TITLE: ***Linear Energy Transfer (LET)-Optimized Intensity Modulated Proton Therapy (IMPT) as a Component of Definitive Chemoradiation for Newly Diagnosed Squamous Cell Carcinoma of the Anal Canal: a Feasibility Trial***

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Short Title: **IMPT for Anal Cancer**

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1.0 TRIAL SUMMARY

1.1 Objectives

1.1.1 Primary objective:

1.) To assess physician-reported acute grade 3 or greater gastrointestinal, genitourinary and hematologic toxicities at 12 weeks post-treatment for patients treated with linear energy transfer (LET)-optimized, intensity-modulated proton therapy (IMPT) and compare to contemporary controls treated with VMAT to determine the feasibility of this outcome for a future randomized trial.

1.1.2 Secondary objectives:

1.) To assess the feasibility of enrolling patients on a prospective trial delivering LET-optimized IMPT for newly diagnosed, non-metastatic anal cancer.
2.) To develop guidelines and workflow to create and deliver anal canal cancer treatments using LET-optimized IMPT.
3.) To evaluate complete response rate at 12 weeks and 24 weeks post-treatment.
4.) To evaluate local progression free survival, distant metastasis-free survival and overall survival at 24 and 48 months.
5.) To evaluate rates of patient-reported acute toxicity, function, distress and QOL at 12 weeks.
6.) To evaluate rates of patient-reported late toxicity, function, distress and QOL every 6 months for 24 months.
7.) To evaluate the value of proton therapy by comparing Time-Driven Activity-Based Costing data from the date of consultation until the date of the 12-week follow up visit post-treatment with contemporary controls treated with VMAT.

1.1.3 Exploratory objectives:

1.) To compare dose to the pelvic bone marrow, bowel, bladder and genitalia between LET-optimized IMPT, traditionally-optimized IMPT and VMAT.
2.) To assess rates of leukopenia, neutropenia and lymphopenia at 12-weeks post-treatment for patients treated with LET-optimized IMPT and compare to contemporary controls treated with VMAT.
3.) To correlate white blood cell counts (WBC), absolute neutrophil counts (ANC) and absolute lymphocyte counts (ALC) with dose to the pelvic bone marrow for patients treated with LET-optimized IMPT.

Abbreviated Title	IMPT for Anal Cancer
Trial Phase	Feasibility
Clinical Indication	Newly diagnosed, non-metastatic squamous cell carcinoma of the anal canal dispositioned to definitive chemoradiation treatment
Trial Type	Treatment
Type of control	None
Route of administration	External beam proton radiation therapy with concurrent chemotherapy
Trial Blinding	NA
Treatment Groups	Single arm, non-randomized
Number of trial subjects	40
Estimated duration of trial	9 years
Duration of Participation	9 years

2.0 TRIAL DESIGN

2.1 Research Questions

There are three main research questions we hope to answer with this study:

2.1.1 Is it feasible to enroll patients only a study that utilizes LET-optimized IMPT as the radiation modality in the delivery of definitive chemoradiation?

2.1.2 Can LET-optimized IMPT reduce biologically effective dose to the bone marrow, bladder, bowel and genitali

2.1.3 Can LET-optimized IMPT decrease acute and chronic gastrointestinal, genitourinary and hematologic toxicities compared to historical controls treated with VMAT?

2.2 Hypothesis

Our primary hypothesis is that LET-optimized IMPT can be used to minimize both dose and LET to OARs, which will reduce toxicities while obtaining similar or superior tumor response in anal canal cancers.

3.0 BACKGROUND & RATIONALE:

3.1 Background

The current standard of care for squamous cell carcinoma of the anal canal was established by RTOG 9811 and includes definitive CRT with 5-fluorouracil (5FU) and mitomycin-C (MMC)[1]. However, studies have shown equivalent results with 5FU with cisplatin as concurrent treatment [2], so that regimen is also used at some centers, including MDACC[3]. Intensity modulated radiotherapy (IMRT) emerged as the new standard of care in the treatment of anal cancer when RTOG 0529, a phase II study, prospectively showed that IMRT was able to significantly reduce G2+ hematologic and G3+ dermatologic toxicity[4].

The landmark studies establishing CRT as the standard of care utilized 3D treatment techniques. As such, large volumes of bowel, bladder, external genitalia and skin received significant doses. On the MMC arm of RTOG 9811, G3-4 hematologic and non-hematologic acute toxicity rates were 61% and 74%, respectively [1]. Although IMRT was shown to reduce toxicity, patients receiving IMRT with concurrent 5FU and MMC on RTOG 0529 still experienced 77% G2+ gastrointestinal, 73% G2+ hematologic and 23% G3+ dermatologic toxicity. Additionally, bone marrow dose is typically higher with IMRT [5], and the importance of bone marrow dose in predicting the risk of hematologic toxicity for patients receiving CRT for anal cancer has been established. Specifically, the volume of pelvic bone marrow receiving 5, 10, 15 and 20 Gy were all significantly associated with decreased WBC and ANC nadirs[6].

The advantages of proton therapy (PT) arise from the physical properties of proton beams[7]. From a physical standpoint, proton beam dose deposition is highly favorable, following a Bragg curve as a function of its depth in tissue. The lower entrance doses and elimination of exit doses can produce superior conformal dose distributions to the target volume compared with radiotherapy with megavoltage (MV) x-rays. Thus, the rationale for using protons lies in reducing the integral dose and sparing surrounding healthy tissues and critical organs, minimizing treatment related complications and reducing the risk of radiation-induced secondary cancers [8,9].

Protons are charged particles that continuously interact with tissue, slowing as they penetrate it, and leaving a track of ionizations in their paths, which are quantified in terms of the linear energy transfer (LET). The LET increases as the energy (or speed) of the proton particle decreases. Consequently, the LET of proton beams increases as a function of depth in tissue. At their entrance to the body, the LET is low (~0.6-0.9 keV/µm) and at the tumor volume and end of the proton beam range, the LET increases by more than an order of magnitude. The biological damage produced by therapeutic proton beams depends on LET in addition to absorbed dose. However, current practice in proton therapy assumes that the relative biological effectiveness (RBE) is independent of LET and equal to 1.1. This is only an approximation, and numerous studies have demonstrated the variable RBE within therapeutic proton beams[10–14]. A recent review of the available proton RBE data demonstrated that, although the data support an average value of ~1.1, considerable deviations from this value can occur with variations in LET. In particular, near the end of the proton range where protons have the highest LET, RBE can increase to as much as 3.5[11]. Thus, these physical properties of proton beams make it possible to precisely deliver a high dose of radiation to a tumor volume while minimizing dose to surrounding normal tissue[7].

3.2 Rationale

3.2.1 Rationale for IMPT

In the current clinical practice of PT, techniques such as passively scattered proton therapy (PSPT) are used. With such techniques, the distal high LET regions of beams are nearly always in normal tissues distal to the target volume. Intensity modulated proton therapy (IMPT) is a newer delivery modality in which magnetically scanned thin pristine proton "beamlets" are used to "paint" radiation dose into the target volumes. For treatment planning purposes, IMPT are categorized into single field optimized (SFO-IMPT) or multi-field optimized (MFO-IMPT). In SFO-IMPT, inverse planning is employed to optimize each individual beam to individually conform to the entire target volume while minimizing dose outside. In MFO-IMPT there is simultaneous optimization of all beamlets of all incident beams to deliver the homogeneous prescription dose to the target while limiting the dose to critical volumes of normal tissues to within tolerance levels. For highly complex target shapes and anatomic geometries, MFO-IMPT frequently allows for the optimal balancing of tumor coverage and normal tissue sparing.

In the pelvis, IMPT has the potential to preferentially spare bone marrow from unnecessary dose, and potentially increase the tolerability of treatment. Additionally, IMPT has the potential to decrease dose to the external genitalia, bowel and bladder, which may further improve acute and long-term quality of life for patients receiving CRT for anal cancer. Hematologic, gastrointestinal and dermatologic toxicity are common reasons patients need breaks during treatment. As it has been demonstrated that prolonging the overall treatment duration with breaks can worsen oncologic outcomes[15], IMPT thus has the potential to improve the efficacy of treatment. Recently we completed a treatment planning study of 10 consecutive patients treated with VMAT for anal cancer, which we generated comparison IMPT plans. We compared doses to the pelvic bone marrow, bowel, bladder and genitalia. We found that IMPT plans resulted in clinically signficiant lower doses (RBE=1.1) to the pelvic bone marrow (mean dose, V10, V20, V30, V40) as well as bladder (V40) and genitalia (V30 and V20) and comparable V45 doses to bowel..

Two small treatment planning studies published in 2015 have also evaluated the potential benefits of IMPT for anal cancer[16,17]. The first, performed at the Mayo Clinic, compared IMPT plans for eight patients with squamous cell carcinoma of the anal canal. They treated the primary site and nodes with individual beams by using sub-target CTVs that represent primary target as anus and the right and left lateral nodes. These sub-target CTVs were then used to establish individual beam assignments. Thus, although they used a MFO algorithm, most of the target received a near-uniform dose from individual fields. The gross tumor volume plus margin received 54-60 Gy (RBE) while the elective nodal volumes received 45-50.4 Gy (RBE). They found no different between target coverage between IMRT and IMPT. The IMPT plans were also robust to uncertainties as evidenced by acceptable coverage even with the worse-case dose values. However, the mean doses to the bone marrow, bladder, small bowel and genitalia were all significantly lower with IMPT as compared to IMRT. Specifically with regard to the bone marrow, IMPT reduced the pelvic bone marrow V10 by 54% (P=.008), V20 by 56% (P=008) and V30 by 44% (P=.008)[17]. Another treatment planning study performed at The University of Pennsylvania also compared IMRT and IMPT plans for patients with anal cancer. Although they used SFO-IMPT, they similarly found no difference in target coverage, but they showed significant reductions of doses up to 35 Gy to the small bowel (P=.008), reductions of doses up to 29 Gy to the genitalia (P=.008), as well as reductions of doses up to 30 Gy to the bone marrow (P=.008) [16]. **Figure 1** shows the bone marrow and anterior viscera sparing achievable with IMPT compared with IMRT for a patient treated in our department.

3.2.2 Rationate for LET-opimization

Although IMPT appears to be quite promising for normal tissue avoidance in the treatment of anal squamous cell carcinomna, with current MFO-IMPT techniques, location of high biological effect regions is uncontrollable. To reduce the probability of normal tissue injury, the strategy is currently to avoid placing such regions in sensitive normal tissues, but sometimes at the cost of compromising tumor dose. However, with MFO-IMPT's ability to control intensities of individual beamlets that incorporate LET and/or RBE, it is possible to place such highly effective regions within the tumor volume and away from normal critical tissues, which would effectively allow for safe prescribed and delivered doses, and improved normal tissue sparing. Evaluation of safety and feasibility of such an approach is one of the secondary objectives of the proposed trial. We have developed novel methods for the incorporation of LET in to the IMPT planning process, which use Fast Monte Carlo (fMC) dose engine to recalculate treatment plans generated using MFO-IMPT in order to obtain LET distributions. In a recent treatment planning study we completed of nine patients with anal cancer treated with VMAT, we observed that IMPT plans reduced the exposure of pelvic bone marrow to higher LET regions while maintaining target coverage compared to the original VMAT plans.

3.2.3 Rationale for use of charges and Time-Driven Activity-Based Costing data to describe value

Healthcare value can be defined as outcomes over costs, but measuring the true costs of delivering care can be imprecise and difficult, particularly when utilizing advanced technology. In the current fee-for-service model, charges and reimbursements are utilized most often utilized as proxies for cost[17]. From the perspective of the healthcare provider, the true cost of delivering a particular treatment is a result of the resources that are required to deliver that care, not necessarily the charges or the reimbursements.

The field of radiation oncology has relied on the development of, sometimes costly, technology in order to advance the field, improve outcomes and decrease toxicities. In the treatment of anal cancer, the more costly IMRT technique was shown to decrease toxicity rates as compared to the traditional 3D conformal radiation techniques[4]. As mentioned above, IMPT has the potential to reduce dose to non-target tissues even more than IMRT and has the subsequent potential to decrease toxicity rates even further. Although the charges and reimbursements for IMPT are undisputedly higher than those for IMRT, reducing the toxicity rates and the subsequent needs and costs of managing those toxicities can dramatically affect the overall cost of delivering care.

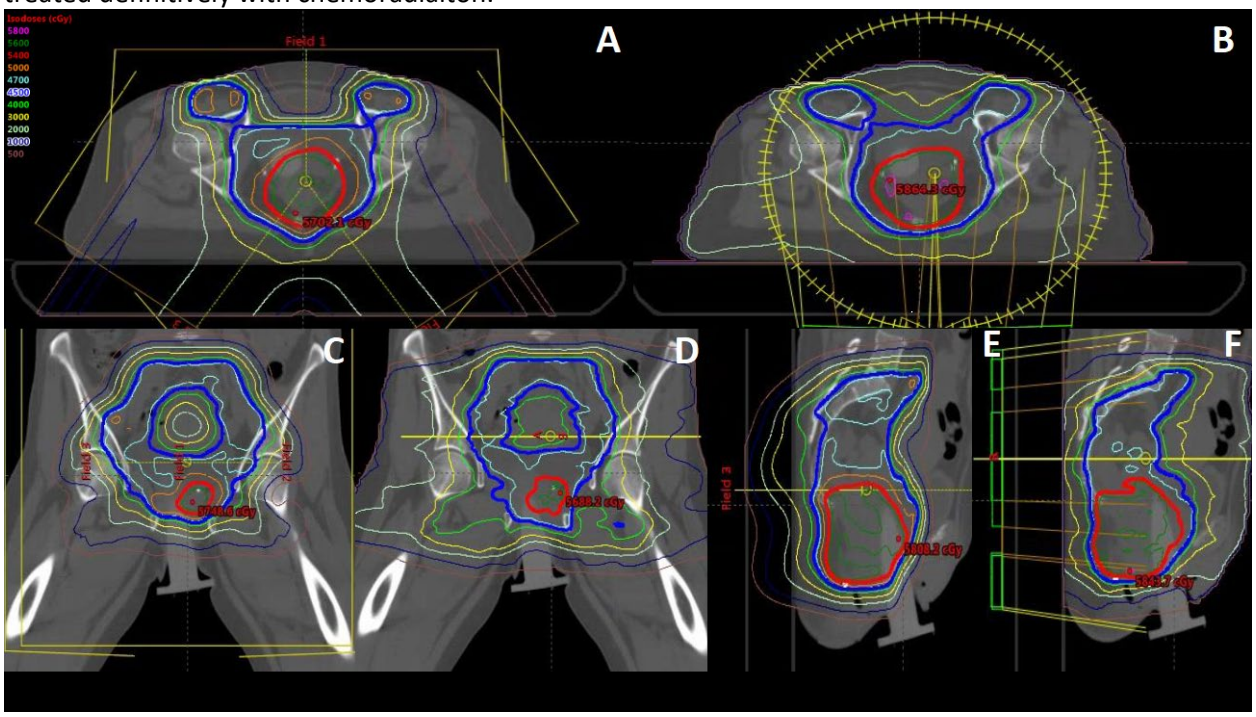
Time-driven activity-based costing (TDABC) is a method that has been used by several fields of business and medicine[19,20] and can provide a "bottom-up" cost accounting methodology used to more accurately measure resources utilized based on the time consumption of constituent activities[21,22]. TDABC analyses require process maps to first be generated including all points of contact the patient has with the radiation oncology department from consultation, to treatment planning and delivery, to follow up and all ancillary services required for the management of radiation-related toxicities. Costs are then assigned to each step of the process map based on multiplying the adjusted average hourly rate for the particular personnel performing the task but the length of time typically required to complete it. Direct and indirect costs of treatment including depreciation of radiation therapy and imaging equipment are also integrated.

Groups at this institution have pioneered the field of utilizing the TDABC method to account for and compare the true costs of delivering radiation therapy as a part of oncologic care. Extensive process mapping and radar chart analyses were performed on a cohort of 238 men treated for prostate

cancer at MD Anderson with prostate brachytherapy showing the true cost and value of that modality[23]. The same group also looked at utilizing TDABC to show the true difference in cost between definitive chemoradiation utilizing either IMRT or IMPT in the treatment of patients with head and neck cancer. They showed that, for a subgroup of patients, the cost savings of reduced toxicity with IMPT offset the increased technical costs associated with delivering this treatment[24].

This study will be the first to gather TDABC data on patients treated with IMPT for squamous cell carcinoma of the anal canal. The radiation oncology department has already developed process maps for the different radiation oncology clinics, and we will be able to adapt these maps for patients treated on study at the proton therapy center. We hope the data collected as a part of this study will better account for the true costs of delivering IMPT for this population of patients so that we can better define the value in the future.

Figure 1- shows representative axial (A & B), coronal (C & D) and sagittal (E & F) images from IMPT (A, C, E) and VMAT (B, D, F) comparison plans for a patient with anal canal squamous cell carcinoma treated definitively with chemoradiaiton.



4.0 INCLUSION AND EXCLUSION CRITERIA

4.1 Inclusion criteria

1.) Histologically-proven, non-metastatic invasive primary squamous cell carcinoma of the anal canal (Stages I, II, and III).
2.) History/physical examination within 60 days prior to registration.
3.) Anal examination with biopsy on either colonoscopy, sigmoidoscopy, rigid proctoscopy or anoscopy.
4.) CT scan of the chest and abdomen with contrast or contrast-enhanced PET/CT scan within 60 days of registration unless the patient has a documented contrast allergy.

5.) CT scan of pelvis with contrast or contrast-enhanced PET/CT scan within 60 days of registration unless the patient has a documented contrast allergy.
6.) Zubrod Performance Status of 0-1 within 60 days prior to registration.
7.) Age >/=18 years – 85 years.
8.) Laboratory data within 30 days prior to study registration showing:
- Absolute neutrophil count (ANC) >/= 1.8 K/µL
  - Cannot be achieved through GCSF use
- Platelets >/= 100 K/µL
  - Cannot be achieved through transfusion.
- Hemoglobin >/= 8g/dL
  - Cannot be achieved through transfusion.
- Serum creatinine </= 1.5mg/dL
- Bilirubin </= 1.4mg/dL
  - Except in the case of patients with Gilberts disease.
- White blood cells (WBC) >/= 3000/microliter
- AST/ALT < 3x the upper limit of normal

9.) HIV test must be done within 90 days of study registration. If HIV positive, CD4 count must be obtained within 90 days of study registration. Note: HIV positive patients are eligible for this study if they have a CD4 count >400 cells/mm3.
10.) The patient must either have insurance authorization or otherwise secure funding to cover IMPT.
11.)The patient must be able to receive concurrent chemotherapy.

4.2 Exclusion criteria

1.) Prior invasive malignancy (except non-melanomatous skin cancer), unless disease free for a minimum of 3 years.
2.) Prior systemic chemotherapy for anal cancer.
3.) Prior radiotherapy to the pelvis that would result in overlap of radiation fields.
4.) Evidence of distant metastatic disease (M1).
5.) Not receiving concurrent chemotherapy
6.) Women of childbearing potential or men who do not agree to use a medically effective form of birth control throughout their participation in the treatment phase of the study.
7.) Severe, active co-morbidity defined as follows:

- unstable angina and/or congestive heart failure requiring hospitalization within the last 6 months.
- transmural myocardial infarction within the last 6 months.
- acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration.
- Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration.
- hepatic insufficiency resulting in clinical jaundice and/or coagulation defects.
- HIV positive with a CD4 count <400 cells/mm3.
- other immunocompromised status.
- women who are pregnant or lactating.
- uncontrolled infection as deemed by the PI.
- patient incarceration.

5.0 TREATMENT DETAILS

5.1 Radiation treatment guidelines

5.1.1 Non-contrast treatment planning CT scans will be used to delineate the gross tumor volume (GTV) and clinical target volume (CTV). The treatment planning CT scan will be obtained with the patient in our standard treatment position: supine with legs in a "frog-legged" position. A custom immobilization device will be created to minimize set-up variability. A radiopaque anal marker will be used to indicate the anal verge. A vaginal dilator will be used during simulation and treatment for all female patients.

5.1.2 IMPT Planning and Plan Evaluation

The following is the series of steps in the IMPT planning process for each patient:

1. An MFO-IMPT plan will be designed using a commercial (clinical) treatment planning system (TPS) based on the current practice of using criteria defined in terms of dose- and dose-volume constraints and RBE = 1.1. (The Fixed RBE-IMPT plan.)
2. For the LET-optimized IMPT plan, dose distribution will be optimized using dose- and dose-volume criteria extended to include terms that increase LET in the target and reduce it in normal tissues. Such planning will require the use of an in-house TPS based on Monte Carlo techniques. Post-processing of scanning spots to ensure deliverability will also be performed as is done in the clinical TPS.
3. Both 1) IMPT and 2) LET-optimized IMPT will be reported in terms of three distributions: a) fixed RBE weighted dose (RBE=1.1), b) dose-averaged LET, and c) variable RBE-weighted dose (employing the RBE model of McNamara et al 2015) computed using Monte Carlo simulations. These distributions, dose- and LET-volume histograms and indices derived therefrom will be compared and presented to physicians to support decision making.
4. For safety considerations and for consistency with traditional practice, all dose distributions expressed in terms of RBE of 1.1 will be used to make final treatment decisions. To accomplish this, LET-optimized IMPT plan will be imported back into the current clinical TPS, Plan 1) and 2) will be renormalized to require the same target coverage and compared.
5. The dose distribution that best meets the dose requirements in the RBE=1.1 domain (Step 4) and maximizes the target LET while minimizes LET to critical structures (Step 3), as judged by the attending physician, will be chosen for treatment. If LET-optimzed IMPT plan is not deemed significantly superior, the conventional IMPT plan will be chosen.
6. The selected plan will be approved in the clinical TPS and processed for quality assurance and treatment delivery.

5.1.3 GTV and CTV should be delineated on each axial slice of the planning CT scan on which they appear.

- GTVp includes the gross primary tumor in the anal canal as documented by physical exam, endoscopic exam, CT, PET and MRI.
- GTVn includes all involved nodes as documented by imaging or biopsy.
- CTVp includes the GTVp with a 1 cm margin except into uninvolved bone.
- CTVn includes the GTVn with a 1 cm margin except into uninvolved bone, muscle, bowel or bladder.
- CTVel includes the elective nodal regions at risk including the perirectal, presacral, right and left internal iliacs, right and left external iliacs and right and left inguinals down to the level of the lesser trochanter of the femur inferiorly.

5.1.4 Planning target volumes (PTV) will provide a margin around the CTV to compensate for variability in set up as well as internal motion. The PTV margin will be 5 mm added to each of the CTV volumes above.

5.1.5 Radiation dosing to the PTVp and PTVel will be based on primary tumor size:

Primary tumor size	**PTVp dose/fractionation**	**PTVel dose/fractionation***
<2cm	50Gy(RBE) in 25 fractions	43Gy in 25 fractions
2-5cm	54Gy(RBE) in 27 fractions	45Gy in 27 fractions
>5cm	58Gy(RBE) in 29 fractions	47Gy in 29 fractions

Nodal size	**PTVn dose/fractionation**
<2cm	50Gy(RBE) in 25 fractions
2-5cm	54Gy(RBE) in 27 fractions
>5cm	58Gy(RBE) in 29 fractions

**In the event that the gross node is larger than the primary tumor, the dose and fractionation chosen for the nodal size will dictate the PTVel dose and fractionation.*

5.1.6 Normal structures that should be contoured prior to treatment planning include the bilateral femoral heads, bladder, external genitalia, bowel bag that contains both small bowel and large bowel outside the CTV (contoured as the entire peritoneal potential space per Kavanaugh BD, Pan CC, Dawson LA, et al. Radiation dose-volume effects in the stomach and small bowel. IJROBP 2010;76:S101-107)[25], and pelvic bone marrow (contoured per Mell LK, Kochanski JD, Roeske JC, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients treated with concurrent cisplatin and intensity-modulated pelvic radiotherapy. IJROBP 2006;66:1356-1365)[26].

5.1.7 Treatment planning directives used for inverse planning should include the following goals and normal tissue constraints. These constraints are only a guideline and the final decision will be made by the treating physician based on individual patient anatomy.

- PTV coverage: V100%>95%, V95%>99%, V105%<10%, Dmax<120% (ideal; the treating MD may accept a plan with less coverage or higher heterogeneity based on individual patient anatomy and proximity to organs at risk).
- Bowel bag: point dose Dmax <54Gy (ideal) V54Gy<1cc (acceptable), V45Gy<195cc (ideal; higher doses are acceptable at treating MD's discretion).

- Femoral heads: V45Gy<20% (ideal; higher doses are acceptable at treating MD's discretion)
- Bladder: V50Gy<30% (ideal; higher doses are acceptable at treating MD's discretion).
- External genitalia: V30Gy<20%, V20Gy<67% (ideal; higher doses are acceptable at treating MD's discretion).
- Pelvic bone marrow: V10Gy <90% (ideal; higher doses are acceptable at treating MD's discretion).

5.1.8 Daily image guidance is required for set-up verification and should include daily orthogonal images utilizing the bony anatomy for daily alignment. At least one verification CT simulations will be obtained in the treatment setup position during treatment to verify setup, dose to targets, and dose to normal organs.

5.1.9 Treatment breaks during radiation therapy are discouraged. Treatment breaks may be considered at the discretion of the treating physicians in the case of acute toxicity.

5.2 Chemotherapy details

5.2.1 Cisplatin

- Chemistry: Cisplatin (cis-diamminedichloroplatinum II) has the empiric formula H6Cl2N2Pt. It is a planar inorganic compound with a molecular weight of 300; soluble in water at a concentration of 1 mg/ml. The (II) nomenclature denotes the (active) valence state of the platinum. The interatomic distance of the chlorides is 3.3 A which is different from the 5-7 A interatomic distance of the classic alkylating agents. Only the cis-isomer is therapeutically active.
- Mechanism of Action: Primarily causes inhibition of DNA synthesis, and to a lesser degree inhibition of RNA and protein. It has not been shown to be cell cycle specific.
- Human Pharmacology: Highest concentrations were found in kidney, liver, and spleen; lowest concentrations were found in the brain. Plasma contained less than 10% of dose at 1 hour and the peak urinary excretion was 22-32% in 48 hours. It has a biphasic mode half-life, the initial phase of 25-49 minutes, and the secondary, 58-73 hours. The drug was 79% protein bound with intracellular leukocyte levels 6.1% of plasma levels.
- Side Effects: Impairment of renal function, myelosuppression, high-frequency hearing loss, nausea and vomiting.

5.2.1 5-Fluorouracil

- Chemistry: 5-fluorouracil is has the empiric forumla C4H3FN2O2. It is a pyrimidine analog and blocks thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid.
- Mechanism of action: 5-fluorouracil (5-FU) is a pyrimidine analog antimetabolite that interferes with DNA and RNA synthesis; after activation, F-UMP (an active metabolite) is incorporated into RNA to replace uracil and inhibit cell growth; the active metabolite F-dUMP, inhibits thymidylate synthetase, depleting thymidine triphosphate (a necessary component of DNA synthesis).
- Human pharmacology: 5-FU Penetrates extracellular fluid, CSF, and third space fluids (eg, pleural effusions and ascitic fluid), marrow, intestinal mucosa, liver and other tissues. It

exhibits primarily Hepatic metabolism (90%) via a dehydrogenase enzyme; FU must be metabolized to be active metabolites, 5-fluoroxyuridine monophosphate (F-UMP) and 5-5-fluoro-2'-deoxyuridine-5'-O-monophosphate (F-dUMP). Half life elimination is 16 minutes (range: 8-20 minutes); two metabolites, F-dUMP and F-UMP, have prolonged half-lives depending on the type of tissue. 5-FU is excreted in lung (as expired CO2) and urine (7% to 20% as unchanged drug within 6 hours; also as metabolites within 9-10 hours).

- Side effects: Primary side effects include nausea, diarrhea, and maculopapular rash. Cardiovascular and hematologic side effects are rare.

6.0 TRIAL PROCEDURES*

Figure 2-

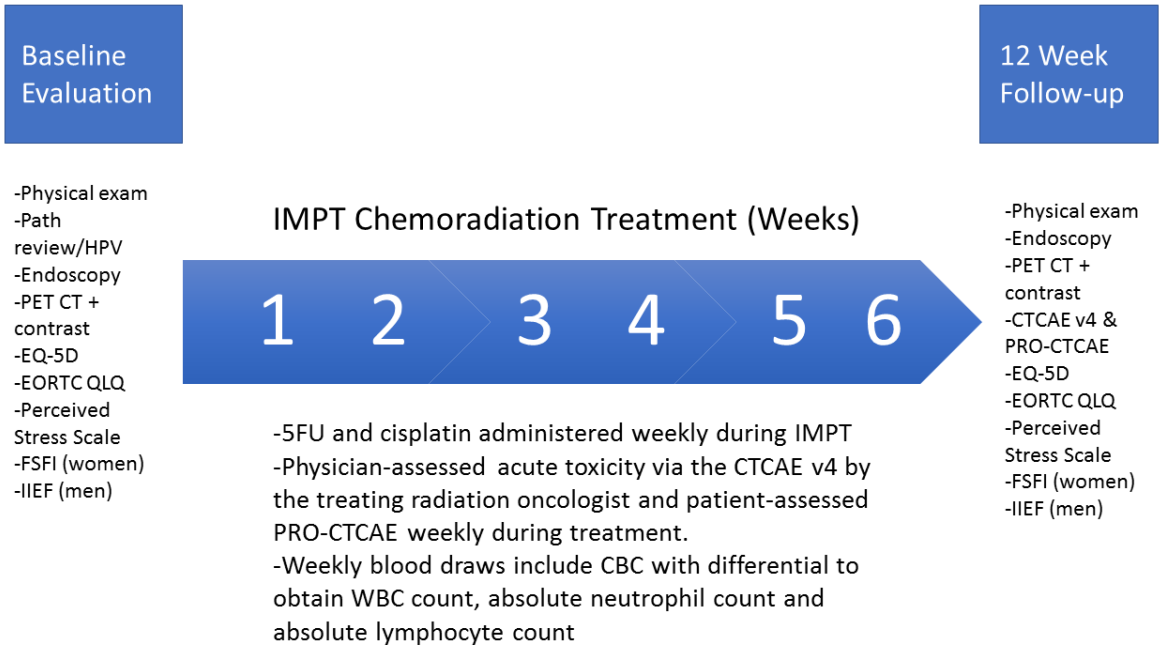


Table 1-

Trial Period:	**Screening Phase**	**On-Treatment Monitoring Visit**						**Post-Treatment**
	Study Screening	Week 1	Week 2	Week 3	Week 4	Week 5	*Week 6*	Survival Follow-Up
Scheduling Window (Days):	4 weeks to 0 days prior to beginning of CRT	Fraction 1-5 of CRT	Fraction 6-10 of CRT	Fraction 11-15 of CRT	Fraction 16-20 of CRT	Fraction 20-25 of CRT	*Fraction 26-29 of CRT if applicable*	12+/-4 wks after end of CRT and then per SOC FU algorithm (Table 2)
Administrative Procedures								
Informed Consent	X							
Medical History	X							
Survival Status								X[c]
Clinical Procedures/Assessments								
Directed Physical Examination	X[a]	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X
ECOG Performance Status	X	X	X	X	X	X	X	X
Radiation CT-Simulation	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	
Physician-assessed CTCAE v4		X	X	X	X	X	X	X[c]
Patient-reported PRO-CTCAE		X	X	X	X	X	X	X
Chemoradiation Treatment		X	X	X	X	X	X	
Laboratory/Imaging/Assessments								
Pregnancy Test – Urine or Serum HCG	X							
CBC with Differential including ANC and ALC	X	X	X	X	X	X	X	X
Quality of Life Questionnaires	X[d]							X[d]
PET/CT + contrast or CT chest/abdomen/pelvis with contrast	X							X
Anoscopy or Flexible Sigmoidoscopy	X							X[e]

Trial Period:	**Screening Phase**	**On-Treatment Monitoring Visit**						**Post-Treatment**
	Study Screening	Week 1	Week 2	Week 3	Week 4	Week 5	*Week 6*	Survival Follow-Up
Scheduling Window (Days):	4 weeks to 0 days prior to beginning of CRT	Fraction 1-5 of CRT	Fraction 6-10 of CRT	Fraction 11-15 of CRT	Fraction 16-20 of CRT	Fraction 20-25 of CRT	*Fraction 26-29 of CRT if applicable*	12+/-4 wks after end of CRT and then per SOC FU algorithm (Table 2)

*All are standard-of-care tests/procedures/charges.

a. The first physical examination during the screening period will be a full examination, all subsequent examinations will be directed

b. Patients will be simulated followed approximately one week or more prior to starting radiation therapy. Initial CT simulation is required for treatment planning, and at least one verification CT simulation is required during treatment, but verification CT simulations can be done as often as weekly at the discretion of the treating physician.

c. In lieu of an in person visit, all subsequent follow ups after the first follow up can be conducted over the phone. In these instances a directed physical examination will not be required and appropriate outside imaging scans must be utilized to assess response.

d. EQ-5D, EORTC QLQ -C30, Perceived Stress Scale, FSFI (women) and IIEF (men).

e. Baseline and 12 week follow up endoscopy must be performed at MD Anderson. Schedule of follow up endoscopies after the 12 week follow up will be decided by the managing team based on findings.

Figure 2 and Table 1 above summarize the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. Patients are strongly encouraged to maintain follow up at MD Anderson. If unable to do so due to unexpected logistical or financial consideration, patients will be advised to maintain the follow up schedule with an outside oncologist as per the current standard of care- and have all imaging and clinical documentation forwarded to MD Anderson. Additional history and/or QOL instrumens will be emailed via REDCap or collected over the phone as needed by the study team. The first follow up study visit as well as all required tests at 12+/-4 weeks after compleition of CRT will be required to be at MD Anderson.

6.1 Pretreatment Procedures

6.1.1 Patients will be screened for eligibility from 30 days prior to the start of CRT to the day of the start of CRT (provided that consents are signed before radiation treatment starts) (see section 4.0). A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

6.1.2 The treating physician or qualified designee will perform a full physical examination at the first pretreatment evaluation including documentation of the primary anal lesion size and distance from the anal verge up to 30 days prior to registration.

6.1.3 The treating physician or qualified designee will record weight at the first pretreatment evaluation, weekly clinic visits and during follow-up).

6.1.4 The investigator or qualified designee will assess performance status using the Eastern Cooperative Oncology Group (ECOG) Performance Scale at screening and at every subsequent study visit.

6.1.5 Informed consent: the investigator must obtain document informed consent from each potential subject prior to participating in a clinical trial (Appendix).

6.1.6 MD Anderson pathology review of the biopsy specimen (if not performed at this institution) for histologic confirmation of squamous cell carcinoma. Human Papilloma Virus (HPV) determination will be noted from the biopsy specimen if available.

6.1.7 Endoscopic examination of the anal canal on either sigmoidoscopy or rigid proctoscopy will be completedprior to initiation of CRT and 7 days after initiation of CRT (although endoscopic examination prior to the start of CRT is strongly encouraged).

6.1.8 Contrast-enhanced PET/CT scan or CT scan of the chest, abdomen and pelvis with contrast up to 60 days prior to initiation of CRT unless the patient has a documented contrast allergy.

6.1.9 Baseline labs will be obtained, including complete blood count (CBC: hemoglobin, hematocrit, WBC with differential blood cell counts (neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils) and platelets. Women of child-bearing potential require a urine pregnancy test (beta-hCG) prior to simulation.

6.1.10 Patients will report baseline symptoms and quality of life with the following instruments as outlined below. Questionnaires will be administered electronically and paper copies of the questionnaires will be available if necessary.

- The EQ-5D-5L
- The EORTC QLQ-C30 will be utilized to report GI-specific quality of life
- The Perceived Stress Scale
- For female patients:
  Female Sexual Function Index (FSFI)- 19 question survey validated in cancer survivors.
- For male patients:
  Individual Items of International Index of Erectile Function Questionnaire (IIEF).

6.2 On-treatment Procedures

6.2.1 Radiation treatment will be precede approximately 1 week or more by radiation treatment planning simulation consisting of a non-contrast CT scan in the treatment position. At least one verification CT scan in the treatment poision is required after the initiation of treatment. The treating radiation oncologist should review the internal anatomy on the verification scan with respect to the initial treatment planning scan and review the target coverage as well as doses to normal organs on the verifcations scan with respect to the initial treatment plan. Adaptive plans can be generated if deemed clinically necessary by the treating radiation oncologist. Additional verifaction scans can be obtained up to weekly if deemed necessary by the treating radiation oncologist. See section 5.1 for further details on radiation treatment.

6.2.2 Chemoradiation will be administered using doses of both radiation and chemotherapy consistent with the current institutional standard of care (further details provided in sections 5.1 and 5.2).

6.2.3 On-treatment evaluation consists of weekly clinic visits with the treating radiation oncologist who will assign CTCAE v4 toxicity grades of hematologic, gastrointestinal, genitourinary and dermatologic side effects GI: nausea, vomiting, diarrhea, constipation, anal pain, proctitis, and fecal incontinence; GU: urinary frequency, urinary urgency, urinary tract pain, urinary incontinence, vaginal pain (women only), and perineal pain; Dermatological: radiation dermatitis). Patients will also report toxicity using the PRO-CTCAE. Weekly blood work will be monitored by both the treating medical and radiation oncologists including: complete blood count (CBC: hemoglobin, hematocrit, WBC with differential blood cell counts (neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils, platelets, and febrile neutropenia). A focused physical examination, weight and ECOG performance status will be assessed at each weekly visit.

6.2.4 For all patients enrolled on study, charges and TDABC data will be collected from the date of initial consultation until the 12 week follow up. Data will capture all visits, treatments, procedures, interventions, emergency room visits and hospital admissions within the MDACC system. Total cost of care will be calculated for each enrolled patient. Total professional and technical charges will also be calculated for each enrolled patient with the assistance of the billing department.

6.3 Post-treatment Follow-up Procedures

6.3.1 The treating physician or qualified designee will perform a focused physical examination at the first follow-up evaluation including documentation of clinical response to treatment (complete clinical response, partial clinical response/residual disease, or progressive disease). .

6.3.2 The treating physician or qualified designee will record weight at the first follow-up evaluation.

6.3.3 The investigator or qualified designee will assess performance status using the Eastern Cooperative Oncology Group (ECOG) Performance Scale at the first follow-up evaluation.

6.3.4 Endoscopic examination of the anal canal on either anoscopy, sigmoidoscopy or rigid proctoscopy at the time of the first follow-up evaluation (12+/-4 weeks from completion of CRT).

6.3.5 Contrast-enhanced CT scan of the chest, abdomen and pelvis with contrast at the time of the first follow-up evaluation (12+/-4 weeks from completion of CRT) unless the patient has a documented contrast allergy or inadequate renal function. The CT will be read by a staff radiologist as per the current standard of care. Dimensions of any residual tumor should be recorded where applicable.

6.3.6 Labs will be obtained at the first follow-up evaluation including complete blood count (CBC: hemoglobin, hematocrit, WBC with differential blood cell counts (neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils) and platelets.

6.3.7 At the first follow-up evaluation, patients will report symptoms and quality of life with the following instruments as outlined below. Questionnaires will be administered electronically and paper copies of the questionnaires will be available if necessary.

- The PRO-CTCAE
- The EQ-5D.
- The EORTC QLQ-C30 will be utilized to report GI-specific quality of life
- The Perceived Stress Scale
- For female patients:
  Female Sexual Function Index (FSFI)- 19 question survey validated in cancer survivors.
- For male patients:
  Individual Items of International Index of Erectile Function Questionnaire (IIEF).

6.3.8 Patients should be followed every 12+/-4 weeks for the first two years per the current standard of care with physical exam, labs, imaging and endoscopy at the discretion of the treating physicians (See Table 2). Only the first follow-up visit will be required on study. However, the questionnaires mentioned in 6.3.7 above will be sent via email using REDCap at 6 month intervals for voluntary participation for 24 months after treatment completion. Patients returning for follow up at MD Anderson will have their medical records queried for response rate, local progression free survival, distant metastasis-free survival and overall survival at each subsequent visit for 48 months after treatment completion.

Table 2

Time from Completion of Treatment	Evaluation	Team Providing Clinical Follow-uo
3 months	PE, CT, scope	MO, XRT, CRS
6 months	PE, scope	MO + CRS*
9 months	PE	XRT
12 months	PE, CT, scope	MO + CRS
15 months	PE	XRT
18 months	PE, CT (CT not needed if <T3 or <N2)	MO
21 months	PE	XRT
24 months	PE, CT, scope	MO + CRS
30 months	PE	XRT
36 months	PE, CT, scope	MO
42 months	PE	XRT
48 months	PE, CT	MO
>48 months		Survivorship or discharge to PCP for routine care

**initial visits with CRS for proctoscopy until complete response or disease persistence is established.*

PE = physical exam, CT = computed tomography, MO = medical oncology, XRT = radiation oncology, CRS = colorectal surgery, PCP = primary care physician.

6.4 Administrative/Other Procedures

6.4.1 Subject Withdrawal/Discontinuation Criteria: Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator if enrollment onto the trial is inappropriate, if the trial plan is violated, or for administrative and/or other safety reasons.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal withdraws consent.
- Inter-current illness that prevents further monitoring.
- Investigator's decision to withdraw the subject.
- The subject has a confirmed positive serum pregnancy test.
- Noncompliance with trial treatment or procedure requirements.
- The subject is lost to follow-up.

Additional subjects may be enrolled on an as-needed basis to substitute for patient's removed prior to the first follow up visit after chemoradiation.

6.4.2 Adverse Event (AE) Monitoring: The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period. Toxicities will be characterized regarding

seriousness, causality, grading, and action taken with regard to trial treatment. Grading will be determined utilizing CTCAE v. 4.0.

6.4.3 Trial Database: All data on trial including toxicities, demographics, medical history, and treatment response will be entered into a trial-specific REDCap database.

6.5 Correlative Analyses

6.5.1 Both traditionally-optimized IMPT and LET-optimized IMPT plans will be generated for each patient enrolled on study. The goal of LET-optimized IMPT is to maximize the LET within the target and minimize it in normal tissues while maintaining the coverage requirements for target volumes and dose volume constraints for OARs expressed in terms of (RBE=1.1) weighted doses. This requirement is imposed to assure safety and for consistency with the current practice. The difference in dose distribution between IMPT and LET-optimized IMPT plans is expected to be minimal to moderate. Even though constraints are fully met, due to possible redistribution of high LET values from normal tissues into target volumes in LET-optimized IMPT plans, for some cases mean dose to surrounding critical organs may be increased. Our goal is to evaluate the safety of such differences in dose distributions.

6.5.2 Blood will be drawn at baseline, weekly during chemoradiation and at the 12-week post-treatment follow up visit as per standard of care (Table 1). WBC, ANC and ALC values will be recorded from the standard of care CBC drawn and their nadirs will be correlated with radiation dose to the pelvic bone marrow.

7.0 STASTICAL PLAN:

7.1 Data Collection, Management and Statistical Summary

Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MD Anderson [27]. REDCap (http://www.project-redcap.org) is a secure, web-based application with controlled access designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless downloads to common statistical packages; and 4) procedures for importing data from external sources. In the case of multi-center studies REDCap uses Data Access Groups (DAGs) to ensure that personnel at each institution are blinded to the data from other institutions. REDCap (https://redcap.mdanderson.org) is hosted on a secure server by MD Anderson Cancer Center's Department of Research Information Systems & Technology Services. REDCap has undergone a Governance Risk & Compliance Assessment (05/14/14) by MD Anderson's Information Security Office and found to be compliant with HIPAA, Texas Administrative Codes 202-203, University of Texas Policy 165, federal regulations outlined in 21CFR Part 11, and UTMDACC Institutional Policy #ADM0335. Those having access to the data file include the study PI and research team personnel. All protected health information (PHI) will be removed from the data when it is exported from REDCap for analysis. All dates for a given patient will be shifted by a randomly generated number between 0 and 364, thus preserving the distance between dates. Dates for each

patient will be shifted by a different randomly generated number. Following publication study data will be archived in REDCap.

General descriptive statistics will be computed to report toxicity and outcome metrics. In addition, descriptive statistics will describe pretreatment patient demographics including age, gender, and performance status as well as disease-related characteristics including disease tumor and nodal stage. All time to event analyses will start from the time of enrollment.

7.2 Enrollment

Anticipated enrollment is 1 patient every other month. We should be able to enroll 48 evaluable patients over 8 years. We anticipate 8 patients may not be evaluable such that we will have a total of 40 evaluable patients on this trial. Following enrollment of the last patient, we anticipate 6 months of follow up to obtain adequate toxicity data for the primary endpoint. Thus, we anticipate that this trial will take approximately 6-8 years to complete.

7.3 Evaluability

To be evaluable for toxicity, patients must have at least one weekly assessments of adverse events (CTCAEv4).

7.4 Statistical Analysis Plan

7.4.1 Primary Clinical Endpoint: *To assess physician-reported acute grade 3 or greater gastrointestinal, genitourinary and hematologic toxicies at 12 weeks post-treatment for patients treated with linear energy transfer (LET)-optimized, intensity-modulated proton therapy (IMPT) and compare to contemporary controls treated with VMAT to determine the feasibility of this outcome for a future randomized trial.*

- At baseline, weekly during treatment, and at the 12 week post-treatment follow up appointment, the treating physician will document physician-assessed acute hematologic, gastrointestinal, genitourinary, dermatologic and other toxicities according to the Common Toxicity Criteria for Adverse Events v-4 (CTCAE v4).
- For each cumulative physician-reported toxicity (hematologic, gastrointestinal, genitourinary, dermatologic and other) at 12-weeks post-treatment, we will tabulate results by type, grade, and attribution and compare with contemporary controls treated with standard of care VMAT-based chemoradiation with 5FU and cisplatin for non-metastatic anal squamous cell carcinoma manually and individually matched for (in order): T-stage, radiation dose, age and gender. We will compare toxicity rates using a McNemar's chi-squared test for paired proportions.
- A separate PA protocol will be submitted to retrospectively review the data from these contemporary controls.
- Subset analyses will be carried out to evaluate the effect of other variables that may also impact toxicity in addition to radiation modality (IMPT vs VMAT).

On RTOG 0529, patients treated with dose-painted IMRT-based concurrent CRT for anal cancer had reported worst overall G3 or greater toxicity rates of 83%. GU/GI G3 or greater toxicity rates were 21%, dermatologic G3 or greater toxicity rates were 23% and hematologic G3 or greater toxicity rates were 58% [4].

To ensure an LET-optimized IMPT-based CRT approach is not overly toxic, we will monitor physician-assessed toxicity using the Bayesian optimal phase 2 (BOP2) design [28]. Specifically, let n denote the interim sample size and N denote the maximum sample size. Let p_{tox} denote the probability of G4 or greater toxicity and define the null hypothesis $H_0: p_{tox} > 0.5$, representing that the treatment is too toxic. We will stop enrolling patients and inspect the safety data for possible trial termination if

$$Pr(p_{tox} \leq 0.5 \mid data) < \lambda(\frac{n}{N})^{\alpha},$$

where λ and α are design parameters optimized to minimize the chance of incorrectly claiming that a safe treatment is unacceptable under the alternative hypothesis $H_1: p_{tox} = 0.3$, while controlling the type I error rate at 0.1 (i.e., the chance of incorrectly claiming that an overly toxic treatment is acceptable is no more than 10%). Assuming a Beta (0.5,0.5) prior distribution for p_{tox}, the above decision rule corresponds to the following stopping boundaries:

Table 3: Optimized stopping boundaries

# patients treated	Stop if # toxicity >=
10	7
20	11
30	14
40	16

Based on Table 3, we perform the interim analysis when the number of enrolled patients reaches 10, 20, 30. When the total number of patients reaches the maximum sample size of 40, we reject the null hypothesis and conclude that the treatment is acceptable if the number of toxicities are less than 16; otherwise we conclude that the treatment is unacceptable. We anticipate we will have to enroll 48 patients to have 40 evaluable patients for this analysis.
Below in Table 4 are the operating characteristics for the safety monitoring based on 10000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org.

Table 4: Operating characteristics

Toxicity rate	Early stopping (%)	Claim acceptable (%)	Sample size
0.5	72.70	7.31	26.7

0.4	30.55	42.83	34.9
0.3	4.93	87.57	39.1
0.2	0.17	99.57	40.0
0.1	0.00	100.00	40.0

7.4.2 Secondary Clinical Endpoints:

- Secondary Endpoint #1: *To assess the feasibility of enrolling patients on a prospective trial delivering LET-optimized IMPT for newly diagnosed, non-metastatic anal cancer.*
  - We will assess accrual at 24 months after the study open to determine feasibility of enrolling 40 patients and obtaining insurance approval for IMPT treatment over the planned 9 years.

- Secondary Endpoint #2: *To develop guidelines and workflow to create and deliver anal canal cancer treatments using LET-optimized IMPT.*
  - We will present the results of our workflows in a descriptive manner.

- Secondary Endpoint #3: *To evaluate complete response rate at 12 weeks and 24 weeks post-treatment.*
  - Complete response will be defined as no evidence of disease by physical exam, endoscopy and cross-sectional imaging.
  - Complete response will be reported at 12 and 24 weeks post-treatment as determined clinically by physical exam, endoscopic examination and imaging.

- Secondary Endpoint #4: *To evaluate local progression-free survival, distant metastasis-free survival and overall survival at 24 and 48 months.*
  - Patients will follow up with their treating physician at every 3-6 months per the current standard of care MD Anderson gastrointestinal center follow-up algorithm (Table 2).
  - Local Failure/Progression: Either recurrence of disease in the anal canal following clearance, progression of disease in the anal canal after completion of treatment or persistence of disease in the anal canal for more than 6 months after completion of treatment.
  - Distant Metastatic Failure: the appearance of distant metastatic disease.
  - Disease-Free Survival: measured as time to locoregional failure, appearance of distant metastases or death due to any cause.
  - Overall Survival: measured as time due to death due to any cause.
    - We will estimate LPFS, DMFS, and OS from time of enrollment using the Kaplan-Meier method and report estimates with appropriate 95% confidence intervals.
    - For local and regional failure, the date of failure will be recorded as 6 months after the completion of treatment in the case of persistent, biopsy-proven disease after 6 months status-post completion of all therapy.

- Secondary Endpoint #5: *To evaluate rates of patient-reported acute toxicity, function, distress and QOL at 12 weeks post-treatment.*

- PRO-CTCAE will be collected weekly during treatment and also at the 12 week post-treatment follow up visit
- EQ-5D will be collected at baseline and also at the 12 week post-treatment follow-up visit
- The EORTC QLQ- C30will be collected at baseline and also at the 12 week post-treatment followup visit
- Treatment-Related Stress Questionnaire and the Perceived Stress Scale will be collected at baseline and also at the 12 week post-treatment followup visit
- For female patients:
    - Female Sexual Function Index (FSFI)- 19 question survey validated in cancer survivors will be collected at baseline and also at the 12 week post-treatment followup visit.
- For male patients:
    - International Index of Erectile Function Questionnaire (IIEF) will be collected at baseline and also at the 12 week post-treatment followup visit
- For patient-reported acute toxicity, function, distress and QOL instruments, we will graph and summarize the distributions of scores for each instrument over time noting how many patients have data at each time point. We will analyze the changes over time using mixed effects linear models.

- Secondary Endpoint #6: *To evaluate rates of patient-reported late toxicity, function, distress and QOL every 6 months for 24 months.*
    - PRO-CTCAE, EQ-5D, EORTC QLQ-C30, treatment-related stress questionnaire, perceived stress scale, female sexual function index, and international index of erectile function questionnaire will be collected every 6 months following treatment until 24 months post-treatment.
    - For patient-reported acute toxicity, function, distress and QOL instruments, we will graph and summarize the distributions of scores for each instrument over time noting how many patients have data at each time point. We will analyze the changes over time using mixed effects linear models.

- Secondary Endpoint #7: *To evaluate the value of proton therapy by comparing Time-Driven Activity-Based Costing data from the date of consultation until the date of the 12-week follow up visit post-treatment with contemporary controls treated with VMAT.*
    - TDABC data will be collected for the entire acute episode of care from the date of consultation until the 12-week follow up post-treatment for all patients using previously generated departmental process maps. Costs will be tabulated as either treatment or toxicity-management related.

  We will then compare these TDABC data with contemporary controls treated with VMAT (as described above) treated during the same fiscal year.

7.4.3 Exploratory Endpoints:

- Exploratory Endpoint #1: *To compare dose to the pelvic bone marrow, bowel, bladder and genitalia between LET-optimized IMPT, traditionally optimized IMPT and VMAT.*
    - Dosimetric studies will be performed comparing the doses from plans generated using the techniques described above.

- We will record dosimetric differences between conventionally optimized (RBE = 1.1) IMPT plans and LET-optimized IMPT plans for analysis using the Wilcoxon rank-sum test. We will also record how often the LET-optimized IMPT plan is chosen by the treating physician over the conventionally optimized (RBE = 1.1) IMPT plan.

- Exploratory Endpoint #2: *To assess rates of leukopenia, neutropenia and lymphopenia at 12-weeks post-treatment for patients treated with LET-optimized IMPT and compare to contemporary controls treated with VMAT.*
  - We will compare the on-treatment blood count nadirs for patients treated with LET-optimized IMPT with matched historical controls treated with VMAT (as described above). Continuous variables will be compared using a Wilcoxon-Rank-Sum test and corresponding p-values will be reported.

- Exploratory Endpoint #3: *To correlate white blood cell counts (WBC), absolute neutrophil counts (ANC) and absolute lymphocyte counts (ALC) with dose to the pelvic bone marrow for patients treated with LET-optimized IMPT.*
  - For each patient treated with LET-optimized IMPT, mean bone marrow dose and bone marrow V10 will be correlated with WBC, ANC and ALC nadir using the Spearman rank correlation coefficient to evaluate for correlation.

- Exploratory Endpoint #4: *To encourage optional co-enrollment on study 2014-0543 so that tumor DNA, rectal microbiome and MRI imaging-based biomarkers can be assessed for patients receiving LET-optimized IMPT and compared with other patients enrolled on 2014-0543 receiving VMAT-based radiation.*
  - Data from tumor swabs, tumor brushings and rectal swabs will be evaluated on 2014-0543 to identify mutations in cancer related genes enriched during the course of chemoradiation. Changes in tumor gene mutations are also correlated with protein phosphorylation status. Additionally, shifts in the microbiome occurring during the course of chemoradiation in the rectal flora.
  - Data from patients coenrolled on 2014-0543 receiving LET-optimized IMPT will be compared with patients enrolled on 2014-0543 only who receive VMAT-based chemoradiation.
  - We will use descriptive statistics and graphical methods illustrate genomic mutations and rectal flora from baseline at each assessment during chemoradiation (week 1, 3 and 5).

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