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RTA 402 suppresses tumor and treatment induced inflammation, sensitizing tumors to and protecting normal tissue from radiation

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RTA 402 is a synthetic triterpenoid in a phase 1 study in patients with solids tumors and lymphoid malignancies at M.D. Anderson and the Dana-Farber. It has demonstrated potent anti-cancer and anti-inflammatory activity *in vitro* and *in vivo*. This compound has induced regression, complete responses, and significant growth suppression in *in vivo* preclinical models. In models of oral mucositis, it has repeatedly decreased the severity and duration of ulceration. To determine whether the drug could enhance the anti-cancer effects of radiation while protecting normal GI mucosal tissue from radiation-induced damage, we developed a model of radiation-induced proctitis in tumor-bearing rats. Athymic rats were injected with NCI-H460 NSCLC cells. Once tumors grew to ~100 mm^3, treatment was initiated with RTA 402, radiation, or the combination. Multiple regimens were tried. RTA 402 was dosed orally BID for the duration of the study in all cases, and radiation was administered in either 2 or 3 fractions of 5 or 4 Gy. Tumor size was measured throughout the study. At sacrifice, rectums were scored blinded for 5 distinct histological parameters of proctitis on a scale of 0 to 4. Tumor and gut specimens were examined for expression of TNFα, IL-1, and IL-6, and the degree of NF-κB active cells. Levels of circulating cytokines were also analyzed. The degree of tumor vascularity was assessed by CD31 and von Willebrand Factor (vWF) staining. RTA 402 caused dose-dependent growth suppression of tumors. Radiation also retarded the growth of the tumors; however, the combination of RTA 402 and radiation resulted in regression in both studies. Blinded scoring of the guts demonstrated that RTA 402 protected guts from radiation damage by reducing all 5 histological endpoints relative to radiation alone. Upon immunohistochemical examination, expression of TNFα, IL-1, IL-6, and VEGF was suppressed in all RTA 402 treated tumors. Circulating levels of many pro-inflammatory cytokines, including VEGF, IL-8, CRP, EMAP-II, MCP-1, and MIP-1α, were also decreased upon treatment with RTA 402 relative to vehicle and radiation treated animals. In the tumors, RTA 402 dose dependently decreased the expression of CD31 and vWF endothelial cells. In both tumors and guts, RTA 402 decreased levels of active NF-κB. In summary, RTA 402 suppresses tumor and treatment associated inflammation resulting in radiation enhancing anti-cancer activity and protection of radiation-induced damage to the GI mucosa.

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Increased radiation susceptibility by poly(ADP-ribose) polymerase inhibitors is restricted to the S phase of the cell cycle and involves loss of control of replication forks

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Aim: To analyze the mechanism of altered radiation recovery by poly(ADP-ribose) polymerase (PARP-1) inhibitors.
Materials and Methods: Clonogenic survival, single-strand and double-strand break repair, cell cycle progression and DNA synthesis were investigated in growing cells exposed to graded doses of gamma-rays without or with 4-amino-1,8-naphthalimide (ANI), a potent PARP inhibitor.
Results: ANI did not significantly enhance radiation susceptibility in human cell lines yet single-strand break rejoining was lengthened by ca. 10-fold in all but PARP-1-defective cells. Substantial radiosensitization was achieved only as the relative S phase content was large enough. Experiments using synchronized HeLa cells confirmed that ANI-induced radiosensitization is specific of the S phase of the cell cycle. The available data suggest that PARP-1 inhibition entails collision of unrepaired DNA single-strand breaks with slowly moving replication forks, ending in the formation of numerous DNA double-strand breaks, induction of futile recombination and immediate cell death putting a stop to repair. The molecular mechanism underlying these processes will be discussed.
Conclusions: The results show that ANI does not affect radiation response in the G1 and G2 phases of the cell cycle. The specificity observed for S phase targeting might be turned to advantage for the treatment of tumors with a high S phase content. On the other hand, it has been known from some time that PARP inhibitors are able to exert vasoactive effects in vivo. This effect reportedly provides increased cure of xenografted tumors by drugs or radiation. To discriminate between this issue and S phase targeting, it is necessary to compare the effect of potent PARP inhibitors on the radiation response of xenografts established from tumors of the same origin but presenting widely different mitotic index.
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Targeting the thioredoxin system in breast cancer treatment

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The thioredoxin system regulates the activity of many important proteins involved in proliferation, DNA synthesis, apoptosis and redox regulation.
To evaluate the suitability of targeting the thioredoxin system in breast cancer treatment, we immunohistochemically stained breast tumour tissue for Thioredoxin (Trx1), Thioredoxin Reductase (TrxR1), and Peroxiredoxins (Prx1–6). We examined, for Trx1 and TrxR1 expression, a cohort of 190 early stage breast cancer patients who were treated with radiotherapy after wide local excision. In those patients under age 40, elevated levels of TrxR1 correlate with increased risk of local recurrence ($p = 0.02$), but show no significance in those over 40. A smaller pilot study, examining 22 of the patients under age 40 for Prx1–6 expression, suggests that high levels of Prx1 may also be predictive of increased risk of local recurrence. A separate pilot study, examining the response of 25 locally advanced breast cancer patients to neoadjuvent anthracycline-based chemotherapy, suggests that patients are more likely to have a complete response to therapy if they are low expressers of TrxR1. Taken together, these results indicate that high expression of redox proteins in early or late stage breast cancer can be predictive of poorer response to radio-/chemotherapy; suggesting that the Trx system is an interesting target for inhibition, especially in combination with conventional R/T or C/T.
We then examined the *in vitro* effects of Trx inhibitors in breast cancer, selecting two novel cyclohexadienone compounds, PMX 464 and PMX 290 which inhibit Trx1 and the 2-imidazolyl disulfide, IV-2, which inhibits both Trx1 and TrxR1. The effect of these drugs, both as single agents and combined with ionising radiation, were evaluated on two breast cancer cell lines (MCF-7 and MDA-MB-231) and two normal cell lines (HUVEC and MRC-V fibroblasts) cultured under normoxic and hypoxic (1% O_2) conditions. Proliferation and clonogenic assays using breast cancer cells show that PMX 464 and PMX 290 are equipotent, with IC_{50} values of ~0.5 μM, while the IC_{50} of IV-2 is ~5−10 μM. For combinational radiation experiments, cells were pre-treated with sub-IC_{50} doses of drug and exposed to 2 Gy of radiation. Clonogenic survival indicates that while the radiosensitivity of the normal cells is unaffected, breast cancer cells are radiosensitised by PMX 464 ($p \leqslant 0.01$). Such increased radiosensitization is apparent even under hypoxia-induced radioresistance.

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Nutlin-3 radiosensitizes prostate cancer cell lines independent of p53 status

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Background: Nutlin-3 is a small molecule inhibitor which acts to inhibit MDM2 binding to p53, thereby stabilizing p53 and potentially altering DNA damage responses. The aim of this study was to investigate the potential radiosensitization properties of Nutlin-3 on prostate cancer cells and normal diploid fibroblasts.
Material and Methods: Two prostate cancer cell lines which differ in their p53 status were selected: 22RV1 (wild type p53; WTp53) and DU145 (one allele presenting with an inactivating mutation of p53; MTp53). GM5757 normal fibroblast (WTp53) cells acted as a normal cellular control. Using a variety of doses and treatment duration, we determined the effects of Nutlin-3 on p53-MDM2 expression and function using Western-blotting and quantification, cell cycle checkpoint control via flow cytometry and cell toxicity via clonogenic and apoptosis assays. For each cell line, the Sensitization Enhancement Ratio (SER) was calculated based on the Mean Inactivation Dose (MID) in the presence and absence of 2–24 hrs of drug after correcting for toxicity due to the drugalone.
Results: Nutlin-3 stabilized p53, p21WAF and MDM2 levels in WTp53 cells, but not in MTp53 cells. As a single agent, Nutlin-3 led to clonogenic cell death in GM5757 (IC50: 1.5 μM), 22RV1 (IC50: 3.7 μM) and DU145 (IC50: 16.0 μM) cells. When combined with radiation, Nutlin-3 radiosensitized all