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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

three cell lines – 22RV1 cells (SER: 1.2), DU145 cells (SER: 1.3) and GM5757 (SER: 2.0). Combined treatment with Nutlin-3 and Radiation (5 μM + 6 Gy) increased apoptotic fraction of 22RV1 from 1% to 21% as compared to radiation (12%) or Nutlin-3 alone (7%), apoptosis was not altered in DU145 and GM5757. In contrast, preliminary data suggests that the mechanisms of sensitization may include increased mitotic catastrophe. Current experiments are determining whether radiosensitzation is also observed under hypoxic conditions prior to tumour xenograft studies.
Conclusion: Nutlin-3 is a potent radiosensitizer of prostate cancer cells regardless of p53 status. This suggests that Nutlin-3 can act via p53- and p21WAF-independent mechanisms. The observed radiosensitization of a normal fibroblast strain suggests that confirmatory studies in vivo be conducted with both tumour and normal tissue endpoints.

Regulatory affairs

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Novel investigational agent clinical trials: cancer therapy evaluation program initiatives to enhance combination studies

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The Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment and Diagnosis, National Cancer Institute has made the evaluation of rational combinations of molecularly targeted agents a high priority. Because most such agents are being developed by the pharmaceutical/biotechnology industry, this often requires the agreement and cooperation of 2 or more pharmaceutical companies. Because CTEP sponsors over 125 active Investigational New Drug applications (INDs), it is in an unique position to facilitate the combination of investigational agents for multiple therapeutic target types. In order to facilitate preclinical and clinical studies involving the novel combinations of promising investigational anticancer agents originating from more than one pharmaceutical collaborator, CTEP has created standard intellectual property agreements. All collaborative clinical agreements between CTEP and pharmaceutical collaborators include provisions to facilitate mutually agreeable combination studies, both preclinical and clinical, sponsored by the NCI without the need to negotiate additional agreements between the collaborators, CTEP, or the investigative site. One of the key provisions that encourages such studies is a modification of the basic Intellectual Property Option to Collaborator (the Option) which provides all collaborators contributing an agent for a combination study with a non-exclusive royalty free license to any invention that might arise using the combination. Furthermore, the Option also applies to preclinical studies designed to provide data in support of a clinical trial. In addition, CTEP includes provisions for data sharing with all collaborators in all collaborative agreements. Thus, the need for collaborators to negotiate cumbersome and time-consuming intellectual property or data sharing agreements with each other or the site conducting the study prior to approving such studies has been eliminated. Such arrangements have led to the initiation of over 100 investigational agent combination protocols. Current data on the volume and frequency of these studies is illustrated, as well as the mechanisms and terms by which these agreements are modeled and implemented.

Signal transduction modulators

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Pulmonary changes in a randomized phase II study of the mTOR inhibitor RAD001C (Everolimus): NCIC CTG IND.163

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Background: mTOR inhibitors may be associated with pulmonary toxicity, particularly pneumonitis (PN). PN is poorly described in the literature. While many patients (pts) are asymptomatic with nonspecific findings noted incidentally on thoracic imaging, others may present with symptoms that include dry cough and/or dyspnea. Severe cases can result in hypoxia necessitating drug discontinuation, corticosteroid therapy and hospitalization. In our ongoing randomized study of an mTOR inhibitor, 3 cases of possible PN were noted, prompting a safety review.
Methods: A review of medical records and radiological reports was conducted on 34 pts enrolled on NCIC CTG IND.163, a single-agent phase II study evaluating two schedules of administration (Arm A: 10 mg p.o. daily; Arm B: 70 mg p.o. weekly) of RAD001C for the treatment of metastatic breast cancer.
Results: From January to November 2005, 34 women with metastatic breast cancer were treated with RAD001C (Arm A: n = 18; Arm B: n = 16) in part 1 of the study. Fifteen pts (44%) developed clinical and/or radiological changes suggestive of possible PN (Arm A: 12, 67%; Arm B: 3, 19%). Of these 15 pts, 11 (73%) received at least one previous anthracycline-containing chemotherapy regimen and 12 (80%) received radiation to the ipsilateral breast or chest wall; 4 pts (27%) were asymptomatic, whereas 11 (73%) had either dry cough and/or dyspnea, usually grade 1 or 2 (G_1 or G_2). Radiological changes consisted of patchy airspace consolidation, ground-glass opacities or diffuse interstitial disease. Of the 15 pts with possible PN, 4 discontinued study drug due to PN (G_2=3, G_3=1), 1 due to G_3 left ventricular dysfunction, 7 due to disease progression (worst PN: G_1=4, G_2=3) while 3 pts continue on treatment (all G_2 PN) without worsening PN. Most pts with G_1 and G_2 PN were managed expectantly whereas 3 women (G_2=2, G_3=1) were treated with corticosteroids. Two pts required hospitalization. Symptoms and radiological changes cleared after drug discontinuation. No pts have been rechallenged.
Conclusions: In this trial, RAD001C appeared to be associated with schedule dependent pulmonary changes consistent, in some cases, with drug-induced PN. The clinical severity of presentation was variable while the findings were, in general, reversible. Protocol modifications for part 2 of the study have been implemented. The potential for pulmonary toxicity should be considered when designing future trials with this class of agent.

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A randomized phase II study of two different schedules of RAD001C in patients with recurrent/metastatic breast cancer

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Background: RAD001C is a macrolide antibiotic that exerts antiproliferative effects by mTOR (mammalian target of rapamycin) inhibition, and antiangiogenic effects by inhibition of endothelial cell proliferation. mTOR is critical in the transduction of proliferative signals mediated via the PI3K/Akt pathway. This signal transduction pathway is relevant to HER2 and ER signaling, and in PTEN mutant tumours, so mTOR may be a central and relevant factor in breast cancer.
Methods: Multi-centre randomized phase II study assessing two oral schedules of RAD001C: Arm A (A): 10 mg daily, or Arm B (B): 70 mg weekly, assessed clinically each 4 weeks, imaged each 8 weeks. Eligibility: Patients (pts) with measurable metastatic breast cancer (MBC) who may have received adjuvant chemotherapy (chemo), with up to one prior chemo for advanced/recurrent disease. Stratification factors: 0 or 1 prior chemo for MBC; presence/absence of visceral metastases. Primary endpoint: clinical/radiologic response and early progression (<8 wks). Two-stage accrual design with 15 evaluable pts in each arm in first phase. If $\geqslant$1 response and <10 early progressors, continue arm and enter 15 more pts. If 1 arm stopped after first phase, continue non-randomized accrual to remaining arm.
Results: First phase of accrual complete with A: 18 and B: 16. Number of prior chemo regimens (0/1/2): A: 3/11/4; B: 3/7/6. Visceral/bone/nodal metastases: A: 11/9/14, B: 17/6/9. Median number of cycles: A: 3 (1–9), B: 2 (2–9). 3 partial responses and 7 early progressors were seen in A and 0 responses and 11 early progressors in B. Median SD duration for A: 5.7 months (range 3–7.1) and B: 5.1 months (range 3.4–7.3). Discontinued therapy: A: 15 off, 9 for PD, 4 for AE (grade 3 pneumonitis (PN), grade 2 PN $\times$ 2 pts, grade 3 left ventricular systolic dysfunction), 2 symptomatic progression. B: 16 off, 13 for PD, 2 for AE (grade 2 PN, grade 4 fatigue), 1 symptomatic progression. Toxicities (nausea, stomatitis, diarrhea, rash, cough, neutropenia) were generally mild, similar between arms, and as expected, except for higher than expected incidence of clinical and/or radiologic PN (A: 67% and B: 19%).
Conclusions: Early findings indicate RAD001C has activity in MBC. Criteria for expanding Arm A were met. PN was more frequent in arm A, the daily schedule. Accrual to Arm A will continue with close monitoring and consideration of early intervention for those patients who develop radiologic changes of PN.