An IRAK1–PIN1 signalling axis drives intrinsic tumour resistance to radiation therapy

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Drug-based strategies to overcome tumour resistance to radiotherapy (R-RT) remain limited by the single-agent toxicity of traditional radiosensitizers (for example, platinums) and a lack of targeted alternatives. In a screen for compounds that restore radiosensitivity in p53 mutant zebrafish while tolerated in non-irradiated wild-type animals, we identified the benzimidazole anthelmintic oxfendazole. Surprisingly, oxfendazole acts via the inhibition of IRAK1, a kinase thus far implicated in interleukin-1 receptor (IL-1R) and Toll-like receptor (TLR) immune responses. IRAK1 drives R-RT in a pathway involving IRAK4 and TRAF6 but not the IL-1R/TLR–IRAK adaptor MyD88. Rather than stimulating nuclear factor-κB, radiation-activated IRAK1 prevented apoptosis mediated by the PIDDosome complex (comprising PIDD, RAIDD and caspase-2). Countering this pathway with IRAK1 inhibitors suppressed R-RT in tumour models derived from cancers in which TP53 mutations predict R-RT. Moreover, IRAK1 inhibitors synergized with inhibitors of PIN1, a prolyl isomerase essential for IRAK1 activation in response to pathogens and, as shown here, in response to ionizing radiation. These data identify an IRAK1 radiation-response pathway as a rational chemoradiation therapy target.

RadiOTHERAPy induces cytotoxic DNA breaks in tumour cells while minimizing damage to healthy tissues, and is given to ~60% of patients with cancer over the course of treatment1–3. Current approaches to overcoming tumour R-RT consist of concurrent systemic chemotherapy with classical anticancer agents such as genotoxins (for example, cisplatin and 5-fluorouracil) and microtubule inhibitors (for example, taxanes). These traditional radiosensitizers primarily act by augmenting DNA damage levels, thus enhancing cell killing within the field of radiation4–6. Radiosensitizers can be effective; for example, cisplatin-based chemoradiation therapy (CRT) improves survival by 10% compared to RT alone in patients with head and neck squamous cell carcinoma (HNSCC) and is the current standard of care in this cancer7. However, tumours recur in a large majority of patients, leading to invariably fatal disease. Further improvements of CRT have remained limited by the toxicity of radiosensitizers as single agents2,4. Moreover, these genotoxic drugs were not designed against—and thus do not necessarily target—the genetic defects or signalling pathways that drive tumour R-RT. Devising targeted strategies to supplant these cytotoxic chemotherapies is a current central focus of the Radiation Therapy Oncology Group of the US National Cancer Institute8 and the Clinical and Translational Radiotherapy Research Working Group of the The National Cancer Research Institute (UK)9.

A candidate and potentially pervasive mechanism of tumour R-RT is mutation of the p53 transcription factor, which occurs in ~50% of solid tumours6. Cells with mutant p53 fail to initiate apoptotic or senescence gene-expression programmes in response to ionizing radiation (IR)-induced DNA breaks6–8. In HNSCC10,11, colorectal cancer (CRC)12,13, breast cancer14, glioblastoma15 and medulloblastoma16, patients with missense TP53 mutations have markedly worse outcomes following RT or CRT compared with patients with wild-type (WT) TP53, with declines in recurrence-free or overall survival ranging from ~33% to 100%. Yet, patients are not stratified by TP53 status and there are currently no drugs reported to improve RT outcomes in TP53 mutant tumours1,2.

Results
In vivo zebrafish radiosensitizer screen identifies oxfendazole. To identify such genotype-directed radiosensitizers while accounting for the problem of systemic toxicity, we developed a whole-animal model of mutant TP53-driven R-RT for use in unbiased genetic and chemical screens17,18. In this model, zebrafish embryos
Fig. 1 In vivo zebrafish drug screen identifies oxfendazole as a radiosensitizer of p53 mutant embryos. a, 37Cs-irradiated p53MK/MK zebrafish embryos (15 Gy total body irradiation (TBI) at 18 h.p.f.) observed at 120 h.p.f. lack the DTC phenotype of WT embryos (arrowheads indicate a late readout of embryonic radiosensitivity). DTCs are restored with a CHK1 inhibitor (Gö6976, 1 μM), mercazole (RAPA), two known clinical radiosensitizers, were also recovered in the blind screen, providing internal validation of the DTC assay. b, Rescreen of the top 136 hits from a primary screen (Supplementary Fig. 1c–g) scored for potency (percentage of DTC after γIR), including in WT animals. DTCs are restored with a CHK1 inhibitor (Gö6976, 1 μM), mercazole (RAPA) and rapamycin (RAPA), two known clinical radiosensitizers, were also recovered in the blind screen, providing internal validation of the DTC assay. c, Doses of oxfendazole (shown here, 63 μM) that suppress R-RT in p53MK/MK embryos are fully tolerated in the absence of γIR, including in WT animals. d, p53MK/MK mutants treated with oxfendazole + IR score positive for acridine orange (AO), a vital marker of cell death, and apoptosis markers (TUNEL and anti-active caspase-3) at 48 h.p.f. e, Structure–activity relationship of benzimidazole tubulin-binding analogues in dose–response curves for 120 h.p.f. p53MK/MK embryos scored for DTCs. The chemical structure of each analogue is shown. Black curves, no IR. Red curves, 15 Gy TBI delivered at 18 h.p.f. Data are shown as the mean of n = 3 independent experiments for oxfendazole without IR (10–40 μM), oxfendazole + IR (0.1–40 μM), flubendazole without IR (0.1–10 μM), flubendazole + IR (0.1–10 μM), albendazole without IR (0.05–0.2 μM), albendazole + IR (0.05–2.0 μM), mebendazole without IR (0.2–2.0 μM), mebendazole + IR (0.1–2.0 μM), fenbendazole without IR (0.05–0.1 μM), fenbendazole + IR (0.025–0.1 μM), miconazole without IR (20–75 μM), miconazole + IR (2 μM), miconazole + IR (2 μM), n = 2 independent experiments for the remaining data points, as indicated by the dot plots and as detailed in Supplementary Fig. 5, with >21 embryos scored per experiment. **P < 0.005, ***P < 0.0005 (two-tailed Student’s t-test, α = 0.05). See Supplementary Table 4 for statistics source data, including precise P values. Scale bars, 0.5 mm (a,c) and 0.2 mm (d).

homozygous for the M214K (MK) mutation in tp53 display fully penetrant R-RT. This is evidenced by a complete lack of cell death induction in response to IR, a phenotype scored in 24–48 h post fertilization (h.p.f.) embryos17,18 (Supplementary Fig. 1a,b). Moreover, p53MK/MK mutants exhibit a complete lack of IR-induced dorsal tail curvatures (DTCs), a morphological manifestation of zebrafish radiosensitivity19 assessable by eye in 96–120 h.p.f. larvae (Fig. 1a). In a pilot candidate gene-based screen, we found that inhibitors of checkpoint kinase 1 (CHK1), such as Gö6976, restore WT levels of IR-induced cell death in p53MK/MK embryos, with minimal toxicity in the absence of IR19 (Supplementary Fig. 1a,b). Such potent radiosensitization by means of CHK1 inhibition was also evident in the late DTC assay, whereby Gö6976 restored DTC formation in ~75% of the mutants with no effects in the absence of IR (Fig. 1a,b; Supplementary Fig. 1d). Gö6976 therefore provided a positive control for large-scale radiosensitizer screens exploiting the morphological DTC phenotype as a readout.

In a screen of 1,151 small molecules (including 640 drugs approved by the US Food and Drug Administration (FDA)), we identified one compound, oxfendazole, which radiosensitized p53MK/MK mutants with both greater potency and lesser toxicity than Gö6976 (Fig. 1b; Supplementary Fig. 1c–g; Supplementary Tables 1 and 2). Importantly, these effects were observed at concentrations tolerated by non-irradiated WT embryos (Fig. 1c). Radiosensitization by oxfendazole was retained in mutant p53-depleted p53MK/MK embryos (Supplementary Fig. 2a–c) and was apoptotic in nature, as evidenced by acridine orange, TUNEL.
Target discovery for oxendazole identifies IRAK1. Oxendazole is a benzimidazole anthelmintic approved for the treatment of worm infections in livestock\textsuperscript{20,21}. Because other microtubule inhibitors, such as taxanes, are commonly used as radiosensitizers\textsuperscript{2}, we initially considered tubulin inhibition as the mechanism for oxendazole-mediated radiosensitization. Unexpectedly, none of seven tubulin-binding analogues of oxendazole, including the classical antimitotic nocodazole, could phenocopy the drug (the in vivo structure–activity relationship is shown in Fig. 1e). Specifically, while the analogues could produce DTCs as efficiently as oxendazole, none showed any selectivity for IR (with the possible exception of robenzodazole) and induced DTCs in the mutants regardless of radiation (Fig. 1e). Thus, tubulin inhibition is unlikely to fully account for, if even involved in, the radiosensitizing properties of oxendazole. It is notable in this regard that of all the benzimidazoles tested, oxendazole and robenzodazole have the lowest affinity for tubulin\textsuperscript{22}.

To identify the novel target (or targets) of oxendazole whose inhibition might drive radiosensitization, we used the similarity ensemble approach (SEA) target-prediction algorithm\textsuperscript{22} (Fig. 2a). SEA yielded 12 candidate targets, which we then tested for their ability to phenocopy oxendazole when inhibited by specific inhibitors in vivo (Fig. 2b; Supplementary Fig. 2j). This analysis identified the IL-1 receptor (IL-1R)-associated kinases IRAK1 and IRAK4\textsuperscript{23–25} as targets, and inhibition of these by an IRAK1/4 kinase inhibitor\textsuperscript{26} radiosensitized \textit{p53<sup>MK/MK</sup>} embryos with a potency nearing that of oxendazole (Figs. 2b–d and 3a; Supplementary Fig. 2j). Kinase-binding and in vitro kinase assays demonstrated that oxendazole is a selective IRAK1 inhibitor (Fig. 2h,i). The observed dissociation constant ($K_d$ of $5.8\,\mu M$) and half-maximum inhibitory concentration ($IC_{50}$ of $38\,\mu M$) were consistent with concentrations of oxendazole that radiosensitize zebrafish $p53^{MK/MK}$ mutants (Fig. 1e). Docketing of oxendazole onto IRAK4-derived zebrafish and human IRAK1 models\textsuperscript{30} predicted drug binding to the ATP-binding site and an interaction with the hinge region via the benzimidazo[1,2-c]pyridine moiety, while the phenylsulfone moiety resides in a pocket adjacent to the DFG motif (Fig. 2e–g).

IRAK1 is known as an effector of the IL-1R and TLRs in innate immune signalling\textsuperscript{31}. It acts through the TRAF6 E3 ubiquitin ligase to stimulate nuclear-factor-κB (NF-κB), p38 MAPK, JNK and ERK prosurvival and inflammatory responses to pathogens\textsuperscript{32–34}. IRAK1 had not been previously implicated in the DNA damage response or cellular response to RT. Yet, inspection of The Cancer Genome Atlas (TCGA) cohort revealed significant overexpression of IRAK1 in HNSCC and breast cancer samples harbouring TP53 mutant genotypes compared to WT, as well as tumours from patients with breast cancer who ultimately received RT as part of their treatment (Supplementary Fig. 3a,i,j; $P < 0.0001$, $P < 0.0001$ and $P < 0.05$, respectively, Wilcoxon rank-sum test). We also found that IRAK1 is commonly activated in response to IR in TP53 mutant HNSCC-, breast cancer- and CRC-derived cell lines, correlating with pronounced R-RT phenotypes (Fig. 5d,g,h). We therefore further investigated IRAK1 as a target for inhibition in tumour R-RT.

We first sought to confirm the oxendazole and IRAK1/4 inhibitor data with additional IRAK1 inhibitors and gene targeting in zebrafish $p53^{MK/MK}$ embryos, as well as TP53 mutant human cancer cells. For IRAK1 inhibition, we selected the tyrosine kinase inhibitor R406, which inhibits IRAK1 with an $IC_{50}$ of 9.7 nM compared with 150 nM for IRAK4\textsuperscript{35}, and ginsenoside-Rb1, a ginseng extract that inhibits IRAK1 but not IRAK4 or IRAK2\textsuperscript{36}. Whether in zebrafish $p53^{MK/MK}$ mutants, HeLa cells (devoid of p53 protein via human papillomavirus E6) or TP53 mutant or null human cancer cell lines, genetic or pharmacological inhibition of IRAK1 was consistently incompatible with cell survival in the presence of IR but tolerated in the absence of IR (Figs. 2j, 3a–f, 4a and 5e; Supplementary Fig. 4a–f). Both the zebrafish and human cell IRAK1 knockdown models were rescued by WT but not kinase dead\textsuperscript{38} (KD) human IRAK1 (Figs. 3e–g and 4c). Importantly, overexpression of WT, but not KD, IRAK1 was sufficient to confer R-RT to otherwise radiosensitive Dasyo MB cells (Fig. 4b). Finally, CRISPR (clustered regularly interspaced short palindromic repeats)–Cas9 gene editing confirmed the essential requirement of the kinase for cell survival specifically after IR (Fig. 4d).

IRAK1 drives R-RT independently of its IL-1R/TLR adaptor MyD88 and of canonical downstream pathways. Both the proximal activator and proximal effector of IRAK1 in IL-1R and TLR signalling—IRAK4 and TRAF6, respectively\textsuperscript{35,36,37}—were also required for cell survival after IR (Fig. 4e). IRAK1 was necessary for IR-induced activation of IRAK1, as assessed by T209 phosphorylation\textsuperscript{35,36} (Fig. 4f), while an IRAK1 mutant deficient in TRAF6-binding, IRAK1<sup>ΔN</sup> (E544A/E587A/E706A)\textsuperscript{38}, afforded only partial rescue of irak1-depleted $p53^{MK/MK}$ embryos (Fig. 3e–g). By contrast, the adaptor protein MyD88—which bridges IRAK1 and IRAK4 to IL-1R and TLRs and is essential for IRAK1 and IRAK4 activation in innate immunity\textsuperscript{39,40,41,42}—was dispensable for both IR-induced IRAK1 T209 phosphorylation and overall R-RT in HeLa cells and $p53^{MK/MK}$ zebrafish (Fig. 4e–i; Supplementary Fig. 4g,k,l). In further contrast to canonical IRAK1 immune signalling, the kinase did not engage NF-κB, p38 MAPK, JNK or ERK signalling in response to IR (Fig. 4f; Supplementary Fig. 4h). Moreover, an IRAK1 mutant deficient in NF-κB essential modulator (NEMO) binding, IRAK1<sup>1128R</sup> (K134R/K180R)\textsuperscript{43}, restored R-RT in IRAK1-depleted $p53^{MK/MK}$ zebrafish as efficiently as WT IRAK1 (Fig. 3e–g).

IR-activated IRAK1 acts to suppress PIDDosome-mediated apoptosis. Instead of acting through its aforementioned canonical signalling pathways, we found that IRAK1 drives survival after IR by preventing PIDDosome (PIDD–RAIDD–caspase–2) signalling, a DNA damage-inducible apoptotic axis that does not require p53 for activation or function after IR\textsuperscript{44–46}. Indeed, deletion or depletion of IRAK1 triggered caspase-2 maturation in irradiated cells in a PIDD– and RAIDD-dependent manner (Fig. 4k,l; Supplementary Fig. 4j). Moreover, IRAK1 inhibitor-mediated radiosensitization was abolished in cells depleted of PIDD, RAIDD or caspase-2\textsuperscript{47} (Fig. 4m,n). As expected from previous studies\textsuperscript{48,49}, PIDDosome-mediated radiosensitization was associated with increased levels of DNA damage (Supplementary Fig. 2f). Finally, IRAK1 inhibition after IR was sufficient to enable ATM-mediated phosphorylation of the PIDD death domain (PIDD-pT788) (Fig. 4k,l; Supplementary Fig. 4i). An event necessary and sufficient for RAIDD recruitment and PIDDosome formation\textsuperscript{49}. Together, these data point to an evolutionarily conserved role for IRAK1 in protecting cells against IR-induced cell death, acting in a pathway related to, but genetically distinct from, IL-1R/TLR signalling.

IRAK1 inhibitors restore radiosensitivity in multiple cell models of tumour R-RT. To evaluate the robustness of the IRAK1-targeting strategy, we analysed the radiosensitizing properties of IRAK1 inhibitors across both tumour and TP53 mutation spectra. We assembled a panel of relevant cancer cell lines based on the following three criteria: they must originate from a tumour type in which TP53 mutations adversely affect patient survival after RT or CRT; they must contain a non-synonymous mutation in TP53; and they must have been previously demonstrated as radioresistant in clonogenic assays. A search of the Cancer Cell Line Encyclopedia\textsuperscript{50}
Fig. 2 | Target discovery for oxendazole identifies IRAK1. a, SEA-predicted targets of oxendazole and analogues. The E-value is colour-coded white to red. Black bars represent predicted targets for oxendazole. b, DTC assay of p53<sup>MK/MK</sup> embryos (120 h.p.f.) treated with inhibitors of SEA-predicted oxendazole targets (a) with or without 15 Gy IR. The dotted line represents the penetrance cut-off at DMSO. Data are presented as the mean ± s.d. of n = 4 independent experiments (DMSO, IRAK1/4 inhibitor (IRAK1/4i)), n = 3 independent experiments (oxendazole, buparvaquone, rebastinib) or n = 2 independent experiments (remaining drugs) with >12 embryos per experiment. **P < 0.005, ***P < 0.0005 relative to DMSO-treated irradiated embryos (bar 2; two-tailed Student’s t-test). c, Chemical structures of indicated drugs. d, Dose-response to IRAK1/4 inhibitor (μg ml<sup>-1</sup>) of p53<sup>MK/MK</sup> embryos scored in DTC assays. Data are presented as the mean ± s.d. of n = 3 independent experiments. *P < 0.05, **P < 0.005, ***P < 0.0005 relative to DMSO-treated irradiated embryos (bar 2; two-tailed Student’s t-test). e, Sequence alignment of human (Hs) and zebrafish (Dr) IRAK1 kinase domains, ATP-binding domain boxed in blue. Id, identity, sim., similarity. f,g, Induced-fit docking of oxendazole to ATP-binding site of zebrafish (f) and human (g) IRAK1 models. Gate-keeper residue, Y255 (Y288 in human). Three residue changes near the proposed docking pose within the binding site is highlighted in cyan in the zebrafish model: M239, Y257 and M258 (V272, F290 and L291 in the human model). h,i, KINOMEScan in vitro kinase capture assay for indicated kinases (h) and in vitro kinase assay versus IRAK1 (i), curved-fit from two and three replicates, respectively, j. The IRAK1 inhibitor R406 (40 μg ml<sup>-1</sup>) phenocopies oxendazole (Fig. 1d) in an AO assay at 48 h.p.f., with images representative of two independent experiments. See Supplementary Table 4 for statistics source data, including precise P values. Scale bar, 0.2 mm.

combined with a literature search identified 12 such lines derived from HNSCC, medulloblastoma, glioblastoma, CRC and breast cancer (Fig. 5d). We also included MCF7 cells, which, while WT for TP53, display profound resistance to IR due to deletion of CASP3<sup>31</sup>. With the exception of the Daoy MB line, all selected cell lines were confirmed as radioresistant in response to 2.5, 5 and up to 7.5 Gy
IR (see Fig. 5b–d and Supplementary Fig. 5 for dimethylsulfoxide (DMSO) columns and corresponding cell viability curves). We therefore screened the panel with the IRAK1 inhibitors R406 and ginsenoside-Rb1 applied at their respective maximum tolerated doses (MTDs), as determined in HeLa cells (Fig. 5a), as well as a twofold higher dose (2xMTD). For comparison, we tested cisplatin (MTD and 2xMTD, as above), whose combination with RT is a standard of care in HNSCC and is commonly used in CRT of many other cancers. Cisplatin failed to sensitize any of the TP53 mutant lines to IR, with only marginal additive effects observed in YD38...

Fig. 3 | Targeting irak1 overcomes R-RT in p53MK/MK zebrafish. a–c, Morpholino (MO) depletion of irak1 phenocopies oxendazole and IRAK1/4 inhibitor (IRAK1/4i) in DTC (a). AO (b, top) and TUNEL (b, bottom) assays (oxendazole shown in Fig. 1d). MO dose-response in p53MK/MK embryos in the DTC assay (c). Data are the mean ± s.d. of n = 3 independent experiments. d, RT-PCR of pooled embryonic extracts showing reduced WT mRNA levels (upper band) and skipping of exon 4 (lower band). rpp0, ribosomal protein P0. e, Representative images (e) and quantification (f) of irak1-depleted p53MK/MK embryos reconstituted with WT versus KD (left), WT versus IRAK1/4i (K2R, middle) or WT versus IRAK1K2A (E3A, right) human IRAK1 (hIRAK1) mRNA scored in AO assays. Number of quantified, independent images (as boxed in e) for each column of images is as follows. Left: std MO + mock: n = 3 (non-irradiated = 14, 3 of non-irradiated = 13, 1 of non-irradiated = 11, 0 of irradiated = 11, 0 irak1 MO + mock: 9 of non-irradiated = 14, 5 of non-irradiated = 13, 1 of irradiated = 13, 1 irak1 MO + WT: n = 1 (non-irradiated = 14, 0 of irradiated = 13, 0 irak1 MO + KD: n = 0 (non-irradiated = 13, 0 of irradiated = 13). Middle: std MO + mock: n = 5 (non-irradiated = 9, 0 of irradiated = 13, 1 irak1 MO + mock: n = 2 (non-irradiated = 13, 0 of irradiated = 13, 1 irak1 MO + WT: n = 1 (non-irradiated = 13, 0 of irradiated = 13, 1 irak1 MO + KD: n = 0 (non-irradiated = 13, 0 of irradiated = 13). Right: std MO + mock: n = 3 (non-irradiated = 13, 0 of irradiated = 13, 1 irak1 MO + mock: n = 2 (non-irradiated = 13, 0 of irradiated = 13, 1 irak1 MO + WT: n = 1 (non-irradiated = 13, 0 of irradiated = 13, 1 irak1 MO + KD: n = 0 (non-irradiated = 13, 0 of irradiated = 13). Raw images of all embryos and cropped spinal chord areas are available at https://doi.org/10.6084/m9.figshare.7427942. g, Western blot of embryonic extracts showing hIRAK1 levels and diagram of hIRAK1, with disrupted residues and interactions shown. All data are presented as the mean ± s.d., *P < 0.05, **P < 0.005, ***P < 0.0005 (two-tailed Student’s t-test). See Supplementary Table 4 for statistics source data, including precise P values, and Supplementary Fig. 8 for unprocessed immunoblots. Scale bars, 0.5 mm (a) and 0.2 mm (b,e).
**Fig. 4 | IRAK1 acts independently of MyD88 and counters PIDDosome signalling.** a–f.j–n, HeLa (a.d.f.j–n), Daoy MB (b) and CAL27 (c) cells were transfected with or treated as follows: indicated siRNA (a.e.f); WT or KD Flag-IRAK1 (b,c); IRAK1 sgRNA plus Cas9 plasmid (d) and/or stably expressing indicated doxycycline (dox)-inducible shRNA (a.c.k) or non-inducible shRNA (l–n), and/or treated with IRAK1 inhibitor ginsenoside-Rb1 (10 μM) (m,n). Cells were then analysed as follows: 5 dpIR (IR doses in Gy) by AlamarBlue cell viability assay (a–e,n); 14 dpIR by clonogenic assay (d); 2 dpIR by TUNEL assay (m) and/or by western blotting with the indicated antibodies (a–f.j–l) at indicated minutes post-IR or IL-1β treatment (j); or 24 hpiR (a–f.k,l). eIRAK1, endogenous IRAK1; pro-C2, procaspase-2; cl-C2 (p35), intermediate cleavage product; cl-C2 (p19), mature cleavage product. Data in a–e and m,n are presented as the mean ± s.d., n = 3 independent experiments performed in triplicate. In j, activation of the following pathways are marked as follows: by a gradual decline in IB levels (NF-κB); p38 T180/Y182 phosphorylation (p38 MAPK); JNK T183/Y185 phosphorylation (JNK); ERK T202/Y204 phosphorylation (ERK). g,h, Representative images (as in Fig. 3b for std and irak1 MOs) and quantification of p53T156/157* embryos injected with indicated MOs and treated with or without 15 Gy IR at 18 h.p.i. and analysed in an AO assay at 24 hpiR. Number of quantified spinal chord images (as boxed in g) from 4 independent experiments (bars 1–4) or 3 independent experiments (bars 5–8) were as follows: std MO: n of non-irradiated = 9, n of irradiated = 11; irak1 MO: n of non-irradiated = 11, n of irradiated = 13; myd88 MO high (hi, 6.4 ng): n of non-irradiated = 7, n of irradiated = 8; myd88 MO low (lo, 3.2 ng): n of non-irradiated = 9, n of irradiated = 11. i, RT-PCR of pooled embryo mRNA extracts shows MO dose-dependent intron retention in myd88 messages. *P < 0.05, **P < 0.005, ***P < 0.0005 throughout the figure (two-tailed Student’s t-test). See Supplementary Table 4 for statistics source data, including precise P values, and Supplementary Fig. 8 for unprocessed immunoblots. Scale bar, 0.2 mm (for all panels).

and T98G cells (Fig. 5d; Supplementary Fig. 5). In stark contrast, ginsenoside-Rb1 and R406 radiosensitized up to 10 and 7 of 11 TP53 mutant lines, respectively (Daoy MB excluded) (see Fig. 5d and Supplementary Fig. 5 for corresponding cell viability curves, and Supplementary Fig. 4d–f for select colony assays). MCF7 radioresistance could not be overcome throughout the screen regardless.
Fig. 5 | IRAK1 inhibitors restore radiosensitivity across TP53 mutant tumour cell models. a, AlamarBlue-based HeLa cell viability assays establish MTDs (green arrowheads) for cisplatin, the IRAK1 inhibitors ginsenoside-Rb1 (Gin-Rb1) and R406, and the PIN1 inhibitors EGCG and buparvaquone. Gin-Rb1 required 2.5 Gy to achieve MTD at doses below the precipitation point of the drug. n = 2 independent experiments in triplicate, data are presented as the means. b, Explanatory schematic for the heatmap shown in d, with R406 on IR-treated HeLa cells as the example. b, Cells treated with MTD (0.5 μM) and 2×MTD (1 μM) with 3 doses of IR represented in a standard dose–response curve (left) and corresponding heatmap representation (right). Cell viability colour code shown from red at 0% to blue at 100%. Note that this example showcases the three main phenotypic classes observed in the screen required 2.5 Gy to achieve MTD at doses below the precipitation point of the drug.

c, IR (Gy): 0, 2.5, 5, 7.5. Data are presented as the mean of 2 independent experiments. *P < 0.05, ***P < 0.0005 (two-tailed Student’s t-test). Source data available in Supplementary Table 4, sheet S5. d, f, AlamarBlue-based cell viability heatmaps of radioresistant cancer cell lines. Cell line names are depicted to the left with TP53 genotype in parentheses and tumour of origin to the right (d), WT and TP53-null HCT116 cells (e) and normal human cells (MCF10A, IMR90; f) treated with cisplatin or IRAK1 inhibitors, Gin-Rb1 and R406, at indicated doses (MTD and 2×MTD, in μg per ml) and IR (0, 2.5, 5 and 7.5 Gy, as indicated) in n = 3 independent experiments performed in triplicate. Corresponding survival curves and P values are shown in Supplementary Fig. 5. g, Indicated HeLa shRNA lines treated with or without IR (10 Gy) and analysed by western blotting at 24 hpiR. h, Cell lines as in d treated with or without IR (10 Gy) and analysed by western blotting 24 hpiR. A total of 7 of 9 lines (HeLa, CAL27, SAS, T47D, MDA-MB-231, SW480 and DLD-1, see d) reliably radiosensitized by IRAK1 inhibitors engage IRAK1 phosphorylation in response to IR, while both resistant lines (BHY, MCF7) do not. See Supplementary Table 4 for statistics source data, including precise P values, and Supplementary Fig. 8 for unprocessed immunoblots.
See Supplementary Table 3 for patient characteristics. Mutant, n = s.d. of ±, shTRIPZ and shPIN1 CAL27 cells reconstituted with Flag-PIN1 constructs exposed to 0 or 7.5 Gy IR and assayed by AlamarBlue at 5 dpIR. Data in ec–ec or catalytically inactive (W34/K63A) PIN1 exposed to 0 or 7.5 Gy IR and assayed by AlamarBlue at 5 dpIR. Western blot of Flag-PIN1 levels also shown.

Daoy MB cells transfected with mock, WT mRNA.

Clonogenic assay of shTRIPZ and shPIN1 HeLa cells, Right: RT–PCR of embryonic mRNA extracts detect nonsense mediated decay of pin1 mRNA. MO depletion of PIN1 but not std MO restores DTCs (baa) after 15 Gy IR. See Supplementary Fig. 6c,d for quantifications.

Fig. 6 | PIN1 inhibition overcomes R-RT in zebrafish and human tumour-cell models while its overexpression associates with TP53 mutant HNSCC recurrence. a, b, MO depletion of PIN1 but not std MO restores DTCs (a) and AO uptake (b) after 15 Gy IR. See Supplementary Fig. 6c,d for quantifications. b, Right: RT–PCR of embryonic mRNA extracts detect nonsense mediated decay of pin1 mRNA. c, Clonogenic assay of shTRIPZ and shPIN1 HeLa cells after up to 5 Gy IR. Surviving fractions to the right. Western blot of PIN1 knockdown also shown in the inset. d, Daoy MB cells transfected with mock, WT or catalytically inactive (W34/K63A) PIN1 exposed to 0 or 7.5 Gy IR and assayed by AlamarBlue at 5 dpIR. Western blot of Flag-PIN1 levels also shown.

e, shTRIPZ and shPIN1 CAL27 cells reconstituted with Flag–PIN1 constructs exposed to 0 or 7.5 Gy IR and assayed by AlamarBlue at 5 dpIR. Data in c–e are the mean ± s.d. of n = 3 independent experiments performed in triplicate. *P < 0.05, **P < 0.005 (two-tailed Student’s t-test). f, MTD and 2xMTD of buparvaquone (Bup.) and EGCG given to indicated cell lines with 0, 2.5 or 5 Gy IR and analysed by western blot at indicated hpIR. See Supplementary Table 4 for statistics source data, including precise P values, and Supplementary Fig. 8 for unprocessed immunoblots.
Fig. 7 | Low-dose IRAK1 and PIN1 inhibitors synergistically suppress R-RT in vitro and in vivo. a,b: HeLa (a) and non-tumorigenic IMR90 and MCF10A cells (b) treated with indicated IRAK1 and PIN1 inhibitors at subtherapeutic doses (buparvaquone (0.5 µg ml⁻¹), R406 (0.1 µg ml⁻¹), ginsenoside-Rb1 (5 µg ml⁻¹) and EGCG (5 µg ml⁻¹); see Fig. 5a) and indicated γIR doses (Gy) and analysed by clonogenic assay at 14 dpiR (a, with representative images to the right) or AlamarBlue at 5 dpiR (b). Data are presented as the mean ± s.d. of n = 3 independent experiments (a, b, left) and n = 4 independent experiments (b, right) performed in triplicate. c, p53MK/MK embryos treated with subtherapeutic doses of EGCG (40 µg ml⁻¹) and R406 (20 µg ml⁻¹) and 0 or 15 Gy TBI at 18 h.p.f. analysed for DTCs at 120 h.p.f. Data are presented as the mean ± s.d. of n = 3 independent experiments, with representative images shown to the right. P value is relative to DMSO-treated irradiated embryos (bar 2). Cl, combination index (see Supplementary Fig. 7f). Scale bar, 0.5 mm. d, Surviving fractions of SAS cells treated with subtherapeutic doses of ginsenoside-Rb1 (5 µg ml⁻¹) and/or EGCG (5 µg ml⁻¹) at indicated IR exposures (Gy), and analysed 14 dpiR. Representative images shown below. Data are presented as the mean ± s.d. of n = 3 independent experiments performed in triplicate. e, SAS (2 x 10⁶ cells)-derived tumour xenografts grown in NSG mice with n = 5 animals per indicated group analysed by haematoxylin and eosin staining (e) and immunofluorescence confocal microscopy with indicated antibodies (f, g) 24 days post-implantation. See Methods for detailed implantation, RT, drug delivery and staining protocols. Scale bars, 100 µm (e) and 80 µm (g). Data in f are from the analysis of n = 4 independent samples per group with 3 independent images (as in g) scored per tumour. Anti-vimentin and anti-active caspase-3 mark human and apoptotic cells, respectively. Unless otherwise indicated, data throughout are expressed as the mean ± s.d., *P < 0.05, **P < 0.005, ***P < 0.0005 (two-tailed Student’s t-test). See Supplementary Table 4 for statistics source data, including precise P values.

of drug, drug dose or IR dose. In the great majority of cases, radiosensitization by the IRAK1 inhibitors occurred in those lines that engaged IRAK1 T209 phosphorylation in response to IR (Fig. 5g,h). Radiosensitization was also obtained at drug doses that were tolerated in the absence of IR (see 0 Gy data points in Fig. 5d and Supplementary Fig. 5) and in irradiated non-tumorigenic fibroblasts (IMR-90) and mammary epithelial cells (MCF10A) (Fig. 5f; Supplementary Fig. 5). Taken together with the zebrafish and HeLa
An additional predicted oxendazole target, PIN1 isomerase, drives R-RT in zebrafish and tumour-cell models and associates with R-RT in HNSCC. Following our analysis of SEA-predicted oxendazole targets, we noted that inhibition of one additional candidate target, the peptidyl-prolyl cis/trans isomerase PIN1, radio-sensitized zebrafish p53<sup>−/−</sup> mutants with a similar potency to that of the IRAK1/4 inhibitor (Fig. 2a,b). While in vitro isomerase and thermal shift assays could not immediately confirm PIN1 as an oxendazole target (Supplementary Fig. 6a,b), genetic or pharmacological inhibition of PIN1 did consistently suppress R-RT in zebrafish (Fig. 6a,b; Supplementary Fig. 6c–g), in HeLa cells (Fig. 6c; Supplementary Fig. 6i,j) and in HNSCC lines (see Fig. 6f and Supplementary Fig. 6n for corresponding cell viability curves, and Supplementary Fig. 6j–m for select colony assays). Similar to IRAK1, PIN1 was sufficient to force R-RT when overexpressed in radiosensitive Daoy MB cells, whereas a catalytically inactive (W34A/K63A) variant<sup>23</sup> was less potent (Fig. 6d). In further support of PIN1 as a driver of tumour R-RT, overexpression of PIN1 was significant- ly associated with locoregional recurrence (LRR; P = 0.006) and reduced overall survival (P = 0.007), but not distant metastases, in patients with TP53 mutant HNSCC who were treated with post-operative RT at the MD Anderson Cancer Center<sup>19,44</sup> (Fig. 6g–j; Supplementary Table 3). Inspection of TCGA HNSCC cohort—whose analysis is however limited by a lack of LRR data<sup>23</sup>—revealed significant upregulation of PIN1 in TP53 mutant tumours that were ultimately treated with RT (Supplementary Fig. 3f–h).

PIN1 inhibition prevents IR-induced IRAK1 activation and synergizes with IRAK1 inhibitors in vitro and in vivo. Interestingly, PIN1 plays an essential and direct role in TLR-induced IRAK1 activation<sup>8</sup>. Similarly, we found that genetic or pharmacological inhibition of PIN1 blocked IR-induced IRAK1 phosphorylation on T209 (Fig. 6k,l). We therefore tested whether IRAK1 and PIN1 inhibitors synergistically suppress R-RT in our various models. We trialled four combinations of IRAK1+PIN1 inhibitors, involving the IRAK1 inhibitors ginsenoside-Rb1 and R406 (see above), and the PIN1 inhibitors epigallocatechin gallate (EGCG), a competitive inhibitor with micromolar efficacy shown to bind the PIN1 catalytic site<sup>40</sup>, and buparvaquone, a repurposed antiparasitic that binds and inhibits PIN1 with nanomolar efficacy<sup>41</sup>. Each inhibitor was titrated to a dose that did not decrease HeLa survival as a single agent, even after 2.5 or 5 Gy IR. Under these subtherapeutic conditions, all four combination treatments produced marked synergistic declines in survival specifically after IR (see Fig. 7a and Supplementary Fig. 7a,e and for combination indexes). These findings were recapitulated in the following models: p53<sup>−/−;MKMK</sup> fish (Fig. 7c; Supplementary Figs. 6h and 7f); TP53 mutant HNSCC cell lines grown in vitro (Fig. 7d; Supplementary Fig. 7b–d,g) or as tumour xenografts in vivo (Fig. 7e–g; Supplementary Fig. 7h); TP53<sup>+/−</sup> as well as WT HCT116 cells (Supplementary Fig. 7i); and one of the cell lines from our radiosensitive panel that best resisted IRAK1 inhibitors as single agents (SW480 cells; Supplementary Fig. 7b,g). All four IRAK1 + PIN1 inhibitor combinations were otherwise tolerated in IMR90 human fibroblasts, including after up to 7.5 Gy IR (Fig. 7b, left). While MCF10A mammary epithelial cells showed some sensitivity (Fig. 7b, right), such toxicity was not fur- ther exacerbated by IR and was marginal compared with the levels of tumour-cell lethality induced by the various drug combinations in the tumorigenic cell lines (for example, compare 5 Gy data points in Fig. 7b to that in Supplementary Fig. 7a,b). Clinical translation will require extending these studies to additional in vivo tumour models and TP53 alleles, including in immunocompetent mice. Together, our data collectively identify IRAK1 and PIN1 as rational targets in radioresistant cancers, with efficacy stemming from single or low-dose combination treatments.

**Discussion**

The data presented here show that IRAK1, a core transducer in innate immune signalling conserved from flies to humans<sup>23,24,27</sup>, plays an additional conserved role in the cell survival response to IR. This pathway involves the IL-1R/TLR pathway members PIN1, IRAK4 and TRAF6. However, its MyD88-independence, full reliance on IRAK1 kinase activity, partial reliance on TRAF6 and divergent downstream target (the PIDDosome) are supportive of a distinct stress-response pathway that diverged from, or possibly preceded, the pathogen response. The pathway may respond to one or more IR-induced primary or secondary ionization events, including DNA breakage, micronucleation or other occurrences of cytosolic DNA, hydroxyl radicals or other reactive oxygen species. Ruptured lipid bilayers and/or so-called danger-associated molecular patterns (that is, DNA or nuclear proteins released into extracellular space) may also trigger the pathway<sup>50,51</sup>. A key feature of this pathway from a therapeutic viewpoint is its IR-induced essentiality, whereby pathway inhibition is lethal to zebrafish or cancer cells exposed to IR but is otherwise tolerated in the absence of IR. Even germline losses of *Irak1* or *Pin1* are viable in mice<sup>23,24</sup>. Thus, IRAK1 inhibitor treatment (alone or with a PIN1 inhibitor) could lead to improved tumour radiosensitization strategies whereby drug-induced cytotoxicity is restricted to the field of RT with minimal effects in unexposed tissues. Additionally, the radiosensitizing properties of IRAK1 and PIN1 inhibitors in TP53 mutant tumour cells are not allele-specific and are largely retained in TP53<sup>−/−</sup> and WT backgrounds. This potentially expands the patient population that might benefit from IRAK1±PIN1 inhibitor-based CRT. Finally, recent reports impli- cate deregulated IRAK1 and PIN1 in tumour progression, main- tenance and metastasis, via stabilization of mutant p53 itself<sup>42</sup> and other pathways<sup>23,24,27,44</sup>. Our discovery of these enzymes as drivers of cellular R-RT calls for further development of IRAK1 and PIN1 inhibitors for therapeutic use.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41556-018-0260-7.

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Author contributions

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Supplementary information

**Additional information**

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Methods

Zebrafish lines and maintenance. Adult zebrafish were maintained on a 14:10 h light:dark cycle at 28 °C in accordance with the regulations and policies of the Mount Sinai Institutional Animal Care and Use Committee. The study is compliant with all relevant ethical regulations regarding zebrafish research. The progeny of p53MK/MK fish were used in most experiments. WT zebrafish were from the AB line. The TILLING-mediated generation of the p53MK/MK line, including allele designation, has been previously described1,2.

Zebrafish drug screen. Live embryos were dechorionated in pronase (2.0 mg ml⁻¹ in egg water) for 7 min and rinsed three times in egg water at 17 h.p.f. At least 15 p53MK/MK embryos were then arrayed into each well of a 24-well plate and treated with drugs from FDA-approved drug library V1 (Enzo Life Sciences) or proprietary kinase inhibitors (Reddy Lab) at a final concentration of 20 ng ml⁻¹ in egg water. In the primary screen, three wells were set aside for controls: one negative control, p53MK/MK + DMSO; and two positive controls, p53MK/MK + Go6956 (1 µM) and p53⁺/− + DMSO. Plates containing drug-treated embryos were y-irradiated at 18 h.p.f. using a ¹³⁷Cs-irradiator (X-ray IR can also be used, but developmental stage and dose differ, presumably due to low-energy electrons). At 6 hPi, embryos were washed three times and scored at 72 h.p.f. and 120 h.p.f. for curved tails and gross morphological changes. If any well lost embryos to necrosis or manipulation such that fewer than 12 embryos were left before 120 h.p.f., that data point was not included in the analysis and the entire condition was repeated. In the secondary screen, the set-up was similar except two 24-well plates were set-up with identical drug treatments identified in the primary screen, with embryos randomly assigned to each plate. Phenotyping was identical to the primary screen. Although at least 12 embryos were required to survive until 120 h.p.f. for the data point to be counted, and the secondary screen was performed in three independent experiments. Pictures were obtained of tricaine-anaesthetized embryos mounted on 2–3% methylcellulose and imaged with a Nikon SMZ 1500 fluorescence microscope.

Acridine orange labelling. Live embryos were dechorionated in pronase (2.0 mg ml⁻¹ in egg water) for 7 min and rinsed three times in egg water at 17 h.p.f. After being arrayed and incubated with drugs, depending on the experiment, embryos were then y-irradiated at 18 h.p.f. using a ¹³⁷Cs-irradiator. At 6 hPi, embryos were labelled live with acridine orange at 10 mg ml⁻¹ in egg water for 10 min, washed three times and analysed using ImageJ as previously described 3.

Whole-mount TUNEL staining and caspase-3 immunohistochemistry. The TUNEL cell death assay was performed according to the manufacturer’s instructions (ApopTag Fluorescein In Situ Apoptosis Detection kit) with zebrafish manipulations as previously described 2. Embryos stained for caspase 3 or yH2AX were fixed in 4% paraformaldehyde overnight at 4 °C and subsequently dehydrated in methanol at −20 °C for at least 2 h. Embryos were then rehydrated three times for 5 min in PBST (1X PBS, 0.1% Tween-20), and permeabilized by treatment with PBT (PBST, 1% DMSO) supplemented with 0.3% Triton-X for 20 min. Embryos were treated with blocking solution (PBT supplemented with 10% heat-inactivated fetal bovine serum (FBS)) for 30 min before the addition of primary antibody (1:500 anti-activated-caspase-3 (StressGen AA-103), 1:200 anti-yH2AX (Millipore, 05–4636)). Embryos were incubated in primary antibody overnight at 4 °C, rinsed three times for 20 min in PBST and then re-blocked for 30 min in blocking solution before the addition of AlexaFluor-conjugated secondary antibody (1:250). Immunohistochemistry for yH2AX and imaging were performed with a Leica SP5 DM confocal microscope.

SEA analysis. Chemical compound SMILES formulas were queried with the online SEA search tool (http://sea.bkslab.org/search/), searching against ChEMBL v.16 Database. Chemicals and inhibitors. Oxendazole, fluocinolone, exemestane, cefepime, pranlukast, amiloride, alfalcacidol and albenzazole were obtained from VWR. Amoxapine, ricobendazole and mercuzazole were obtained from Sigma-Aldrich. Salmeterol, indomethacin, canthin-6, bile, rimonapine, mependezol, fenbendazole and flubendazole were obtained from Santa Cruz Biotechnology. Misoprostol was obtained from Thermo Fisher, while rebastinib and regorafenib were obtained from Selleck Chem. Go6956 was obtained from Calbiochem, while lapatinib, SB203580 and SB202190 were gifts from J.A.A.-G. Dabrafenib, sorafenib and dabrafenib-flurbiprofen were gifts from A. Dar. Recombinant human interleukin-10 (10 µg ml⁻¹) from Peprotech (200-01B). Doxycycline hyclate was purchased from Sigma (D9891-1G). The following inhibitors were used in this study: IRAK1/4 inhibitor (Sigma, I5409); R406 (Selleck Chemicals, S2194); ginsenoside-Rb1 (Ammoc, ab142646, and J&K Scientific, 11217); buparvaquone (Santa Cruz Biotechnology, sc-210970); and EGCg (Sigma, E4268 and E1443). The IRAK1/4 inhibitor was originally characterized in a high-throughput in vitro screening assay and contained with IC₅₀ values of 300 nM and 200 nM for IRAK1 and IRAK4, respectively. For 27 other kinases tested during discovery, IC₅₀ values were >10,000 nM. Ginsenoside-Rb1 was originally isolated from ginseng extract and identified as a saponin among other components extracted from ginseng. It inhibits IRAK1 in an in vitro kinase assay with an IC₅₀ of ~10 µM, with other reported activities against PI3K, AKT, ERK and inhibition of the NRF2–HO-1 pathway, among others. R406 was originally found to be a SYK inhibitor with an inhibition constant (Kᵢ) of 30 nM, although DiscoverX assays (described below) showed it to also inhibit FLT3, as well as IRAK1 and IRAK4. EGCg was identified as a polyphenolic compound found in green tea, with its earliest effects proposed to be protective against the carcinogenic effects of telodzin and oicadonic acid. It was later found to directly bind and inhibit PI3N with a Kᵢ of 20 µM per litre. Buparvaquone was derived from a series of anti- Thielierea (a cattle parasite) compounds, with its primary proposed mechanism as inhibiting the parasite cytosome b. It was recently found to also inhibit Thielierea amunale PIN1 in vitro (no reported IC₅₀ or IC₃₀ values).

In vitro kinase-binding assays (KINOMEScan). KINOMEScan assays (DiscoverX) were performed as follows: kinase-tagged T7 phage strains were prepared in an Escherichia coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage and incubated with shaking at 32 °C until lysis. The lysates were centrifuged and filtered to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with T7 signal for quantitative PCRs (qQPCR) detection. Streptavidin-conjugated magnetic beads were treated with biotinylated small-molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SealBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM dithiothreitol (DTT)) to remove unbound ligand and to reduce nonspecific binding. Binding reactions were performed by combining kinases, liganded affinity beads and test compounds in 1X binding buffer (20% SealBlock, 0.17X PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 111X stocks in 100% DMSO. Kᵢ values were determined using an 11-point threefold compound dilution series with three DMSO control points. A minimum of 10 independent experiments were performed for each enzyme and compound combination. The enzyme and compound combination with the smallest percentage of control was chosen as the compound concentration for the assay with an IC₅₀ of ~10 µM.

In vitro kinase assays. In vitro kinase assays were performed by Reaction Biology Corporation. Briefly, substrate (myelin basic protein (MBP), 20 µM final) was freshly prepared in reaction buffer (20 mM HEPES, pH 7.5, 10 mM MgCl₂, 1 mM EGTA, 0.02% Brij35, 0.02 mg ml⁻¹ BSA, 0.1 mM NaVO₃, 2 mM DTT, 1% DMSO). Human IRAK1 kinase (4 nM) was added to the substrate solution, followed by oxendazole by acoustical technology (Echo550; nanolitre range). Samples were incubated for 20 min at room temperature before addition of P-ATP (10 µM final concentration, with specific activity of 10 µCi µl⁻¹), then incubated for 2 h at room temperature. Radioactivity was detected using the filter-binding method. Kinase activity data are expressed as the percentage remaining kinase activity in test samples compared to vehicle (DMSO) reactions. Oxendazole was tested in 10-fold IC₅₀ mode in triplicate with threefold serial dilution starting at 200 µM, combining compound and substrate was performed in 10-fold serial dilution starting at 20 µM. IC₅₀ values and curve fits were obtained using Prism (GraphPad Software).

Molecular docking. Protein sequences of the IRAK1 and IRAK4 catalytic domains were aligned with T-Coffee’s structure-based alignment mode. Expresso 4. The IRAK1 kinase structure (2nru) 5 was used as the template for homology modelling of IRAK1. The homology modelling program MODELLER v9.15 6 was used to generate ten IRAK1 models with an average ZDOPE score of ~0.64 ± 0.06,
suggestions that at least 70% of the atoms are within 3.5 Å of the native IRAK1 structure. Molecular docking to the IRAK1 models was performed with Glide, using the Standard Precision mode and a hydrogen-bond constraint at the hinge region. The docking results from the models were combined to generate the consensus docking result. Oxfordazo and analogues are predicted to dock to the DFG motif. The method for zebrafish IRAK1 homology modelling was the same as described previously and used for induced-fit docking of oxfordazo. Specifically, the scaling of van der Waals radii for the receptor atom was changed to 0.75 for atoms with a charge larger than ±0.15 e−.

Microinjections into zebrafish embryos. Human IRAK1 WT, KD and K184R/K186R were subcloned into the pcDNA3.1 plasmid using restriction enzymes EcoRI and XhoI. The IRAK1 E3A-PC2-2 construct was generated using a Q5 Site-Directed Mutagenesis Kit (NEB, E2553) and the E3A variant. All plasmids were linearized by SacI single enzyme treatment at 37 °C for 4 h. Digests were stopped by adding 1/20 volume 0.5 M EDTA, 1/10 volume of 3 M sodium acetate and 2 volumes ethanol. Samples were mixed and chilled at −20 °C for 15 min, washed and resuspended in TE buffer. Sense-capped mRNAs were synthesized for injection using a mMESSAGE mMACHINE SP6 kit (Ambion, AM1340) following the manufacturer’s instructions. mRNA concentrations were determined using a Nanodrop spectrophotometer and by RNA gel electrophoresis. The following mRNAs were co-injected with irak1 mRNA: 500 pg of control mRNA, 25–30 pg of IRAK1 KD, 25–30 pg of K184R/K186R or 25–30 pg of E3A mutant mRNAs. The zp53-ATG mRNA and zp53-specific mRNA were as previously described and used for induced-fit docking of oxfordazo. Protein extractions from embryos were performed as previously described and analysed by western blotting as described below. Morphinol RNA oligonucleotides were obtained from Gene Tools. Sequences are as follows: Standard control (std): 5′-CCTCTAACCTCAGTTACATTATA-3′. pitc-e1: 5′-CAGCTGTCACATGGATGAG-3′. irak1-si6e4: 5′-AATCCCTGCAACAAACACCCAT-3′. irak1-e4: 5′-GTGACAGGATTAGACCTGGATGTG-3′. zp53-ATG: 5′-GGCCCTTTGTTTGAAGAGGG-3′. Mydd8: 5′-TTGCTGACGCTGAGAATCC-3′. Morphinol RNA oligonucleotides were suspended in sterile water to a stock concentration of 2 mM and delivered into one-cell stage zebrafish embryos by microinjection at various final concentrations with 0.1% phenol red (Sigma).

Sequence alignments and multiple sequence alignments were performed using ClustalW, and the sequences were deposited in GenBank. Sequences are as follows: 5′-ACCTAGGGTATGGAGTGTCAC-3′. Mydd8: 5′-TTGCTGACGCTGAGAATCC-3′. Mydd8R: 5′-GATTGTGAGCAGACGAGTACC-3′.

PCR with reverse transcription (RT–PCR) and protein extraction from zebrafish embryos. Embryonic RNA was isolated from 24–48 h.p.f. embryos (>15 embryos per sample) using a standard TRIzol method (250 μl TRizol (Invitrogen), 50 μl chloroform, 150 μl isopropanol). One microgram of purified RNA was used to generate complimentary DNA using an Invitrogen SuperScript First strand III RT–PCR kit, with oligo–dT primers. Two micrograms of the cDNA product was loaded on a 1% agarose gel. Pooled embryo protein lysates were used for 15 min, washed and resuspended in TE buffer. Sense-capped mRNAs were co-injected with irak1 mRNA. Morpholin RNA oligonucleotides were obtained from Gene Tools. Sequences are as follows: Standard control (std): 5′-CCTCTAACCTCAGTTACATTATA-3′. pitc-e1: 5′-CAGCTGTCACATGGATGAG-3′. irak1-si6e4: 5′-AATCCCTGCAACAAACACCCAT-3′. irak1-e4: 5′-GTGACAGGATTAGACCTGGATGTG-3′. zp53-ATG: 5′-GGCCCTTTGTTTGAAGAGGG-3′. Mydd8: 5′-TTGCTGACGCTGAGAATCC-3′. Morphinol RNA oligonucleotides were suspended in sterile water to a stock concentration of 2 mM and delivered into one-cell stage zebrafish embryos by microinjection at various final concentrations with 0.1% phenol red (Sigma). Sequencing primers for confirming knockdown were as follows: rpp-F: TACCGGATCCGGCAAGAACAC. rpp-R: CTGAAACTCTGCTGCCCTTCT. pinil-e1-F: GAAAGATACGACGGCCAAAG. pinil-e3-R: GTGGAAATGAGGCGTTCTTCAA. irak1-e1-F: GGTTATGATGATGCTGCTTCA. irak1-e11-R: CATGGACGATGCTTCCAG. Mydd8F: TCTGTCAGCGACTGGAATACGTG. Mydd8R: GATTGTGAGCAGACGAGTACC.

PCR with reverse transcription (RT–PCR) and protein extraction from zebrafish embryos. Embryonic RNA was isolated from 24–48 h.p.f. embryos (>15 embryos per sample) using a standard TRIzol method (250 μl TRizol (Invitrogen), 50 μl chloroform, 150 μl isopropanol). One microgram of purified RNA was used to generate complimentary DNA using an Invitrogen SuperScript First strand III RT–PCR kit, with oligo–dT primers. Two micrograms of the cDNA product was loaded on a 1% agarose gel. Pooled embryo protein lysates were collected as previously described 41.

Cell culture and cell lines. HeLa, HCT116, CAL27, SAS, BHY, and Detroit562 cell lines were cultured in DMEM medium (Gibco) supplemented with 10% FBS and 1% penicillin–streptomycin. YD38 cells were cultured in RPMI medium, with the addition of 10% FBS and 1% penicillin–streptomycin. YD38 cells were cultured in RPMI medium, with the addition of 10% FBS and 1% penicillin–streptomycin. For infection, the samples were centrifuged at 1,500 g for 45 min at 4 °C, supernatant aspirated, and the cell pellet resuspended in HeLa and CAL27 cell growth media. For infection, 2×105 HeLa and CAL27 cells were plated into 6-well plates. The next day, 5 μl polybrene (Millipore, tr-1003-g) and 200 μl of concentrated viral particles were added per well. The medium was replaced the next day with medium containing 1 μg/ml puromycin for selection.

The IRAK1 sgRNA oligonucleotides (5′ > 3′) were as follows: sgRNA1: 5′-CACCCGACACGGGTTATGACGTTAAG-3′. sgRNA2: 5′-AAACCTTCAACGATCACGGGTC-3′. sgRNA3: 5′-CACCCGAGGAGGTCTTTCTGTGGGATAAATC-3′. sgRNA4: 5′-AAACCTTCACAGCATACACCGTGTC-3′.

AlamarBlue cell survival assay. Cells were seeded into 96-well plates at densities ranging from 400 to 1,500 cells per well (see Supplementary Table 3 for seeding density and time). Eight hours after transfection, drugs were performed on cells in which shRNA was not previously transfected, drugs were added to the medium, incubated for 1 h, then irradiated. If shRNA lines were used, cells were treated with 1 μg/ml doxycycline for 24 h before irradiation. At 3–4 days post IR, cells were incubated with AlamarBlue (ThermoFisher) at a final concentration of 10%. Absorbance was measured at a wavelength of 570 nm with a 600 nm reference wavelength. Relative fluorescence was calculated using cell-free wells as a control reference, and percentage survival was calculated by comparing with DMSO-treated, non-IR controls.

References
Clonogenic assay. Single-cell suspensions were seeded into 6-well plates (30–200 cells per well). Drugs were added to the medium after 8 h and irradiated in a 10-cm2 irradiator. After being cultured for 14 days, plates were rinsed with PBS, incubated with fixing solution (75% methanol, 25% acetic acid) and stained with 0.5% crystal violet (Sigma-Aldrich) in methanol for 30 min at room temperature. Colonies consisting of at least 50 cells were scored. Clonogenic assays performed on mRNA-transfected lines required media to be refreshed with 1 μg/mL doxycycline every 48 h.

TUNEL assay (cell lines). TUNEL assays in HeLa cells were performed using an APO-BRDU kit (BD Biosciences) as described previously.6

Western blotting and antibodies. Whole-cell lysates were prepared in RIPA buffer or 1% NP-40 buffer (Boston BioProducts). Lysate (25–200 μg) was added to NuPAGE LDS Sample Buffer (4 ×), Invitrogen) and 5% mercaptoethanol (Sigma). Samples were boiled for 5 min. Samples were then run on a Tris-acetate gel in MES running buffer (Invitrogen). After electrophoresis, membranes were then blocked with 5% BSA in Tris-buffered saline with 0.1% Tween (TBST) and probed overnight at 4 °C with primary antibodies. Membranes were then washed in TBST and probed with anti-rabbit, -rat or -mouse (Cell Signaling Technology) horseradish peroxidase-linked antibodies at 1:2,000–4,000 dilution after washing. The membranes were then washed in 0.1% SDS, 5% BSA, 1% Tween 20 (Thermo Scientific) using a submerged transfer apparatus (Bio-Rad). Membranes were then transferred for 2 h (90 V, 150–200 mA) to a nitrocellulose membrane (Thermo Scientific, 4204) and probed with horseradish peroxidase-linked antibodies at a 1:2,000–4,000 dilution after washing. Membranes were then washed in TBST and probed with anti-rabbit, -rat or -mouse (Cell Signaling Technology) horseradish peroxidase-linked antibodies at 1:2,000–4,000 dilution after washing.

Quantification of synergistic drug interactions. The synergy experiments were performed in both cell culture AlamarBlue survival and zebrafish DTC assays as described above. Subtherapeutic doses were chosen based on dose–response curves with the highest possible dose that produced <10% decrease in cell viability or ≤20% DTGs after 4 h in cell culture and zebrafish assays, respectively. This established our MTD. We then chose a dose at a fivefold decrease from the MTD and used both doses for synergy experiments, with one IRAK1 inhibitor and one PIN1 inhibitor. The analysis of synergy was done using the isobologram and combination-index methods, derived from the Chou–Talalay median-effect principle using Compusyn software.7

Clinical samples. Pretreatment HNSCC tumours were examined. All patients were treated with complete surgical resection followed by post-operative RT at the MD Anderson Cancer Center. Tumour characteristics are shown in Supplementary Table 3. All studies involving human samples were approved by the MD Anderson Cancer Center Institutional Review Board in accordance with appropriate ethical regulations regarding research involving patient samples. Patient samples were collected as part of clinical protocol approved by the institutional review board of the participating institution.

For the outcomes analysis, patients were first placed into two groups (36 in each group) according to the expression level of IRAK1, IRAK4, TRAF6 or PIN1. Data were then compared with Student’s t test. Kaplan–Meier curves expressing LRR, time to distant metastasis, disease-free survival and overall survival were generated, and log rank statistics were used to determine significance between groups. R software, SPSS statistical software (v.20) and GraphPad Prism were used. For the multivariate analysis for the entire patient population with no clinical characteristic or gene examined excluding expression of TLR4 and LRR following RT. However, when LRR was examined in only those patients whose tumours harboured a TP53 mutation, nodal stage trended towards (P = 0.057) and PIN1 expression was significantly (P = 0.018) associated with LRR. Indeed, 64% of patients with a mutation in TP53 and high levels of PIN1 had a LRR at 5 years compared with ~25% in patients in all other groups (Fig. 6g, P = 0.006).

Xenograft studies. NSG mice (Jackson Laboratories) were used for in vivo studies and were cared for in accordance with guidelines approved by the Institutional Animal Care and Use Committee and Research Animal Resource Center. The study was compliant with all relevant ethical regulations regarding mouse research. Female mice (6–8 weeks old) were injected subcutaneously with 2 million SAS cells (6 × 10⁶ per mouse) and implanted with an Argon–helium laser. Once tumours reached an average volume of 100 mm³ (that is, 6 days post-implantation), mice were randomized into one of the four following treatment groups: (1) Control (saline); (2) localized radiation; (3) ginsenoside-Rb1 + EGCG; (4) radiation + ginsenoside-Rb1 + EGCG. The dosing schedule was as follows: ginsenoside-Rb1 (20 mg per kg) given daily for 3 weeks, EGCG (50 mg per kg) intra peritoneally daily, three times for 3 weeks, localized radiation (2.5 Gy) once on day 1. The drug + RT combination group, RT was delivered 30 min after dosing the mice with ginsenoside-Rb1 + EGCG. Mice were observed daily throughout the treatment period for signs of morbidity or mortality. Tumours were measured twice weekly using calipers, and the volume was calculated using the following formula: length × width² × 0.52. Body weight was also assessed twice weekly. After 3 weeks of treatment, tumour samples were collected for immunoblotting and immunohistochemistry analyses.

Staining of xenograft sections. For immunofluorescence staining, paraffin-embedded sections were deparaffinized, followed by rehydration and antigen retrieval as described below. Paraffin-embedded tissue sections were kept at 60 °C for 15 min, followed by slide hydration in xylene and a graded alcohol series. For antigen retrieval, heat-induced epitope retrieval was performed using a vegetable steamer by incubating slides in 10 mM citrate buffer for 40 min at 60 °C, followed by a wash in H2O and PBS. Post washes, cell membranes were permeabilized in 0.2% Triton X-100 in PBS for 5 min at room temperature for peptide antibody staining. Sections were incubated with 50 mL NHCl for 15 min at room temperature. Following blocking with 3% normal goat serum and 3% BSA in PBS for 60 min at room temperature. Binding of the primary antibodies against cleaved caspase-3 (Asp175) rabbit antibody (Cell Signaling Technology, 9661) and vimentin rabbit antibody (R&D systems, MAB2015) was carried out at 4 °C overnight, followed by PBS washes. Primary antibody-stained sections were incubated with 3% normal goat serum and 3% BSA in PBS for 30 min at room temperature. Detection by secondary antibodies, AlexaFluor488 goat anti-rabbit (Invitrogen, A11008) and AlexaFluor 568 goat anti-rat (Invitrogen, A11077), was carried out at room temperature for 1 h in the dark. Slides were mounted in Vectorshield (Vector Laboratories, 700) containing 1% DAPI (0.1 mg/mL) and 0.1% sodium azide for 30 min at room temperature. Staining of xenograft sections.
of each tumour section were imaged at ×60 magnification. For haematoxylin and eosin stainings, images were acquired using a Leica brightfield microscope at a magnification of ×40.

**Image analysis using Fiji software.** Images were analysed using the open-source processing software Fiji (ImageJ). Vimentin-positive cells were scored for positive cleaved caspase-3 staining. For scoring caspase-3-positive cells, images of samples incubated with secondary antibody, but no primary antibody, were used as background. The brightness and contrast of images was adjusted relative to primary antibody control stainings to identify caspase-3-positive cells in all treated conditions. For this analysis, 3 fields of each tumour section and a total of 12 images, comprising 4 tumour samples for each treated condition, were analysed. Total cell numbers per image were counted and the percentages of caspase-3-positive vimentin cells were calculated. Graphs were plotted using Excel, and P values were calculated using Student's t-test.

**Statistics and reproducibility.** With the exception of TCGA and MD Anderson Cancer Center patient cohort studies (see TCGA analysis and Clinical samples sections above), paired two-tailed Student's t-tests were used to determine P values (α = 0.05). The log rank test was used to determine P values for survival curves. Data in bar graphs are presented as the mean ± s.d. or s.e.m., as indicated in legends, and statistical significance is expressed as follows: *P < 0.05, **P < 0.005, ***P < 0.0005, NS, not significant. Embryos from WT or p53<sup>mk/mk</sup> zebrafish group matings were randomly allocated into experimental groups for irradiation, injections and/or drug treatments. Most experiments were carried out at least twice, and the findings of all key experiments were reliably reproduced. All replicates and precise P values are documented in Supplementary Table 4, which states the number of independent samples, embryos and independent experiments.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Code availability**

All codes used are referenced in the Methods sections above.

**Data availability**

The whole-genome expression array data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE67614. The human HNSCC and breast cancer data were derived from TCGA Research Network: http://cancergenome.nih.gov/. The dataset derived from this resource that supports the findings of this study is available in Supplementary Table 4 (see sheet S3). Source data for Figs. 1e, 2b,d, 3c,e,f, 4a–e,g,h,m,n, 5b,d, 6c–j, 7a–d,f,g and Supplementary Figs. 1b, 2a,b, 3, 4a–f,k,l, 5c–n and 7c,d are provided as Supplementary Table 4. The full image dataset that supports the findings in Fig. 3c,f has been deposited in Figshare at https://doi.org/10.6084/m9.figshare.7427942.

All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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|     | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated |
|     | Clearly defined error bars |
|     | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection: No custom code was used to collect data in this study.

Data analysis: All data were analyzed with Microsoft Excel 2016, GraphPad Prism 6, R v3.4.2, MODELLER v9.15, Glide, ImageJ, Fiji and CompuSyn. No custom code was used in our analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
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The data generated from the primary and secondary screen performed in this study are available from the corresponding author upon reasonable request. All
materials used were commercially available except for 511 unique kinase inhibitors belonging to the OncoNova library, provided by the E. Premkumar Reddy Lab. Sources for all other materials are described in the supplemental materials.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

The sample size for the zebrafish drug screen and mechanistic zebrafish experiments was determined with a power analysis based on preliminary experiments. Irradiated p53M214K/M214K embryos treated with DMSO served as the negative control group with a mean penetrance of curved tails of 5% with a standard deviation of 2.8%. Our positive control used irradiated embryos treated with Go6976, which produced curved tails with a mean penetrance of 86% and a standard deviation of 7.6%. This produced a calculated effect size D of 10.6, which was too high for standard power tables. However, in our preliminary experiments, we observed that our negative control embryos never exceeded 20% penetrance, and positive control group never fell below 75% penetrance, so these were used as the difference in mean, with a maximum standard deviation from those preliminary experiments of 28%, establishing a D of 1.6. For two-tailed t-tests with a significance level of 5% and a power of 90%, the number per group required a sample size of n=10. To account for attrition of embryos due to washing, pronase, or developmental loss as well as to account for pipetting discrepancies, our protocol established that at least 12 embryos be used for condition.

Data exclusions

Exclusion criteria for each zebrafish experiment were as follows: any embryos that were damaged or destroyed during manipulation by washing, pronase treatment, or live imaging were excluded from analysis. Our overall attrition rate due to these manipulations was less than 3% of all embryos used. If any experimental condition (i.e. individual well of zebrafish) yielded exclusion of embryos due to attrition such that the well contained <8 embryos for analysis, the entire condition was excluded due to lack of statistical significance. This condition was then repeated in a new independent experiment. If any 24- or 48-well plate yielded an average attrition rate greater than 3% for all wells in the plate, the experiment was considered compromised and the entire experiment was repeated. Each experiment also contained both negative and positive controls. Negative controls were either nonirradiated AB or p53M214K/M214K for any plate not receiving radiation. For irradiated plates, negative controls were p53M214K/M214K treated with DMSO, and positive controls were p53M214K/M214K embryos treated with Go6976 or oxefendazole in later experiments. Plates were excluded if negative controls yielded phenotypes of >25% AO positivity at 48 hpf or curved tails at 120 hpf OR if positive controls yielded phenotypes of <75% AO positivity or curved tails.

For AlamarBlue cell viability experiments, each plate of selected radioresistant cells treated with drugs and subjected to IR contained negative control wells of each cell line treated with DMSO. If IR alone caused a decreased in cell survival relative to non-irradiated controls, with the exception of IR-dose finding experiments or Daoy (described in "Replication" below), the experiment was considered invalid and was repeated.

Replication

All attempts at replication were successful. The primary screen of 640 FDA-approved drugs in zebrafish was not replicated due to its nature as an initial screen. However, if any condition met exclusion criteria, the individual condition was repeated. The secondary screen and all other zebrafish experiments were successfully replicated. The medulloblastoma cell line Daoy were selected based on literature suggesting it was radioresistant, which we could not replicate in AlamarBlue cell viabilities studies. Our data for Daoy were included regardless.

Randomization

For all zebrafish experiments, each independent experiment utilized embryos collected on different days from randomly selected mating pairs according to parental genotype (e.g. AB wild-type or p53M214K/M214K). Fertilized embryos were then pooled before randomly allocating into experimental conditions (e.g. IR vs no-IR and drug treatments).

Blinding

The zebrafish drug screen described in Fig. 1b and Supplemental Figs. 2-3 was blinded during data acquisition. The identity of the 640 FDA-approved drugs were blinded to experimenters during both primary and secondary screens. After the top hits were selected based on potency and selectivity, the drugs were unblinded for further study and target discovery.

Reporting for specific materials, systems and methods
Materials & experimental systems

| Involved in the study | n/a |
|-----------------------|-----|
| Unique biological materials | X |
| Antibodies | X |
| Eukaryotic cell lines | X |
| Palaeontology | X |
| Animals and other organisms | X |
| Human research participants | X |

Methods

| Involved in the study | n/a |
|-----------------------|-----|
| ChIP-seq | X |
| Flow cytometry | X |
| MRI-based neuroimaging | X |

Antibodies

| Antibodies used |
|------------------|
| anti-γH2AX (Ser139) (Cell Signaling Technology #9718); anti-Chk1 (clone G-4, sc-8408); anti-Chk1pSer345 (clone 13303, Cell Signaling Technology #2348); anti-PIDD (anti-LRRD, clone Anti1, Novus Biologicals NBP1-97595); anti-RAIDD (clone 4B12, MBL); anti-PIDDpT788 (custom-generated, ref.31); anti-caspase-2 (clone 1184, EMD Millipore MAB3507); anti-IRAK1 (Cell Signaling Technology #4504); anti-IRAK1pT209 (Assay Biotech, #A1074); anti-IRAK4 (Cell Signaling Technology #4363); anti-ERKpT202/Y204 (Cell Signaling Technology #4370); anti-ERK1/2 (Cell Signaling Technology #4696); anti-p38pT180/Y182 (Cell Signaling Technology #4511); anti-p38 (BD Biosciences #610168); anti-INKpT183/Y185 (Santa Cruz sc-6254); anti-IκBα (Cell Signaling Technology #4814); anti-TRA6 (Santa Cruz sc-8409); anti-MyD88 (Cell Signaling #4283); anti-PIN1 (Abcam ab53350); anti-actin (Abcam ab8227); anti-Chk2 (clone 7, Millipore); anti-p-Chk2 (T68) (#2661, Cell Signaling); anti-GAPDH (Cell Signaling #2118); anti-FLAG (DYKDDDDK Tag antibody, Cell Signaling Technology #2368); Anti-Cleaved Caspase-3 Rabbit pAb, StressGen AA-103 Anti-Vimentin rat antibody, R&D systems, catalog no. MAB2105 Anti-zp53, Genetex, GTX128135 |

Validation

Antibodies were validated by western blots of control vs. KO or knockdown cells, and/or by functional assays repeating previously reported experiments making use of the antibodies. Additional supporting documentation, references, and validation statements are available at the manufacturer’s website.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) |
|---------------------|
| HeLa: A. Thomas Look Lab, Dana-Farber CAL27: ATCC SAS: HSRBB BHY: D5M2 YD58: KCLB Detroit562: ATCC MDA-MB-231: Dr. Doris Germain Lab, ISMMS MCF7: Dr. Doris Germain Lab, ISMMS T47D: Dr. Doris Germain Lab, ISMMS SW480: ATCC DLD-1: ATCC HT-29: ATCC Daoy: ATCC T98G: ATCC IMR90: Dr. Julio Aguirre-Ghiso Lab, ISMMS MCF10: Dr. Julio Aguirre-Ghiso Lab, ISMMS HCT116 p53 WT: A. Thomas Look Lab, Dana-Farber HCT116 p53 null: A. Thomas Look Lab, Dana-Farber |

Authentication

The cells lines were not authenticated.
### Mycoplasma contamination

All the cell lines were tested for mycoplasma contamination using Lonza MycoAlert Assay (Cat# LT07-418) using the company's protocol. The cell lines that were mycoplasma-positive were treated with 1 ul of 25mg/ml solution of Plasmocin per 1 ml of medium in the plate. Media was changed every 3 days and plasmocin was added. This process was repeated for 14 days. After the completion of the treatment, the cells were again tested for mycoplasma contamination following the protocol.

### Commonly misidentified lines

Of the cell lines used in this study, only MCF7 cells were listed in ICLAC. Our rationale for their use relates to its utility as a commonly used radio-resistant breast cancer cell lines. We chose MBA-MB-231 and T47D as breast cancer cell lines demonstrated to be TP53 mutant as well as radio-resistant. MCF7 cells were also identified as radio-resistant but TP53 wild-type.

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### Animals and other organisms

#### Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

**Laboratory animals**
Details regarding animals used in this study can be found in Methods under "Zebrafish maintenance" and "Xenograft studies". MSG mice (Jackson Laboratories) were used for in vivo xenograft studies and were cared for in accordance with the guidelines approved by the MSKCC IUCAC. Zebrafish were cared for in accordance with the ISMMS IACUC.

**Wild animals**
This study did not involve wild animals.

**Field-collected samples**
This study did not involve field-collected samples.

### Human research participants

#### Policy information about studies involving human research participants

**Population characteristics**
Details regarding patient characteristics in this study, including T stage, N stage, tumor site (oral cavity, oropharynx, hypopharynx or larynx) and TP53 genotype (WT vs. mutant) can be found in Methods under "Clinical Samples", Supplementary Table 3 (Patient Characteristics) and Supplementary Table 4 (sheets 6g-j). All patients were treated with a complete surgical resection followed by post-operative RT at MDACC.

**Recruitment**
All studies involving human samples were approved by the MD Anderson Cancer Center Institutional Review Board in accordance with appropriate ethical regulations regarding research involving patient samples. Patient samples were collected as part of clinical protocols with appropriate consent.