastatin
ABT-751	Canertinib	GDC-0980	Nelarabine	Sorafenib
ABT-869_Linifanib	Capecitabine	Gefitinib	Nilotinib	Sotrastaurin
ABT-888 (Veliparib)	Carboplatin	Gemcitabine HCl	Nilutamide	SR1 HCl
AC220_Quizartinib	Carfilzomib	Goserelin acetate	Nitrogen mustard	Stattic
Acrichine	Carmustine	GSK 269962	Nutlin-3	Streptozocin

Table of compounds tested from the combined TDI Extended Oncology Drugs Library (ODL) and the NCI Developmental Therapeutics Program (DTP) Approved Oncology Drug Library.

Continued

Continued

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	NVP-LDE225 (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	I-BET151 (GSK1210151A)	OSI-906_Linsitinib	Teniposide
Anastrozole	Clafen (Cyclophos-phamide, Endoxan)	Idarubicin HCl	Oxaliplatin	Tetramisole HCl
AP24534 (Ponatinib)	Clofarabine	Ifosfamide	PAC-1	TGX-221
ARQ 197_Tivantinib	Clomifene citrate	Imatinib	Paclitaxel	Thalidomide
ARRY-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_Ibrutinib	Thiotepa
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	Tretinoin
Atorvastatin Ca	Dabrafenib Mesylate	JNJ 26854165 (Serdemetan)	PF 431396	Triethylenemelamine
Auranofin	Dacarbazine	JNJ_26481585_Quisinostat	PF 477736	Tubacin
AV-951 (Tivozanib)	Dacomitinib (monohydrate) (PF-00299804)	KX2-391	PF-04691502	Tubastatin A HCl
AVN944	Dactinomycin	Lapatinib, di-p-toluenesulfonate salt	PF-04708671	UCN-01
Axitinib	Dasatinib	Lasofoxifene	PF-2341066 (Crizotinib)	Uracil mustard
AZ 3146	Daunorubicin HCl	Lenalidomide	PF-3845	Valproic acid
Azacitidine	DCC-2036_Rebastinib	Lestaurtinib	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

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AZD6244 (Selumetinib)	Dinaciclib (SCH727965)	LY2228820 (CP868569)	PKC412_Midostaurin	Vinblastine sulfate
AZD8055	Docetaxel	LY2603618_Rabusertib	Plerixafor	Vincristine Sulfate (Oncovin)
BAY 73-4506_Regorafenib	Doxorubicin	LY2784544_Gandotinib	Plicamycin	Vinorelbine tartrate
Belinostat (PXD101)	Doxorubicin HCl	Masitinib	PLX4032_Vemurafenib	Vismodegib
Bendamustine HCl	EMD1214063	MDV3100_Enzaluamide	Pralatrexate	Vorinostat
Bexarotene	Entinostat	Megestrol acetate	Pravastatin	VX-11e
BI 2536	Enzastaurin	Melphalan	Prednisolone	XAV-939
BI 6727_Volasertib	Epothilone B (Patupilone)	Mercaptopurine	Prednisone	XL-147
BIBF 1120_Nintedanib	Erlotinib HCl	Metformin hydrochloride aq	Prima-1 Met	XL184_Cabozantinib
BIBW2992 (Tovok)_Afatinib	Estramustine sodium phosphate	Methotrexate	Procarbazine	XL880 (Foretinib)
Bicalutamide	Etoposide	Methoxsalen	PX-866_Sonolisib	YM155
BIIB021	Everolimus	Methylprednisolone	Quinacrine HCl	Zolendronic acid
Bimatoprost	Exemestane	MGCD-265	R406_Tamatinib	ZSTK474
BIRB 796 (Doramapimod)	FG-4592	MGCD0103_Mocetinostat	RAF265	
BKM-120_Buparlisib	Finasteride	Mithramycin A	Raloxifene HCl	

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

Cell line	5-FU IC_{50}	Vorinostat IC_{50}	PI-103 IC_{50}	Olaparib IC_{50}	Rucaparib IC_{50}	Mitoxantrone IC_{50}
LS411 0 Gy	24.67 (17.58 to 35.46)	6.79 (3.99 to 11.91)	5.23 (3.47 to 8.12)	24.46 (15.71 to 38.89)	*62.18	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)	1.72 (0.49 to 8.54)	7.8 (5.63 to 10.89)
VACO5 0 Gy	2.54 (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)
VACO5 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)	8.63 (4.32 to 17.22)	61.23 (30.07 to 167.4)	9.75 (6.21 to 15.44)
RKO 4 Gy	1.15 (0.73 to 1.90)	2.14 (1.2 to 4.15)	0.33 (0.20 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_{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_{50} in Graphpad, IC_{50} was calculated manually by interpolation. * > indicates the highest concentration tested in cell lines where the IC_{50} was not reached.

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Cell line	5-FU IC_{50}	Vorinostat IC_{50}	PI-103 IC_{50}	Olaparib IC_{50}	Rucaparib IC_{50}	Mitoxantrone IC_{50}
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 4Gy	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)
COLO678 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)
COLO678 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 (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}	PF4777 IC_{50}	AZD-6244 IC_{50}	Trametinib IC_{50}	Vemurafenib IC_{50}	
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)	

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Cell line	5-FU IC_{50}	Vorinostat IC_{50}	PI-103 IC_{50}	Olaparib IC_{50}	Rucaparib IC_{50}	Mitoxantrone IC_{50}
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)	
VACO5 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)	
VACO5 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 (2.36 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 4Gy	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)	

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Cell line	5-FU IC_{50}	Vorinostat IC_{50}	PI-103 IC_{50}	Olaparib IC_{50}	Rucaparib IC_{50}	Mitoxantrone IC_{50}
HCA7 0 Gy	0.06 (0.01 to 0.47)	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	