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National Cancer Institute (NCI) State of the Science: Targeted Radiosensitizers in Colorectal Cancer

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Abstract

Background—Colorectal cancer (CRC) represents a major public health problem as the second leading cause of cancer-related mortality in the United States. Of an estimated 140,000 newly diagnosed CRC cases in 2018, roughly one-third of these patients will have a primary tumor located in the distal large bowel or rectum. The current standard of care approach includes curative-intent surgery, often following preoperative (neoadjuvant) radiotherapy (RT) to increase rates of tumor downstaging, clinical and pathologic response, as well as improving surgical resection quality. However, despite advancements in surgical techniques, as well as sharpened precision of dosimetry offered by contemporary RT delivery platforms, the oncology community continues to face challenges related to disease relapse.

Methods—Ongoing investigations are aimed at testing novel radiosensitizing agents and treatments that might exploit the systemic antitumor effects of RT utilizing immunotherapies. If successful, these treatments may usher in a new curative paradigm for rectal cancers such that surgical interventions may be avoided. Importantly, this disease offers an opportunity to correlate

matched paired biopsies, radiographic response and molecular mechanisms of treatment sensitivity and resistance with clinical outcomes.

Results—Herein, the authors highlight the available evidence from preclinical models and early-phase studies, with an emphasis on promising developmental therapeutics undergoing prospective validation in larger-scale clinical trials.

Conclusions—This review by the NCI’s Radiation Research Program Colorectal Cancer Working Group provides an updated comprehensive examination of the continuously evolving State of the Science regarding radiosensitizer drug development in the curative treatment of CRC.

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This review by the NCI’s Radiation Research Program Colorectal Cancer Working Group provides an updated comprehensive examination of the continuously evolving State of the Science regarding radiosensitizer drug development in the curative treatment of CRC. Herein, the authors highlight the available evidence from preclinical models and early-phase studies, with an emphasis on promising developmental therapeutics undergoing prospective validation in larger-scale clinical trials.

Keywords

precision radiation medicine; radiation therapy; radiosensitization; chemoradiotherapy; radiation biology; rectal cancer; immunotherapy; targeted therapeutics; abscopal effect; colorectal cancer

Background

Colorectal cancer (CRC) represents the second leading cause of cancer-associated deaths in the United States, with an estimated 135,430 new cases and 50,260 cancer-related deaths in 2018.^[1] Of these cases, nearly one-third represent tumors arising in the distal portion of the large bowel, the rectum, where surgical removal may require a permanent colostomy. In many patients, pre-operative treatment with chemoradiotherapy (chemoRT) is a mainstay of therapy that supports increased tumor downstaging, fewer colostomies and reduced local recurrence. Previous attempts to intensify therapy through radiosensitization with resultant improvement in tumor sterilization have failed to improve outcomes in comparison to concurrent fluoropyrimidine use.

Strategic development of novel radiosensitizers represents a clinical unmet need and has been a focus of the National Cancer Institute’s (NCI) Radiation Research Program.^[2] The NCI’s Radiation Research Program has organized disease-specific Working Groups comprised of experts from across academics, industry, government, cancer disciplines, clinical care and basic cancer biology. The Colorectal Cancer Working Group has systematically catalogued and prioritized agents and interventions that may help improve outcomes for patients with rectal cancer. These efforts provide guidance to investigators involved in pre-clinical testing and have minimized duplication of effort in clinical trial design and development.

This manuscript provides a summary update of the State of the Science related to radiosensitizer development in clinical trials for CRC. Importantly, this field has expanded to include both the traditional sensitizer of radiation for improved local response, as well as agents that can be systemically catalyzed by radiation. This latter group includes immunotherapies, vaccines and immune checkpoint inhibitors that have the potential to revolutionize the management of many diseases. Given the rapidly changing landscape of discovery and development, this manuscript provides a contemporary vantage point of the field and relevant clinical studies that form the basis for ongoing and future clinical trials.

Principles of Radiosensitization

Refinements in surgical technique with the adoption of the total mesorectal excision (TME), incorporation of modern chemotherapy, and advances in timing and dosimetry of radiotherapy (RT), have demonstrated a meaningful impact on local tumor control, however, distant relapse remains the leading cause of mortality in this patient population, with approximately 35% developing metastatic relapse within 5 years of trimodality treatment.^[3]

Following neoadjuvant chemoRT, pathological complete response (pCR), defined as no histopathologic evidence of residual cancer cells, has been extensively studied as a standard measurement tool of tumor regression. In the current era of consistently low local tumor recurrence rates, the goal of increasing the pCR rate is driven from where we aspire to see the field move towards. First, the ability to achieve a pCR serves as an easily defined and pragmatic metric of anticancer activity. Inherent to achieving a pCR, we infer that the tumor and/or the treatment provided limited opportunities for chemo- and radioresistance mechanisms to develop. As such, a higher pCR rate is a useful short-term signal of anti-cancer activity involving novel treatment combinations and sequencing approaches in the neoadjuvant setting. However, it remains a poor surrogate for long term outcomes including survival, which is why more continuous variables of tumor downstaging such as the neoadjuvant rectal (NAR) score, more accurately predict long-term outcomes.^[4]

Second, a growing area of investigation and clinical practice involves non-operative management (NOM). In these situations, patients who achieve a clinical complete response (cCR) after neoadjuvant therapy, as determined by physical exam, radiological and endoscopic evaluation, might be given the opportunity for a delayed or deferred operation. Only the subset of cCR patients who actually have a pCR can be legitimately spared an operation. Thus, the use of pCR as an endpoint in the testing of neoadjuvant strategies may help establish which patient population or treatment approach could more legitimately support a non-operative approach as part of future trials and clinical care.

The goal of combining systemic agents with radiation is multifold. In addition to sensitizing the effects of radiation, the ideal drug combination would result in maximizing tumor cell kill while simultaneously minimizing the effects on surrounding normal tissues. This concept of achieving a high therapeutic index is critical in the design of novel drug-radiation combinations.

Improving therapeutic ratio can be accomplished using three general principles: (1) spatial cooperation (2) synergistic tumor cell kill (3) normal tissue protection.^[5-7] Spatial cooperation involves using radiation to control local disease and chemotherapy to control distant micrometastatic disease. Synergistic tumor cell kill involves enhancing the tumoricidal effects of radiation (radiosensitizers) through additive or synergistic inclusion with another agent (e.g., concurrent continuous infusion 5-fluorouracil [5-FU] or capecitabine). Normal tissue protection can be accomplished by modulating the cellular response to RT and mitigating radiation injury to normal tissues, thereby allowing a higher cumulative RT dose to be delivered. The FDA approved radioprotector, amifostine, is an organic thiophosphate compound that acts as a free radical scavenger.^[8] Although phase II trials in rectal cancer suggest that each of these approaches are feasible, only synergistic tumor cell killing with concurrent chemoRT is routinely practiced.^[9-11]

Major classes of radiosensitizers currently used in clinical practice include cell cycle modulators, signal transduction inhibitors and DNA damaging agents. 5-FU was one of the first drugs to be combined with RT and remains the backbone of chemoRT therapy in rectal cancer. Large randomized trials have established that it is superior to either chemotherapy or RT alone.^[12, 13] The synergistic effect of fluoropyrimidines with radiation is due to its ability to redistribute cells into S-phase, as well as deplete nucleotide pools, which decrease the capacity for DNA repair. The radiosensitizing effects of fluoropyrimidines depend on continuous exposure of tumor cells to the drug. Continuous intravenous infusion of 5-FU is non-inferior to capecitabine, an orally bioavailable fluoropyridine, with either being used with RT in the neoadjuvant treatment of rectal cancer.^[14] Capecitabine is extensively absorbed from the intestine to undergo a three-step enzymatic conversion to 5-FU and avoids the need for an indwelling venous catheter.

There is a diversity of new agents in development that are significantly different than historical radiosensitizers (Figure 1). Some include those that focus on hypoxia modification or change the intrinsic radiosensitivity of the tumor. Another new paradigm involves immunotherapy whereby RT is being used to potentiate the effects of the drug.^[15] Because ionizing radiation can cause immunogenic cell death, modulate antigen presentation by cancer cells; and alter the microenvironment within the radiated field, it has been demonstrated that local radiation can enhance responses to immunomodulating agents at sites distant from the area being treated (i.e., the “abscopal effect”).^[16-18] These are exciting areas of drug development, a number of which are detailed below.

Rationale for Radiation-Targeted Therapy Combinations

MEK inhibition

The Cancer Genome Atlas and other studies have demonstrated the importance of the canonical RAS-RAF-MAPK pathway in CRC with frequencies of KRAS, NRAS, and BRAF gain of function mutations being ~40%, 10%, and 5%, respectively.^[19, 20] RAS isoforms (e.g. K-, N-, and H-RAS) encode small (~21 kD), membrane-bound GTP-binding proteins that are involved in promoting proliferation, migration, invasion, de-differentiation, and inhibition of apoptosis.^[21, 22] Preclinical studies demonstrate that hyper-activation or overexpression of RAS can lead to development of intrinsic radioresistance, defined through

clonogenic cell survival assays. Conversely, KRAS downregulation by siRNA or chemical inhibitors of farnesylation, radiosensitize tumor cells.^[23–25] While RT activates RAS-MAPK signaling in KRAS mutant cells, inhibition of MAPK signaling by MEK inhibition can attenuate survival after RT.^[26–28] Emerging clinical evidence also supports the finding of relative RT resistance with tumors harboring KRAS mutations.^[20, 29–31] [32] Trametinib, a potent and selective MEK1/2 inhibitor that is FDA-approved for metastatic or unresectable BRAF^{V600E/K} mutant melanoma, has been shown to have anti-proliferative activity in human KRAS or BRAF mutant CRC cell lines with reductions in ERK activation.^[33] Multiple groups have shown that MEK1/2 inhibitors in RAS mutant carcinoma preclinical models result in effective radiosensitization (with or without additive effects of 5FU), resulting in tumor cell death and tumor growth delay through increased apoptosis, DNA damage induced cell death, reduced DNA repair, and reduction in hypoxic response.^[26, 34–37] [33, 38] However, MEK inhibition benefits do not appear to be dictated exclusively by genomic subtype (e.g. specifically KRAS or BRAF mutant).^[39]

As such, phase I clinical studies have been developed. In the first, a clinical trial testing AZD6244 in rectal cancer with chemoRT was terminated early due to unspecified dose-limiting toxicities (NCT01160926).^[40] However, a second study using trametinib was designed in conjunction with standard chemoRT with 5-FU for patients with locally advanced rectal cancer (NCT01740648). There was a 5-day lead-in of single-agent trametinib followed by the combination of trametinib and infusional 5-FU (225 mg/m²/day, Monday to Friday) with RT (50.4Gy) using 3 dose cohorts of trametinib: 0.5 mg, 1 mg, and 2 mg (oral, daily Monday to Friday). A total of 18 evaluable patients demonstrated no grade 4 toxicities, with only 1 patient having a dose-limiting toxicity of diarrhea, which was attributed to chemoRT. Twelve patients received the maximum tolerated dose (MTD) of 2 mg. Analysis of the whole patient cohort showed tumor downstaging of 72%, with pathological complete response (pCR) seen in 17% patients. In the cohort of patients treated at the MTD (2 mg), 75% had tumor down-staging and 25% achieved pCR suggesting at least additive benefit and good tolerance.^[41] Correlative analysis was performed on serial tumor biopsies obtained at pre-treatment, after the 5-day trametinib lead-in, and the surgical specimen. Preliminary data shows a trend for dose-dependent decrease in the level of phosphorylated-ERK protein level in tumor cells, as detected by immunohistochemistry, confirming pharmacodynamic inhibition of the RAS-ERK pathway. Future studies in larger patient cohorts are proposed to determine if this activity is clinically meaningful.

Protein Kinase C

Midostaurin is a multi-target kinase inhibitor and a staurosporine analog. It was initially developed as a protein kinase C inhibitor in solid tumors and more recently obtained FDA-approval in fms-related tyrosine kinase 3 (FLT3) mutated acute myeloid leukemia.^[42] In a study by Liu and colleagues,^[43] 32 cancer cell lines were screened for radiosensitization effects using 18 targeted therapeutic agents. Short-term radiosensitization factors (SRF2Gy) and calculated clonogenic survival assay-based dose enhancement factors (DEFSF0.1) were derived. Midostaurin was found to increase radiosensitization in KRAS mutant cell lines. Interestingly, greater sensitization was seen in codon 12/13 mutations, rather than codon 61. The radiosensitization effects were also more pronounced in cells with high expression of

the stem cell marker CD133, typically deemed a more radioresistant phenotype. The efficacy appeared to be mediated through PKC α , as similar in vitro results were seen with a pure PKC inhibitor.

Based on this data, a phase I study was initiated whereby patients with MRI or ultrasound defined T3 or T4 or node+ adenocarcinoma of the rectum without evidence of metastatic disease were treated with neoadjuvant chemoRT (50.4 Gy in 28 fractions combined with infusional 5-FU [225 mg/m²/day]) continuously with midostaurin twice daily (BID) on days of RT (NCT01282502). The midostaurin dose was escalated in a 3+3 design: dose level 1 was 50 mg BID, dose level 2 was 75 mg BID followed by a dose expansion cohort. Surgery was performed 6–9 weeks following the completion of chemoRT. Genotyping was performed using whole exome sequencing, with either blood or adjacent normal tissue normal control.

This phase Ib study enrolled 19 patients with clinical stage II and III rectal cancer. All patients completed therapy and underwent surgery achieving an R0 resection with a pCR rate of 16% (3/19). Maximum tolerated dose (MTD) was 75 mg BID. At least one grade 3–4 toxicity occurred in 9/19 (47%) of patients, with the most common grade 3 events observed being lymphopenia (26%, 5/19) and rash (16%, 3/19). With a median follow up of 3 years, two patients had recurrence- both had synchronous local and distant. DFS at 3 years was 89.5% (CI 75.7–100%), and 3 year OS was 87.7% (CI 71.6–100%). Sixteen tumors underwent mutational sequencing with the genotype of the pCR cases being RAS (2/3) mutant or unknown. Overall, the combination of midostaurin with chemoRT was well tolerated, and final genotyping results and correlative analysis of RAS status and pCR are eagerly awaited.^[44]

Heat Shock Protein 90

Heat Shock Protein 90 (HSP90) is a chaperone involved in folding, stabilization and intracellular trafficking of client proteins.^[45] HSP90 client proteins include key proteins involved in cellular growth, apoptosis, immune function, epithelial to mesenchymal transition (EMT), and angiogenesis. HSP90 is overexpressed in several malignancies; hence the interest in HSP90 in cancer biology.^[45] It has been demonstrated that HSP90 protein is upregulated in CRC tissue as compared to matched normal colon tissue.^[46] HIF-1 α , STAT-3, and NF- κ B are transcriptional factors that influence radioresistance and are also chaperone proteins of HSP90. Agents in this class (i.e., ganetespib) are small molecule inhibitors of HSP90.^[47] The addition of ganetespib to 5-FU and RT significantly inhibited growth of CRC cell lines, decreased the colony formation and inhibited in vivo tumor growth as compared to untreated controls as well as those treated with 5-FU plus radiation.^[48] Ganetespib inhibited EMT, invasion and angiogenesis through blocked activation of transcriptional factors HIF-1 α , STAT-3, and NF- κ B.^[46, 49, 50]

With this pre-clinical data, a phase I study of ganetespib in combination with chemoRT was conducted in patients with stage II-III rectal cancer.^[51] Ganetespib run-in was started at day –14, given twice weekly for 2 weeks and accompanied by a pre- and post run-in biopsies. Patients then completed standard chemoRT (50.4 Gy in 28 fractions over 5.5 to 6 weeks administered with concurrent oral capecitabine at 825 mg/m² PO BID) with ganetespib

followed by surgery. Four dose levels of ganetespib were studied from 60 mg/m² to 120 mg/m². The recommended phase II dose was established at 100 mg/m² once weekly given on days 1, 8, 15, 29, and 36 concurrently with chemoRT. Fifteen patients were enrolled on the trial with baseline endoscopic staging of T3N0 (5), T3N1 (5), and T3N2 (5). Fourteen patients underwent successful surgical resection (3 APR and 11 LAR). The only Grade 3 toxicity seen was diarrhea, and Grade 2 toxicities were fatigue, nausea/vomiting, radiation dermatitis, elevated AST/ALT, and hand/foot syndrome. Pharmacokinetics did not reveal any drug-drug interaction between ganetespib and capecitabine. The pCR was 21% (3/14 patients), and 2 patients had <1cm tumor at surgery, with an overall Neoadjuvant Rectal (NAR) Score of 7.9. By comparison, the NAR score for capecitabine and radiation is approximately 15, with lower scores indicating more treatment activity.^[52] Correlative studies analyzing post-treatment tumor tissue showed down-regulation of mRNA of HIF-1 α , STAT-3, and VEGF.^[46] Despite these encouraging early results, ganetespib is no longer being developed; however, there are several other HSP90 inhibitors in early stages of clinical development. Identification of another HSP90 inhibitor for incorporation into the setting of rectal cancer is a rational future pursuit.

Epidermal and Vascular Endothelial Growth Factor Pathways

Simultaneous targeting of the vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) pathways could potentially enhance the effects of RT. In a study on human head and neck cancer xenografts, the combination of the VEGF inhibitor, AZD2171, the EGFR tyrosine kinase inhibitor (TKI), gefitinib, with RT led to much greater inhibition of tumor growth compared to RT alone, or compared to the two-drug combination.^[53] This study provided a sound rationale for dual inhibition of the VEGF and EGFR pathways in combination with RT, particularly in CRC where both pathways are effective targets in advanced disease.

Investigators at the University of Texas MD Anderson Cancer Center conducted a phase I trial in patients with clinical stage II-III rectal adenocarcinoma, evaluating preoperative chemoRT (50.4 Gy in 28 fractions with concurrent capecitabine), the anti-VEGF-A antibody, bevacizumab, and the EGFR TKI, erlotinib.^[54] Patients were administered bevacizumab (5 mg/kg every two weeks) with escalating dose levels of capecitabine (650–825 mg/m² BID) and erlotinib (50–100 mg daily). The combination was well-tolerated with only 1 Grade 3 acute toxicity (hypertension), and 3 Grade 3 post-operative complications. Among 18 evaluable patients, the pCR rate was 44% with an additional 44% of patients with 10% viable tumor in the surgical specimen.

In a similar phase I/II trial at the Massachusetts General Hospital, 32 patients with stage II-III (T3–4 and/or node-positive) rectal cancer were treated with chemoRT (50.4 Gy in 28 fractions combined with infusional 5-FU [225 mg/m²/day]), bevacizumab (5 mg/kg every two weeks), and escalating doses of erlotinib (50–100 mg daily).^[55] The rate of Grade 3–4 toxicity was higher in this trial (47%), however, the pCR rate was similarly robust at 33%.

Taken together, the consistent results of high pCR rates from these two independent clinical trials makes the data on EGFR and VEGF dual inhibition particularly interesting. However, proposals to develop these agents further were met with little enthusiasm given that a major

limitation to these early clinical trials related to the lack of biomarker or correlative studies. While promising, future studies are needed to evaluate the clinical and biologic predictors of response to dual VEGF/EGFR targeting in combination with chemoRT.

Poly(ADP-ribose) polymerase (PARP)

DNA damage is continuously repaired by an integrated network of repair mechanisms. Double-stranded DNA breaks, which typically occur with radiation, recruit homologous recombination and non-homologous end-joining pathways for repair.^[56] Single-strand breaks utilize another pathway including nucleotide-excision repair, base-excision repair and mismatch repair. Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme that rapidly recognizes and binds to single strand breaks and facilitates single strand DNA repair. Since there are at least two sets of mechanisms leading to DNA repair, the disruption of one pathway may not lead to cell death; however, when both pathways are disrupted the accumulated genomic instability leads to loss of viability. This concept, known as synthetic lethality, is supported by clinical studies in BRCA deficient cancers and triple negative breast cancer.^[57, 58] PARP-1 inhibitors have been shown to enhance the efficacy of RT by interfering with compensatory DNA repair.^[59, 60] The PARP inhibitor, veliparib (ABT-888) in combination with RT in HCT-116 CRC xenograft model, showed a near doubling in median survival compared to RT alone while ABT-888 alone was no better than vehicle.^[61] A similar synergistic effect was demonstrated when veliparib was combined with 5-FU and RT compared to 5FU and RT alone in preclinical CRC xenografts.^[62]

A phase I study testing the safety and tolerability of veliparib with capecitabine and RT in locally advanced rectal cancer (NCT01589419) enrolled patients with stage II-III rectal cancer. They received standard neoadjuvant chemoRT with capecitabine (825 mg/m² BID) on days of RT (50.4 Gy in 28 fractions) with veliparib added at varying dose cohorts. Surgery was performed 5–10 weeks after completion of therapy. Of the 32 patients who received treatment per study protocol, 30 were included in the final analysis. Seventy-three percent of patients had histologic downstaging at the time of surgery, including a pCR rate of 28%. The NAR score for patients treated in the full dose cohort was 12.8. There were two documented DLTs (diarrhea and skin toxicity), neither of which compromised therapy. Twenty-eight percent of patients experienced any Grade 3 or 4 AE with diarrhea (9%) being the most common. There were no surgical complications attributed to the treatment. There was no clinical benefit to dose escalation beyond the recommended for phase II dose level (400mg PO BID) and pharmacokinetic data confirmed no interference with fluoropyrimidine therapy.^[63] Importantly, there were no data provided on differential responses or toxicities based upon patient homologous recombination deficiency (HRD). Based on the promising phase I study results, veliparib as a radiosensitizer is the first experimental arm being explored in the NRG-GI002 randomized phase II trial platform in neoadjuvant treatment of locally advanced rectal cancer (NCT02921256) (Figure 2 and section below). Of note, more potent PARP inhibitors have recently become clinically available and may offer additional opportunities to refine this hypothesis in rectal cancer.

Ropidoxuridine (IPdR)

5-iodo-2-pyrimidinone-2'-deoxyribose (Ropidoxuridine; IPdR) is a halogenated pyrimidine nucleoside that is a prodrug of the halogenated pyrimidine analog, iododeoxyuridine (IUdR). IPdR is administered orally with high bioavailability, and is metabolized to the active drug, IUdR, by an aldehyde oxidase principally in the liver. This metabolic profile is crucial to the improved therapeutic index of oral IPdR compared to continuous infusions of IUdR. The biochemical and cellular interactions of RT and fluoropyrimidines are felt to result from inhibition of the enzyme thymidylate synthetase (TS) by the fluoropyrimidine monophosphate metabolite, FdUMP, leading to decreased (or unbalanced) nucleotide pools needed for DNA synthesis and decreased DNA repair following RT damage. The intracellular IPdR monophosphate metabolite, IdUMP, can inhibit (by binding) TS, leading to increased fluoropyrimidine-mediated radiosensitization as well as enhancing IPdR-mediated radiosensitization secondary to IUdR-DNA incorporation. Thus, oral IPdR added to chemoRT is postulated to increase the pCR rate and overcome radioresistance.

After a first-in-human Phase 0 trial of a single dose of IPdR in patients with advanced malignancies confirmed the presence of adequate plasma levels of the active drug, IUdR, from the oral prodrug, IPdR, were achieved, the Phase I treatment trial was developed.^[64] This dose finding study includes patients with GI cancers (mostly CRC) being treated with palliative RT. Patients receive escalating doses of once daily IPdR for 28 days with RT to 37.5 Gy (2.5 Gy daily fractions) starting on Day 8 of IPdR administration. Thus far, 16 patients have been treated without dose-limiting systemic toxicity.

Interestingly, in prior clinical trials of continuous infusion IUdR, steady-state IUdR plasma levels of $>1\mu\text{M}$ were associated with myelosuppression and GI toxicities. In the current Phase I study (NCT02381561), patients who received 28 days of IPdR (1200 mg daily) had plasma levels of IUdR in the range of 2–5 μM . While pharmacodynamic assessments are ongoing, the lack of toxicity seen with IPdR doses that achieve IUdR plasma levels of $>1\mu\text{M}$ supports the observed improved therapeutic index seen in pre-clinical studies. Final results from this study are anxiously awaited to determine the clinical activity of this new biochemical intervention.

Rationale for Radiation-Immunotherapy Combination

Increasing evidence has been mounting to suggest that the immune system plays a major role in controlling tumor progression in CRC.^[65] Microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic CRC is highly sensitive to checkpoint inhibition alone.^[66, 67] Thus, current CRC combinatorial studies using RT with immunotherapy has focused on microsatellite stable (MSS) patients. The Immunoscore®, first described by Galon et al, was calculated based on the combined analysis of CD8+ plus CD45RO+ cells in the center of the tumors and at the invasive margins, and was found to correlate with prognosis.^[68–70] Interestingly, patients with greater numbers of tumor infiltrating CD8 and granzyme B-expressing T-cells (activated T-cells) had better survival compared to those with tumors that had lower numbers of infiltrating CD8 cells. Chemotherapy and RT can increase the immunogenic properties of tumor cells by enhancing MHC class I expression, thereby increasing their vulnerability to cytotoxic lymphocytes.

Another frequent effect of treatment-associated DNA damage is the increased expression of death receptors, enabling lysis of the tumor cells by Fas/CD95 ligand and TRAIL-positive immune effectors.^[71] Immunogenic cell death induced by RT involves the cell surface exposure of calreticulin, the release of the TLR4 agonist HMGB1, and ATP that trigger dendritic cell engulfment of dying cells, antigen presentation, and production of interleukin (IL)-1 β , ultimately leading to activation of CD8+ T cells.^[72–74] RT can also program the differentiation of iNOS+ M1 macrophages in the tumor microenvironment, supporting T cell recruitment into tumor tissue and successful tumor immune rejection through an NO-dependent mechanism.^[75] [74, 76] Thus, the synergy between RT and immunomodulatory therapies is based on RT's own immunomodulatory effects including inducing immunogenic cell death, releasing antigens for T cell priming, increasing MHC expression and antigen processing, upregulation of immunogenic cell surface markers, improving T cell homing to tumor sites, shifting the polarization of tumor associated macrophages, and destruction of immunosuppressive stromal cells in the tumor microenvironment.^[72]

Anti-PD-1/PD-L1

Strong PD-L1 expression was observed in 30% of CRC and was found to correlate with infiltration by CD8 (+) lymphocytes which did not express PD-1.^[77] Both RT and 5-FU induce the expression of PD-L1 on tumor cells through IFN γ production by CD8(+) T cells leading to an immune-suppressive environment and promoting PD-L1-mediated T-cell apoptosis.^[78, 79] In a CRC xenograft model, concomitant but not sequential administration of fractionated RT in combination with anti-PD-1 or PD-L1 antibodies generated efficacious CD8(+) T-cell responses leading to better tumor response and survival compared to either modality alone.^[78] These results could explain the adaptive RT resistance by tumors through expression of PD-L1. In fact, pre and post-chemoRT pathology from 93 matched-pair rectal cancer patients demonstrated CD8+ stromal tumor infiltrating lymphocyte (STL) density doubled after chemoRT, whereas FOXP3+ STL counts remained stable. High post-chemoRT CD8 + STL density was associated with better prognosis and a high pre-treatment CD8/FOXP3 intraepithelial tumor infiltrating lymphocyte ratio was a predictor of favorable tumor regression.^[80] Additional pre-clinical studies have highlighted the immunomodulatory enhancing effects of RT with PD-1/PD-L1 checkpoint blockade demonstrating improved local, disease free and abscopal effects.^[81–85]

Segal NH, et al. conducted a non-randomized phase II study of pembrolizumab (anti-PD-1 antibody) plus RT in MSS CRC (NCT02437071).^[86] Patients with refractory metastatic disease underwent palliative RT followed by pembrolizumab (200 mg IV every 3 weeks). The primary objective was overall response rate (ORR) in a non-radiated lesion. Twenty-two patients were enrolled and one partial response was observed (ORR, 4.5%). The combination was very well tolerated.

There are currently two active multi-center clinical trials investigating PD-1/PD-L1 blockade as an immunomodulator leveraging radiation as the immune sensitizer in MSS rectal cancer. The NSABP FR-2 study is a phase II, open label study testing the safety and efficacy of durvalumab (anti-PD-L1) monotherapy following standard chemoRT in patients with stages II-IV rectal cancer (NCT03102047). This study uses a “window of opportunity” design

whereby the immunotherapy is incorporated into the space where no treatment traditionally takes place after chemoRT and before surgical resection. This window represents the sweet spot for maximal tumor cytoreduction, optimal neoantigenic expression, and immunologic capacity when concurrent suppressive therapy is absent. Pembrolizumab is being tested as a concurrent adjunct to chemoRT in an experimental arm of the NRG-GI002 clinical trial (NCT02921256) (Figure 2 and section below). Pembrolizumab is administered concurrently with and following chemoRT. The primary endpoint for both studies is tumor downstaging (NAR Score) with correlative analyses to assess immunogenic response and tumor infiltrating lymphocyte (TIL) density.

The complimentary designs of these studies should allow determination of the optimal sequencing of immunotherapy with RT as well as the assessment of many potential biomarkers in the tumor microenvironment and the periphery. If successful, these studies may serve as a foundation to build upon given the rapid development of immune checkpoint inhibitors and alternate co-stimulatory and inhibitory molecules. Importantly, these studies include a number of exploratory analyses of immune biomarkers in an effort to inform the design of future studies and identify patients who may derive the most benefit from immunotherapy and RT.

Dual Inhibition of PD-(L)1 and CTLA-4

Some data suggests that the combination of RT with a single checkpoint inhibitor may not be adequate to activate or maintain activation of the immune system in MSS CRC. Anti-CTLA4 predominantly inhibits Treg cells, thereby increasing the CD8+ T cell to Treg ratio (CD8+/Treg). RT enhances the diversity of the T-cell receptor (TCR) repertoire of intratumoral T cells. This preclinical observation suggested that dual immune checkpoint blockade is required to best induce synergistic antitumor immunity with RT. Twyman-Saint Victor and colleagues reported that resistance to RT with anti-CTLA4 treatment was due to upregulation of PD-L1 on tumor cells, resulting in T-cell exhaustion in a murine melanoma model.^[87] RT in combination with dual checkpoint blockade with anti-CTLA4 and anti-PD-L1/PD-1 showed significant synergistic antitumor activity in murine melanoma and pancreatic cancer models.^[87] Importantly, sequencing of these biologic interventions may matter and need to be fully explored to optimize clinical outcomes.

There are two studies currently testing the role of dual immunotherapy checkpoint inhibitors with RT in advanced pMMR/MSS CRC. NSABP FC-9 is an ongoing phase II study of durvalumab plus tremelimumab following palliative hypofractionated RT in patients with refractory metastatic CRC (NCT03007407). The primary objective is to determine the anti-tumor efficacy of the dual immune checkpoint blockade via assessment of tumor response at unirradiated target lesions (i.e., a quantifiable abscopal effect). Following three doses of hypofractionated palliative RT (9 Gy x 3 on days -2, -1, and day 0 prior to cycle 1), patients receive both tremelimumab (75 mg IV) and durvalumab (1500 mg IV) on Day 1 for 4 cycles. Beginning with cycles 5 through 12, patients receive durvalumab alone (1500 mg IV) on Day 1 of each 28-day cycle. The sample size requires 21 evaluable patients to demonstrate a statistically significant increase in the objective response rate. This trial is nearly done enrolling patients.

Similarly, the NCI-sponsored ETCTN 10021 study is a phase II trial of durvalumab and tremelimumab with high or low-dose RT in patients with metastatic CRC or non-small cell lung cancer (NCT02888743). The CRC cohort of this trial will enroll similar patients as NSABP FC-9, but randomizes between provision of low-dose RT (0.5 Gy twice a day for 2 days) and 8 Gy daily for 3 days. Both modalities are followed by durvalumab and tremelimumab for cycles 1–4 and durvalumab monotherapy from cycle 5 onward. Together, these two trials are particularly well poised to explore the relative contributions of dual checkpoint inhibitor and perhaps an optimal way to deliver the antigenic release from RT.

Oncolytic Virus (T-VEC)

Talimogene laherparepvec (T-VEC; Imlygic™) is a herpes simplex-1 (HSV-1) derived oncolytic virus designed to selectively replicate in tumor cells causing cell lysis and to provoke anti-tumor immunity.^[88, 89] T-VEC was modified from a clinical isolate of HSV-1 by disrupting the genes for neurovirulence (ICP34.5) and for immune-evasion (ICP47) allowing for tumor specific replication and increasing the local immune reaction, respectively. The latter is further increased by addition of a transgene for GM-CSF. T-VEC is administered as an intralesional injection by an initial priming dose for seroconversion followed by serial injections every 2 weeks at higher doses.^[89] After injection and subsequent replication of T-VEC, cell death and lysis of cancer cells occurs with infection of surrounding cells in subsequent waves. The addition of GM-CSF leads to recruitment of immune cells and induction of T-cell mediated antitumor immunity while decreasing the number of regulatory T cells. Accumulating data also suggest the possibility of abscopal effects in distant metastatic lesions resulting from sustained systemic antitumor immunity from cytotoxic T cells that recognize tumor-specific antigens at the site of injection.^[88] In an initial phase I study demonstrating safety, T-VEC obtained approval by multiple regulatory agencies for stages III and IVM1a melanoma showing improvement in overall survival.^[90] Early clinical trials have combined T-VEC with RT in various tumor types, including a phase I/II study in stages III-IVB squamous cell cancer of the head and neck with promising outcomes.^[91] Preclinical data showed increased activity of T-VEC in CRC cell lines compared to those from other primary sites (Kaufman et al, unpublished data). Furthermore, data suggests that RAS-driven cancer cells are more permissive to viral entry, thus offering a potential therapeutic advantage for this approach in a cohort of CRC with such mutations.^[88, 92, 93]

In an ongoing NCI-sponsored (ETCTN 10058) dose-finding phase I trial (NCT03300544), patients with low-lying rectal adenocarcinomas (≤ 6 cm of anal verge) requiring neoadjuvant treatment are administered serial intratumoral T-VEC injections (x 4) via endoscopy in two dose escalation cohorts in combination with neoadjuvant chemotherapy (FOLFOX) followed by chemoRT (50.4 Gy in 28 fractions with capecitabine 825 mg/m² bid on days of RT) (Figure 3). An expansion cohort is planned at the MTD with a total accrual of up to 18 patients. The primary objective is to determine the MTD of the combination and key secondary objectives include determining NARI score and MRI-response of the combination. Paired biopsies and blood samples will be obtained for correlative studies. Future studies may evaluate the role of T-VEC in combination with checkpoint inhibitors resectable rectal adenocarcinoma given strong data suggesting up regulation of PD-L1 after

T-VEC injection.^[94] Together, these novel approaches will further expand our collective understanding of how CRC immunogenicity might be successfully induced by RT, checkpoint inhibitors and oncolytic viruses.

Additional Agents of Clinical Interest

Table 1 includes a number of candidate agents under clinical investigation to further improve outcomes in rectal cancer. Each of these agents is being tested in conjunction with the current chemoRT standard of care in rectal cancer (i.e., concurrent fluoropyrimidine with standard doses and fractionation of RT) and directly compared to that standard approach or to historical outcomes using that standard.

Future Directions in the Clinical Development of Novel Sensitizers

Despite the last few decades of clinical trials, pCR rates following neoadjuvant chemoRT in rectal cancer continue to hover in the low 20% range. To improve outcomes for patients and truly offer organ preservation or non-operative management, higher complete tumor sterilization rates are needed. Better understanding of the molecular signals, oncogenic drivers, and the surrounding tumor microenvironment involved in treatment sensitivity and persistent disease resistance may hold the key to future progress. Areas of fruitful exploration include investigating combinations of DNA damage response inhibitors for tumors with DNA damage repair defects, as well as combinations of immune enabling drugs concurrently or after radiation in a pre-op window of opportunity approach. Incorporation of radiogenomics and more novel functional imaging techniques to assist in maximizing cancer control while minimizing acute and chronic toxicity are poorly resourced priorities. As such, pre-clinical hypothesis driven discovery is essential to support a subsequent scientifically rational clinical trial, but early collaboration between industry and academic partners can leverage the NCI Formulary and established CTEP CRADAs as part of a longitudinal effort towards clinical testing and ultimate FDA registration.

In order to facilitate development of novel radiosensitizers in rectal cancer, several barriers must be overcome. Table 2 identifies some of these challenges and offers potential solutions. While not exhaustive, if adequately resourced this inventory could significantly accelerate progress. For example, development of robust translational research requires access to match-paired tumor samples, serial collection of blood specimens and high quality radiographic imaging. Overcoming data sharing restrictions and biospecimen unavailability represents a major research infrastructure need that can be facilitated through coordination and collaboration of the above referenced clinical trials and with NCI support and resources. Future trials testing innovative neoadjuvant rectal cancer hypotheses should incorporate standardized data collection, patient reported outcomes and biospecimen procurement at consistent time points with centralized public access to the data and molecular determinants. Such guidance could be organized and resourced by the NCI through the NCTN, ETCTN and extramural research administrative supplement mechanisms. This centrally coordinated approach across trials will help to accelerate discoveries and validate mechanisms of treatment resistance that can be further exploited. Lastly, drug development with RT must have a clear pathway towards FDA registration to support industry investment in this

treatment priority. The incorporation of high quality RT delivery as a component of a registrational pathway for new therapies is essential and can only be done in conjunction with high quality control, provision of RT delivery review, and traditional data monitoring of investigational agent toxicity in the context of radiation effects. Such quality control represents critical safeguards so that outcomes in a clinical trial are attributable to the novel investigation rather than differences in treatment delivery.

To help facilitate coordinated and rapid clinical testing, NRG-GI002 was developed by the NCI (NCT02921256). This NCTN multi-arm randomized phase II trial is designed as a master protocol with parallel experimental arms and correlative biomarkers. The study allows testing of a variety of novel hypotheses with neoadjuvant chemo and chemoRT in a consistent and homogenous high-risk patient population. Patients with locally advanced rectal cancer undergo total neoadjuvant therapy (TNT) with systemic chemotherapy (FOLFOX x 8 cycles) followed by chemoRT and then surgical resection (Figure 2). Each hypothesis is added as a protocol amendment as an experimental arm directly compared to a continuously enrolling control arm. The primary endpoint is a reduction in the Neoadjuvant Rectal (NAR) Cancer Score, an externally validated pathologic surrogate endpoint for OS and DFS in rectal cancer.^[52, 95]

Conclusions

Rectal cancer represents nearly a third of CRC patients and has the added challenge of optimizing treatment to achieve organ preservation. The incorporation of novel radiosensitizers to increase the rate of tumor sterilization and/or induce an immunogenic response to improve overall survival represents contemporary efforts of investigators. The NCI's Radiation Research Program Colorectal Cancer Working Group represents a collaborative unit of content experts and stakeholders that facilitate the recognition, prioritization and development of new therapies in this regard. Patients, and the field, will benefit from a coordinated approach to identify and test hypotheses with the most promising pre-clinical rational coupled with well controlled and randomized clinical validation.

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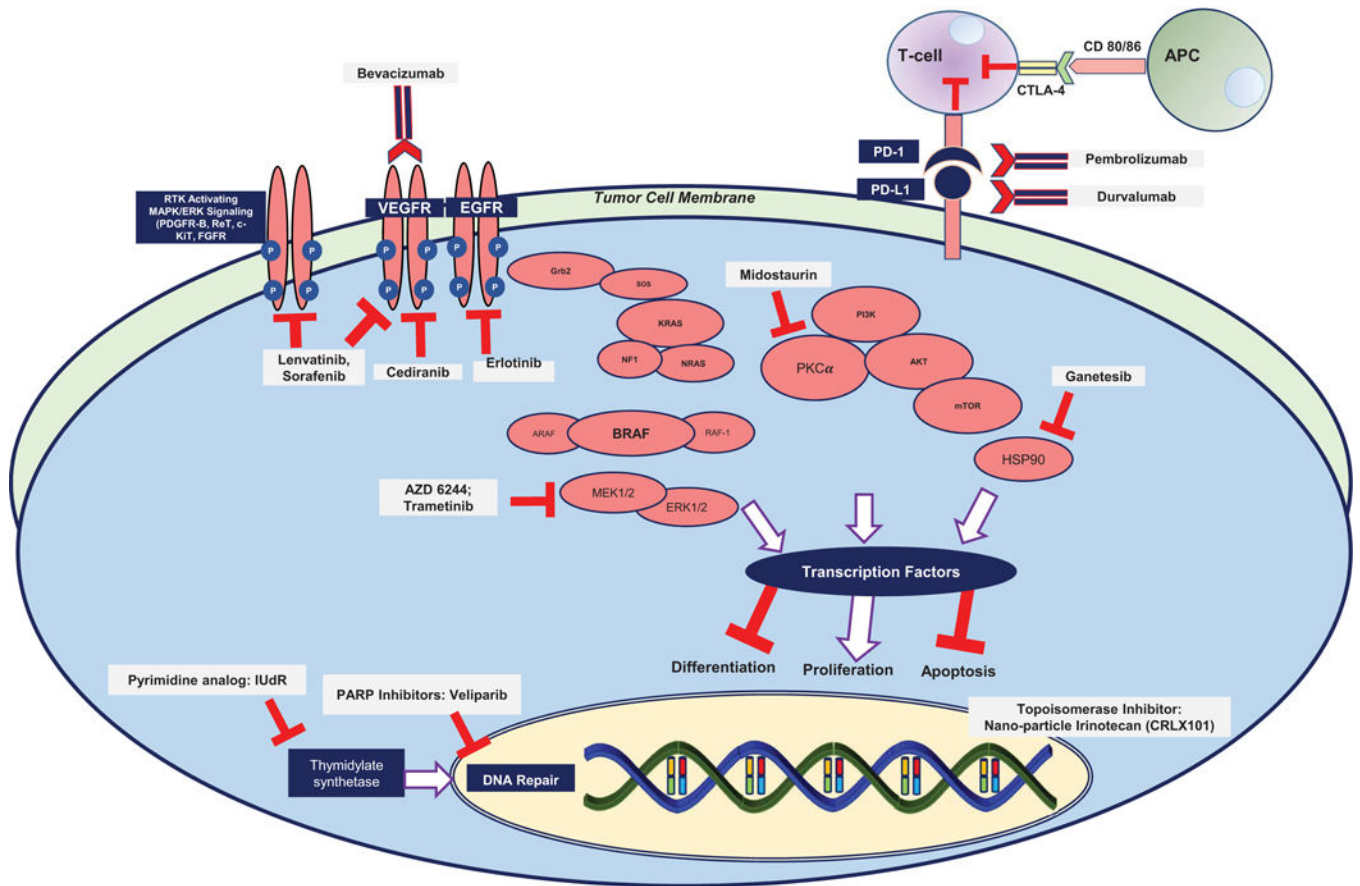


Figure 1. Targeted cellular pathways and new radiosensitizers in development.

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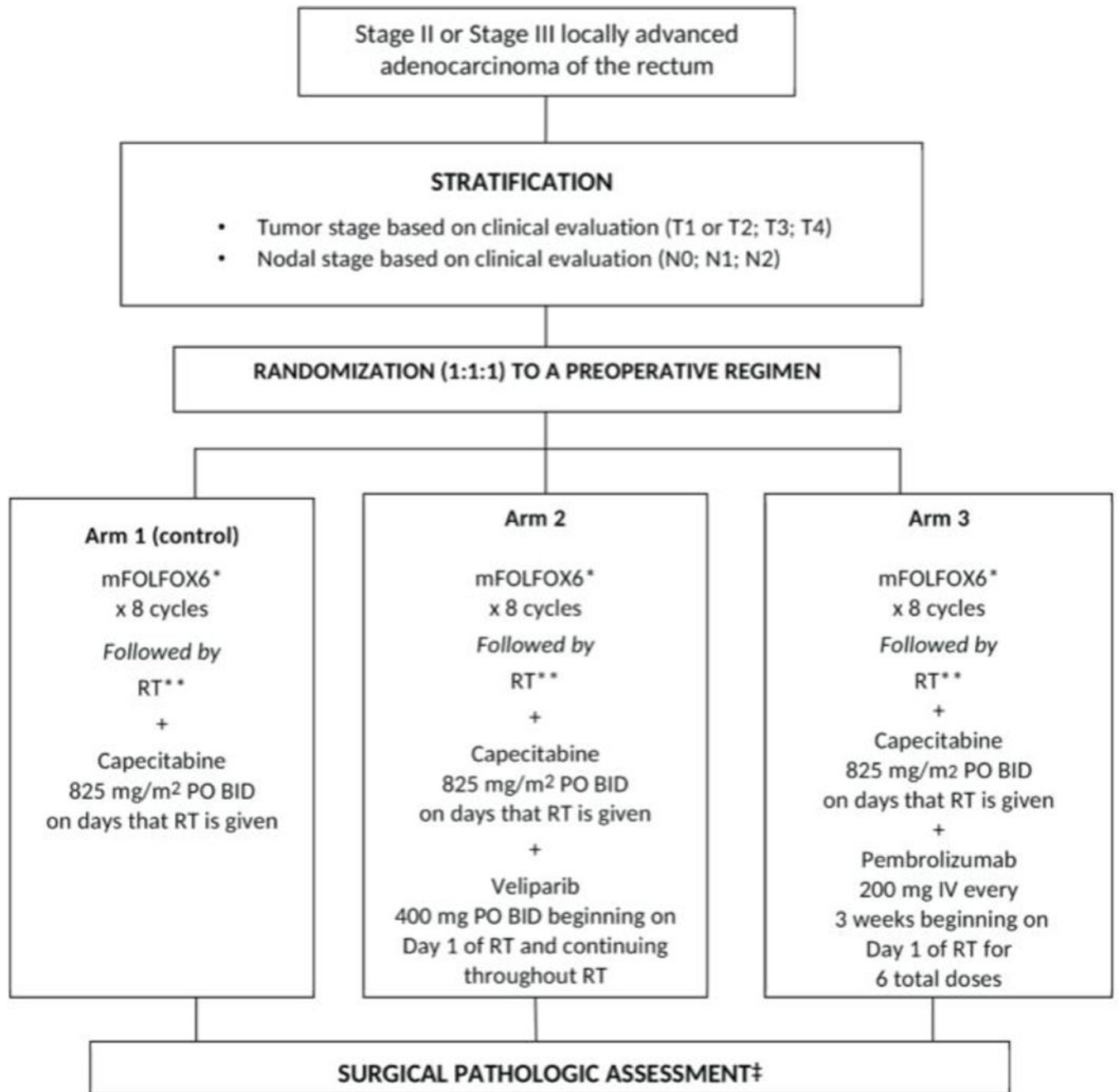


Figure 2. Study schema for randomized phase II arms of NRG-GI002 (NCT02921256). Additional arms added through protocol amendments with comparison to the ongoing control arm.

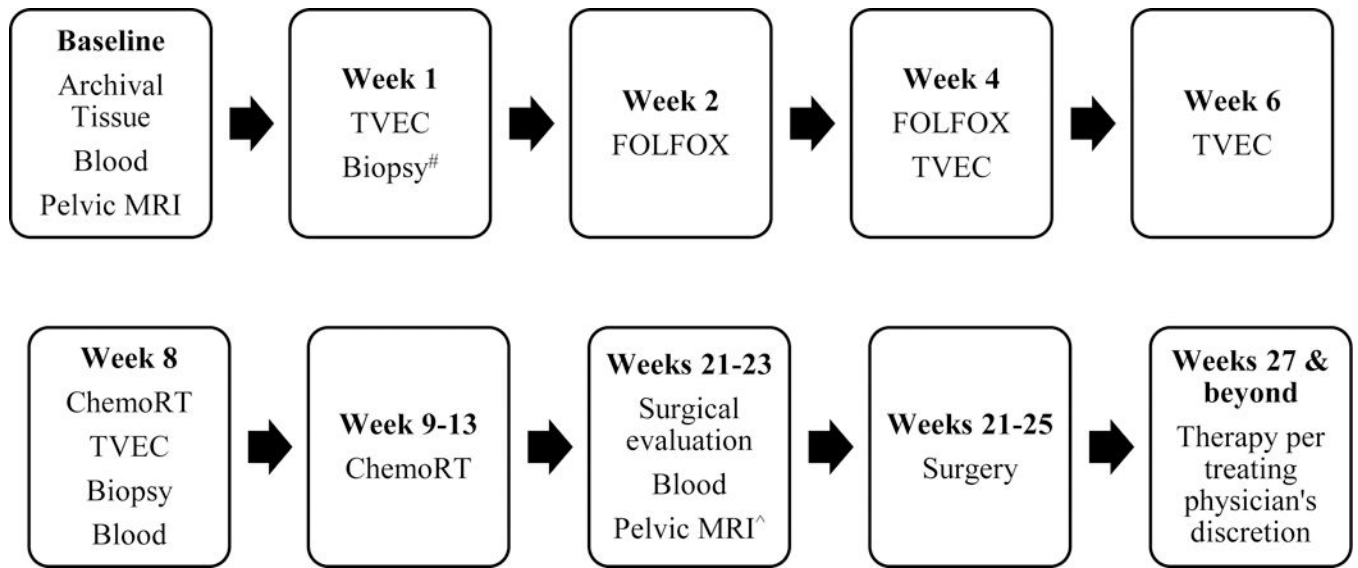


Figure 3.
Sequence of interventions associated with neoadjuvant rectal cancer clinical trial (NCT03300544) incorporating TVEC.

Table 1.

Contemporary clinical trials incorporating novel agents in combination with radiation in the treatment of colorectal cancer

Target / Mechanism	Agent(s)	ClinicalTrial.gov identifier
VEGF TKI	Cediranib	NCT01160926
VEGF MoAb + EGFR TKI	Bevacizumab + Erlotinib	NCT00307736
Pan-target (VEGF) TKI	Sorafenib	NCT01376453
Pan-target (VEGF) TKI	Lenvatinib	NCT02935309
Protein kinase C	Midostaurin	NCT01282502
Heat shock protein 90	Ganetespib	NCT01554969
PARP	Veliparib	NCT01589419; NCT02921256
MEK	AZD 6244; Trametinib	NCT01160926; NCT01740648
Pyrimidine analog	IUdR	NCT02381561
Topoisomerase	Nano-particle Irinotecan (CRLX101)	NCT02010567
Programmed death receptor 1 (PD-1)	Pembrolizumab	NCT02921256
Programmed death receptor ligand 1 (PD-L1)	Durvalumab	NCT03102047
Programmed death receptor ligand 1 (PD-L1) and CTLA4	Durvalumab + Tremilimumab	NCT02888743 NCT03007407
Oncolytic virus	T-VEC	NCT03300544

Abbreviations: VEGF = vascular endothelial growth factor; TKI = tyrosine kinase inhibitor; MoAb = monoclonal antibody; EGFR = epidermal growth factor receptor; PARP = poly (ADP-ribose) polymerase; MEK = mitogen-activated protein kinase enzyme; IUdR = iododeoxyuridine; PD-1 = programmed death receptor 1; PD-L1 = programmed death receptor ligand 1; CTLA4 = cytotoxic T-lymphocyte-associated protein 4; T-VEC = talimogene laherparepvec

Table 2.

Select barriers and potential solutions to accelerate rectal cancer treatment outcomes as a component of neoadjuvant clinical trials.

Barrier	Potential Solution(s)	Comments
Lack of translational and clinically annotated biospecimens for discovery and biomarker development	Collection of matched paired biospecimens including primary tumor	Mandate and resource tissue collection in all neoadjuvant clinical trials at key time points with central catalogue for investigator use
Limited ability to monitor treatment response or detect minimal residual disease	Circulating tumor DNA analyses	Mandate and resource blood collection for all neoadjuvant clinical trials at key time points
Limited ability to monitor treatment response or confirm tumor resolution	Radiographic imaging, including radiomic assessments	I. Establish and mandate standard imaging requirements and protocols for imaging performance, acquisition and centralized collection
		II. NCI sponsored RAVE data collection and TRIAD imaging storage supports these functions, when utilized
Data silos	Data sharing requirements and use of NCI-sponsored metadatabases	I. This is being actively addressed through data sharing requirements for NCI-sponsored trials including utilization of RAVE
		II. Other silos include individual academic center and industry-sponsored studies
Quality control in radiotherapy delivery	I. Consistently required technology and dosimetry standards built into trial protocols	The NCI supported Imaging and Radiation Oncology Core (IROC) supports these functions, when utilized
	II. Central review of treatment plans	
	III. Clinical Trial site credentialing	
Perceived lack of clear pathway for FDA registration	Template guidelines for registration with FDA and industry stakeholders	Precedent exists with temozolomide and cetuximab