TITLE: Phase 1-2 study of individualized bone marrow sparing image-guided radiotherapy incorporating novel use of granulocyte-colony stimulating factor and FDG-PET imaging

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Document History	Notes
Version 2 20 May 2020	Initial version
Version 3 25 August 2020	Revision includes: • Calendar changes: Timepoints, Removing serum chemistry, phosphorus labs
Amendment Date not updated	Revision includes: • Table of content changes • Section 2.1.3:Removed additional information • Version date not addended in protocol
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Version 4 4 March 2021	• Protocol stayed the same as 19 January 2021, but version numbers were updated to v4, 3/4/21
Version 5 6 Nov 2021	Revision includes: • Contact changes • Revised eligibility criteria for inclusion 1 • Section 5.1: Cohort size change from 4 to 3 • Section 10.1: Statistics edited based on changes in section 5.1
Version 6 26 May 2022	Revision includes: • Contact changes • Individual patient course schema on page 5 updated to align with revised calendar • Section 2.2: Updated to remove ITA • Section 9: Calendar updated to include AE assessments and updated timepoints
Version 7 10 January 2023	Revision includes: • Contact changes • Section 3.5: Study completion windows extended • Section 7.2: SAE reporting criteria revised • Section 8: Study calendar revised to remove LDH and off study evaluation • Section 9: Study completion windows changed to align with section 3.5
Version 8 21 September 2023	• Contact changes • Added table of changes • Section 9: Post DSMC changes to clarify AE windows and post treatment timepoints

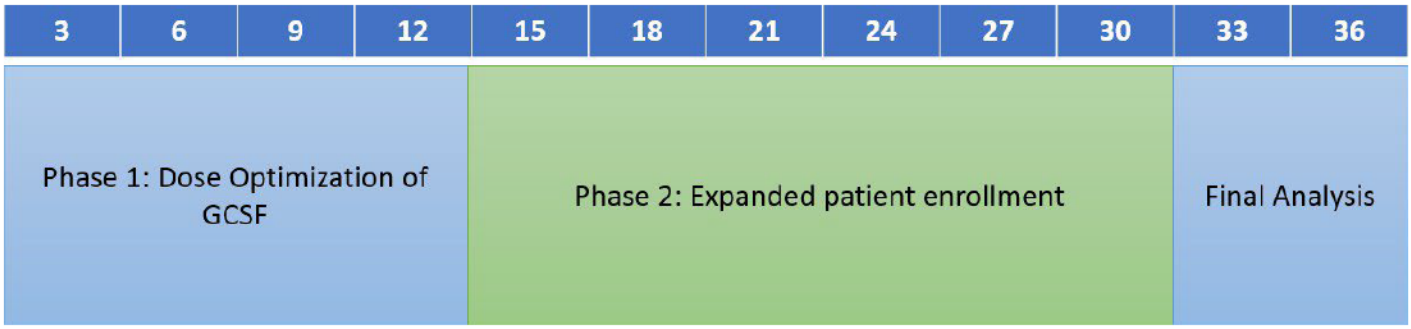
TABLE OF CONTENTS

PROTOCOL SYNOPSIS

TITLE	Phase 1-2 study of individualized bone marrow sparing image-guided radiotherapy incorporating novel use of granulocyte-colony stimulating factor and FDG-PET imaging
STUDY PHASE	Phase 1-2
INDICATION	Gynecologic cancer patients receiving pelvic radiation and chemotherapy, specifically: 1) Stage I-IIIC1 cervix cancer, 2) stage I-III primary vaginal cancer, 3) stage IIIA-IIIC1 endometrial cancer patients status post hysterectomy and lymph node assessment, and 4) recurrent endometrial cancer patients with pelvic confined disease
INVESTIGATIONAL PRODUCT OR PROCEDURE	Using Granulocyte-Colony Stimulating Factor (GCSF) as an imaging bone marrow stimulating agent
PRIMARY OBJECTIVE(S)	1) Determine the optimal dose of GCSF 2) Determine the rate of grade 3 or greater neutropenia during the course of treatment
SECONDARY OBJECTIVE(S)	1) Rate of grade 3 or greater hematologic toxicity; 2) Median time to ANC nadir; 3) Proportion of prescribed chemotherapy cycles completed; 4) Correlation of GCSF-ABM volume sparred ($V_{10\,Gy}$ and $V_{20\,Gy}$) to time to ANC nadir; 5) Correlation of cumulative GCSF-ABM activity spared ($cSUV\,(10\,Gy)$ and $cSUV\,(20\,Gy)$, to time to ANC nadir.
TREATMENT SUMMARY	Participants will have 1-3 doses of GCSF prior to their radiation treatment planning PET/CT. This GCSF stimulated FDG-PET will be used to identify the most relevant active bone marrow to spare with their intensity modulated radiation therapy pelvic radiation. During the chemoradiation +/- adjuvant chemotherapy, participant blood counts will be checked, as per standard of care, and monitored for neutropenia and other hematologic toxicity. The number of chemotherapy cycles completed will also be tracked.
SAMPLE SIZE	6-9 for phase I, 24 for phase II (using 3-9 from phase 1)
STATISTICAL CONSIDERATIONS	Phase I – dose finding with 3 patients per cohort Phase II - sample size goal of 24 patients (including phase I patients who achieve target SUV) to evaluate a reduction in the neutropenia toxicity rate. With this sample size, the trial has 80% power at a 5% significance level to test if the toxicity rate is significantly less than 30%. This calculation assumes an effect size of -0.52 using an arcsine transformation (e.g. target toxicity rate is 10%) and was done in R version 3.5.3 using the function *pwr.p.test()* in the R package 'pwr'.

SCHEMA

Study Timeline by Months



Individual Patient Course

	Enrollment	Day -3	Day -2	Day -1	Day 0	Day 5-10 (prior to Radiation)	Week 2 to 8	Post-tx Week 2 to 6	Post-tx Month 3 to 4
Labs	CBC w/ diff Chem panel		CBC w/ diff	CBC w/ diff	CBC w/ diff	CBC w/ diff	CBC w/ diff (weekly)	CBC w/ diff	CBC w/ diff
Imaging	Diagnostic FDG PET/CT				Treatment Planning FDG-PET/CT				
Intervention		GCSF dose per de-escalation regimen	GCSF dose per de-escalation regimen	GCSF dose per de-escalation regimen					

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABM	Active Bone Marrow
ANC	Absolute Neutrophil Count
BM	Bone Marrow
CBC	Complete blood count
CI	Confidence interval
CRF	Case report/Record form
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
Diff	Differential
DSMB	Data Safety Monitoring Board
FDG	Fluorodeoxyglucose
FLT	Fluorothymidine
GCSF	Granulocyte Colony-Stimulating Factor
Hgb	Hemoglobin
IMRT	Intensity Modulated Radiation Therapy
IRB	Institutional Review Board
IV	Intravenous
OS	Overall survival
PLT	Platelet
PET	Positron Emission Tomography
PFS	Progression free survival
SAE	Serious adverse event
SUV	Standardized Uptake Value
WBC	White blood cell

1. OBJECTIVES

1.1. Primary Objective

1.1.1 Determine optimal dose and duration of GCSF administration to assess and define active pelvic and lumbar bone marrow on PET imaging (GCSF-ABM)

1.1.2 Determine the rate of grade 3 or greater neutropenia during the course of treatment

1.2. Secondary Objectives

1.2 Rate of grade 3 or greater hematologic toxicity;

1.3 Median time to ANC nadir;

1.4 Proportion of prescribed chemotherapy cycles completed;

1.5 Correlation of GCSF-ABM volume sparred ($V_{10\,Gy}$ and $V_{20\,Gy}$) to time to ANC nadir;

1.6 Correlation of cumulative GCSF-ABM activity spared ($cSUV(10\,Gy)$ and $cSUV(20\,Gy)$, to time to ANC nadir.

2. BACKGROUND

2.1 Study Disease

In 2019 75,050 American women are expected to be diagnosed with cervical and endometrial cancer and 16,410 will succumb to the disease [1]. Endometrial cancer, the most common gynecological malignancy in the United States, is one of the few cancers that shows both increasing incidence and worsening survival [1]. Similarly, cervical cancer 5-year survival has shown little improvement over the last 30 years at around 70% [2]. Improving survival for these women remains an unmet clinical challenge.

2.1.1 Chemoradiation-induced hematologic toxicity, in particular grade 3-4 neutropenia, is a major obstacle for promising therapeutic approaches with potential for significant improvement in survival.

One of the most promising treatment approaches to improving survival for cervical and advanced endometrial cancers relates to adding adjuvant chemotherapy following standard chemoradiation. A meta-analysis of 18 chemoradiotherapy studies for cervical cancer found the greatest improvement in survival with the two studies that added adjuvant chemotherapy following chemoradiotherapy [3]. A more recent study showed 9% improvement in progression free survival (PFS) and overall survival (OS) with concurrent and adjuvant cisplatin and gemcitabine [4]. Similarly, for women with high-risk endometrial cancer a Randomized Phase III Trial Comparing Concurrent Chemoradiation and Adjuvant Chemotherapy with Pelvic Radiation Alone in High Risk and Advanced Stage Endometrial Carcinoma (PORTEC-3) showed a ~7% improvement on PFS and 5% in OS [5]. However, elevated Grade 3-4 hematologic toxicity with reported rates of 25% to 67% [4-6]) represents one of the critical clinical challenges for gynecologic patients receiving chemoradiation and adjuvant chemotherapy.

Grade 3-4 neutropenia in particular is a major contributor to hematological toxicity with rates ranging from 20% to 51.2% [4,5,7]. A substantial percentage of hematopoietic function resides in the bone marrow (BM) in the pelvic and lower lumbar areas [8,9]. BM is highly sensitive to radiation [10] and external beam radiation, utilized in the treatment of various abdominopelvic malignancies, results in decreased production of hematopoietic cells. The resultant cytopenias, specifically neutropenia and thrombocytopenia [3,5,11] often cause chemotherapy to not be given. For cervical cancer, concurrent cisplatin will often be stopped in the final weeks if patients develop neutropenia. Similarly, endometrial cancer patients may not be able to receive all four cycles of adjuvant chemotherapy due to hematologic toxicity. All in all, only 70%-75% of the gynecological patients complete their full course of therapy [4,5]. These data underline the importance of optimizing treatment approaches to decrease neutropenia and hematologic toxicity to allow more patients to complete their treatments with correspondingly increased likelihood of cure.

2.1.2. Intensity modulated radiation therapy (IMRT) can potentially lower the risk of neutropenia in individual patients by lowering BM dose but using IMRT to its full benefit requires accessible imaging specific to active BM which is currently lacking.

Existing data suggests that the total pelvic BM volume, proportion of pelvic BM irradiated and type of chemotherapy all impact blood counts and hematologic toxicity [12-14] IMRT represents a way of sculpting radiation dose distributions in order to decrease dose to normal tissues, including BM, while maintaining adequate dose to the tumor [6,15]. In RTOG 0413 (A Phase II Study of IMRT to the Pelvis +/ Chemotherapy for Postoperative Patients with either Endometrial or Cervical Carcinoma) cervical cancer patients in whom 37% of the pelvic bone received dose less than 40 Gy showed significantly less grade 2 or higher hematologic toxicity [6]. This finding strongly suggests that IMRT can indeed be used to spare BM and lower risk of neutropenia. However, given the lymph node regions that need to be treated with radiation, their close proximity to pelvic bones and the other normal tissues (bowel, rectum, bladder) that need to be spared using pelvic IMRT to treat gynecologic cancer, only a relatively small portion of pelvic BM can be kept below the critically low dose thresholds associated with impairment of BM function. *Thus, to optimize IMRT to its full benefit it seems critical to identify and spare the most relevant active BM (**ABM**) portion for preventing or delaying neutropenia. However, as discussed below accessible imaging specific to ABM is lacking.*

Efforts have been made to identify ABM reservoirs of marrow at greatest risk of exposure to pelvic radiotherapy with both fluorodeoxyglucose (FDG) and fluorothymidine (FLT) positron emission tomography (PET) imaging [12,16-20].

FLT-PET ABM imaging is based on the FLT phosphorylation by the enzyme thymidine kinase 1, which leads to intracellular trapping. Because the thymidine kinase 1 concentration increases almost 10-fold during the S phase of the cell cycle, during which DNA synthesis occurs, the FLT uptake intensity on PET bone marrow imaging reflects the proliferation rate of bone marrow [20-22]. Even though FLT-PET is suitable for imaging the distribution of BM proliferative activity FLT is not widely accessible and only available for clinical trials under

Investigational New Device (IND) FDA approval. Moreover, despite being investigated for a number of years it has not yet seen routine clinical adoption because of cost and limited demand.

As an alternative FDG-PET has been suggested to help identify active BM for sparing with IMRT and for potentially decreasing neutropenia [23]. However, BM FDG uptake in the lumber bone as measured by standardized uptake value (SUV) is low (SUV_{mean} ± SD ~ 1.6 ± 0.6) graded as "none" (SUV_{mean} smaller than that of the aortic blood pool) in 57% of the patients, and at most "mild" (SUV_{mean} greater than that of aortic blood pool but less than 2.5) in additional 40% [24]. Furthermore FDG-PET shows both proliferating cells and metabolically active non-proliferating cells. Thus, the low FDG uptake is not specific to active BM and may be misdirecting BM sparing.

Clinical data supports this observation [20]. A comparison of FLT and FDG BM uptake in 9 patients has demonstrated that the discrepancy between FLT and FDG-defined active BM as measured by the Dice similarity coefficient increase with the threshold selected being already low 0.683±0.029 (0.606–0.725) for the top 40% SUV values of the uptake [20]. Furthermore, the inter-patient variability at high SUVs was much higher for FDG-PET in comparison to FLT-PET, as would be expected because of the lower specificity of FDG-PET. Based on these findings Wyss et al. have concluded that *"…FLT-PET is likely superior for BM-sparing strategies, by virtue of its higher interpatient consistency and tendency to identify a more highly concentrated BM sub-volume [20]*".

2.1.3 Administration of Granulocyte Colony-Stimulating Factor (GCSF) prior to FDG-PET increases the specificity of FDG imaging to the depots of precursor cells in the bone marrow and can form the basis for individualized image-guided ABM-sparing IMRT.

Recombinant GCSF (filgrastim) is a known standard agent approved by the United States Food and Drug Administration (FDA) in 1991 to decrease the incidence of infection in patients with non-myeloid malignancies receiving myelosuppressive therapy associated with a significant incidence of febrile neutropenia. Filgrastim and biosimilars have since become mainstays in the supportive care of oncology patients as their safety and efficacy have improved outcomes for high-risk patients receiving dose-intense therapy [25-27]. *GCSF specifically stimulates the proliferation, differentiation, and function of neutrophil precursors and mature neutrophils*. The increased metabolic activity of proliferating and differentiating neutrophil precursors results in corresponding increase of the FDG uptake in the depots of precursor cells in the bone marrow with corresponding manifestation on FGD-PET images. This has been observed consistently both in preclinical and clinical studies [28-32].

The reported increase in uptake is consistent with our own observations, as illustrated in Figure 1 (C) which demonstrates ~2-fold increase in FDG uptake for a patient who received GCSF as supportive therapy during a treatment for Hodgkin lymphoma. ***Error! Reference source not found.*** *(A) and* ***Error! Reference source not found.****(B) demonstrate visually and quantitatively that in the richest ABM depots (****Error! Reference source not found.*** *(D)) there is significant uptake heterogeneity and important discordance (Dice Similarity Coefficient =0.7) between what FDG and GCSF-FDG depict as ABM depots*.

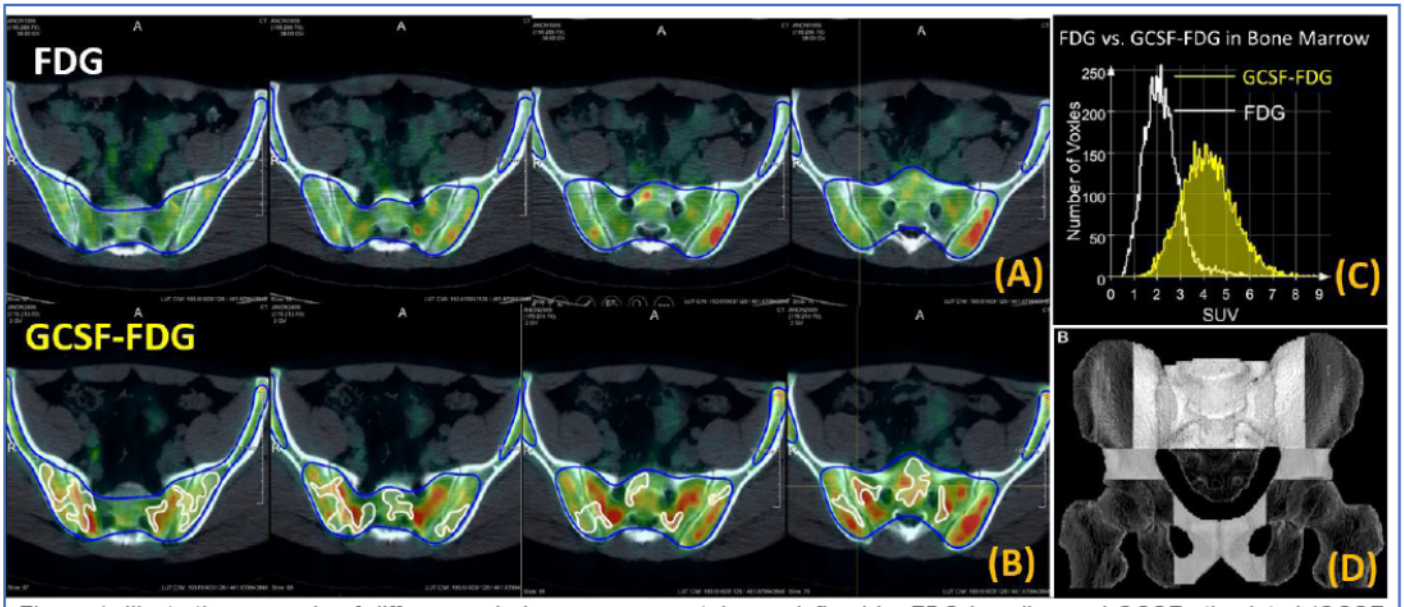


Figure 1. Illustrative example of differences in bone marrow uptake as defined by FDG baseline and GCSF stimulated (GCSF-FDG) mid-treatment PET-CT scans for a patient with Hodgkin lymphoma receiving Stanford V treatment regimen with supportive therapy (GCSF). (A) Representative axial slices from the FDG-baseline scan. (B) Corresponding axial slices from the GCSF-FDG scan. The visualized regions in (A) and (B) correspond to the anatomical area where a previous FLT study of active BM [9] has shown the largest mean SUV uptake (shown in D). Regions of discordance between the volumes encompassing 40% or higher of the respective maximum SUV for each scan are shown with white contours indicating large discrepancies between FDG and GCSF-FDG in depicting the richest active BM depot (Dice Similarity coefficient =0.7). (C) Histogram of the SUV values for both scans evaluated over segmented bone marrow shown with blue contours in (A) and (B). (D) Bone marrow regions grouped by similarity in FLT uptake. The subject average SUV mean is largest in the group identified in white. The group identified in grey has the second largest average SUV mean. The group identified in black has the lowest uptake [9].

These findings form the basis for a novel image-guided therapy strategy we propose to develop and assess in a phase I/II study. Given the ability of GCSF to stimulate the cellular component of marrow - rendering it FDG-avid - we propose to use time and dose optimized GCSF stimulation in combination with accessible FDG-PET imaging to identify the specific locations of neutrophil precursors and thereby localize the most important regions of bone marrow to spare using IMRT.

2.2 Study Agent/Device/Procedure

Administration of Granulocyte Colony-Stimulating Factor (GCSF) prior to FDG-PET increases the specificity of FDG imaging to the depots of precursor cells in the bone marrow and can form the basis for individualized image-guided ABM-sparing IMRT.

Recombinant GCSF (filgrastim) is a known standard agent approved by the United States Food and Drug Administration (FDA) in 1991 to decrease the incidence of infection in patients with non-myeloid malignancies receiving myelosuppressive therapy associated with a significant incidence of febrile neutropenia. Filgrastim and biosimilars have since become mainstays in the supportive care of oncology patients as their safety and efficacy have improved outcomes for high-risk patients receiving dose-intense therapy [25-27]. *GCSF specifically stimulates the proliferation, differentiation, and function of neutrophil precursors and mature neutrophils*. The increased metabolic activity of proliferating and differentiating neutrophil precursors results in corresponding increase of the FDG uptake in the depots of precursor cells in the bone marrow

with corresponding manifestation on FGD-PET images. This has been observed consistently both in preclinical and clinical studies [28-32].

For this study, we aim to enroll 27-33 patients over a span of 4 years with 2018 FIGO stage I-IIIC1 cervix cancer patients without prior treatment and IIIA-IIIC1 endometrial cancer patients status post hysterectomy and lymph node assessment undergoing concurrent chemotherapy with pelvic radiation +/- adjuvant chemotherapy. Patients will undergo the standard of care sequence of lab tests, imaging and interventional procedures with the addition of GCSF administration prior to their treatment planning FDG-PET/CT scan and 3-4 additional CBC lab tests.

Stage 1: Determine the GCSF optimal dose and duration for GCSF-FDG-PET ABM assessment with an initial (minimum of 6 patients and a maximum of 9 patients). Develop IMRT planning tools to spare the maximum cumulative amount of GCSF-ABM imaged by GCSF-FDG-PET.

Stage 2: Expand enrollment to treat a cohort of patients with GCSF-dose optimized, GCSF-ABM sparing IMRT.

GCSF is FDA approved and specifically stimulates the proliferation and differentiation, of neutrophil precursors leading to increased FDG uptake that can be observed on FDG-PET.

The Clinic at Women's Cancer Center at 900 Blake Wilbur will administer GCSF injection (to the first group of patients) subcutaneously on day -3, -2, -1 before initiation of radiation planning scan (the next 2 groups get it less frequently). It will be given in Clinic at Women's Cancer Center. The patient will have a choice about self-administering the GCSF dose for Day -2 and Day -1 or having all three GCSF doses for Day -3, Day -2, and Day -1 administered in Clinic at Women's Cancer Center. Coordinator will call patient to confirm self-administration and collect time dose was administered.

2.3 Rationale

Previous studies show nearly 50% of the total ABM is contained in pelvis and low lumbar spine regions, which receive substantial doses during concurrent chemo-radiotherapy for gynecologic cancer. The extreme radio-sensitivity of ABM necessitates imaging and radiation delivery approaches that uncover and spare the most significant BM depots in the pelvis and the lower lumbar spine regions.
GCSF specifically stimulates the proliferation and differentiation of neutrophil precursors and mature neutrophils. The increased metabolic activity of proliferating and differentiating neutrophil precursors results in corresponding increase of the FDG uptake in the depots of precursor cells in the bone marrow with corresponding manifestation on FGD-PET images. This has been observed consistently both in preclinical and clinical studies [28-32].

2.4 Study Design

- Treatment
- Sequential
- 1 intervention
- Open: no masking is used
- Non-randomized
- Safety/Efficacy

2.5 Correlative Studies Background

No planned correlative studies

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Refer to the Participant Eligibility Checklist in Appendix A.

3.1 Inclusion Criteria

3.1.1 Gynecologic cancer patients receiving pelvic radiation and chemotherapy, specifically: 1) Stage I-IIIC1 cervix cancer, 2) stage I-III primary vaginal cancer, 3) stage IIIA-IIIC1 endometrial cancer patients status post hysterectomy and lymph node assessment, and 4) recurrent endometrial cancer patients with pelvic confined disease No required para-aortic or extended field radiation

3.1.2 Age > 18 years

3.1.3 ECOG performance status 0-2

3.1.4 Adequate kidney function (serum Cr <1.5 or creatinine clearance >50 mg/dl)

3.1.5 Adequate bone marrow function (white blood cells > 3.0 X 10^9/L, platelets >100 x 10^9/L)

3.1.6 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Treatment for other cancer in the past 2 years

3.2.2 Previous pelvic radiation

3.2.3 Medical condition that prevents receiving chemotherapy

3.3 Informed Consent Process

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

3.4 Randomization Procedures

No randomization will be used. Patients will sequentially be entered in the GCSF dosing cohorts, and once optimal dose is determined all participants will receive that designated dose of GCSF.

Dosing will occur in cohorts of 3 patients. Dosing will occur in cohorts of 3 patients and start at dose 780 mcg x 3 days. After the first cohort of patients, the trial design will de-escalate

as follows:

If 3 out of 3 patients achieve the target SUV_{mean}, the next cohort of patients will de-escalate to 780 mcg/d x 2 days (Day -2, Day -3). If target SUV_{mean} is reached in 3 out of 3 patients for this cohort, the last cohort will receive 780 mcg/d x 1 day (Day -2). De-escalation continues until the target SUV_{mean} is no longer met in 3 out of 3 patients, and the previous dose achieving the target SUV_{mean} in 3 out of 3 patients will be RP2D.

3.5 Study Timeline

Primary Completion:

The study will reach primary completion 48 months from the time the study opens to accrual.

Study Completion:

The study will reach study completion 60 months from the time the study opens to accrual.

4. TREATMENT PLAN

Participants will undergo standard of care diagnostic imaging, CBC with differential and chemistry labs initially. While receiving the GCSF, participants will have a CBC with differential checked a day after each dose and prior to radiation. The treatment planning FDG-PET/CT will be performed shortly after the GCSF, as detailed below. During chemoradiation, participants will have weekly CBC with differential checked and other labs as standard of care.

	Enrollment	Day -3	Day -2	Day -1	Day 0	Day 5-10 (prior to Radiation)	Week 2 to 8	Post-tx Week 2 to 6	Post-tx Month 3 to 4
Labs	CBC w/ diff Chem panel		CBC w/ diff	CBC w/ diff	CBC w/ diff	CBC w/ diff	CBC w/ diff (weekly)	CBC w/ diff	CBC w/ diff
Imaging	Diagnostic FDG PET/CT				Treatment Planning FDG-PET/CT				
Intervention		GCSF dose per de-escalation regimen	GCSF dose per de-escalation regimen	GCSF dose per de-escalation regimen					

4.1 General Concomitant Medication and Supportive Care Guidelines

Medication or treatments will be given as per standard of care. Radiation treatment doses will be as per standard of care for pelvic radiation.

Participants of the study will receive 1-3 doses of GCSF and 3-4 additional CBC with differential. Otherwise, treatments and labs are all per standard of care management.

4.2 Criteria for Removal from Study

If participants had unexpected adverse events or poor tolerance to the GCSF they would be removed from the study. If more than one patient experienced the same adverse event from the GCSF we would re-evaluate the treatment plan and could consider lowering the GCSF dose.

4.3 Alternatives

GCSF is a growth factor approved by the FDA close to 30 years ago and generally well

tolerated. In undergoing standard of care chemoradiation patients receive regular blood draws, and 3-4 additional blood draws would be done as part of this study.

Patients may decline participation in the study and receive standard of care treatment.

5. INVESTIGATIONAL AGENT/DEVICE/PROCEDURE INFORMATION

5.1 Investigational Agent/Device/Procedure

GCSF bind to the GCSF receptors present on neutrophil precursors, these cells proliferate and differentiate into mature neutrophils. This activity leads to increased uptake on FDG-PET/CT.

The initial cohort will receive 780 mcg GCSF (approximately 10 mcg/kg) for three consecutive days, starting Day -3. If 3 out of 3 patients achieve the target SUVmean, the next cohort of patients will de-escalate to 780 mcg/d x 2 days (Day -2, Day -3). If target SUVmean is reached in this cohort, the last cohort will receive 780 mcg/d x 1 day (Day -2). De-escalation will continue until the target SUVmean is no longer met in all 3 patients, and the previous dose achieving the target SUVmean will be declared RP2D.

If in the first cohort, 1 or more patients (out of 3) does not achieve the target SUVmean, the next cohort of patients will escalate by adding additional days of GCSF injection with 780 mcg/d. Escalation will continue until the target SUVmean is met in all 3 patients or the highest dose of 5 d x 780 mcg/d is reached.

Phase 2 participants will be treated with the optimal dose found in the phase 1 portion of the study.

5.2 Availability

GCSF will be provided by the Stanford pharmacy.

6. DOSE MODIFICATIONS

We do not anticipate the need for dose modification, except as per the dose finding protocol as discussed above, as similar dose regimens are given in the supportive setting for 5 or more days with good tolerability.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Potential Adverse Events

Research procedures added to the standard of care pathway for participants include: (1) GCSF administration of 780 mcg/d in 1-3 days prior to start of chemoradiation and (2) 2-4 additional blood draws during the course of the study.

The risk to human subjects from GCSF is low as this is an FDA approved growth factor that has been safely used in cancer patients for close to 30 years. The possible side effects participants could experience include possible bone pain that can be controlled with Tylenol or Claritin, increased white blood cell counts and possible tiredness. The timing of GCSF administration is selected to allow for blood counts post-GCSF to return to baseline normal values prior to the

commencement of chemoradiation without disrupting the standard treatment timeline.

Blood draws have the risk of patient discomfort and very low risk of infection. Patients undergoing standard chemotherapy and radiation also routinely undergo blood draws.

7.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE v4.03. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation. Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen over time are not considered adverse events.

All Serious Adverse Events (SAEs) within 30 days after the last dose of GCSF will be tracked until resolution.

SAEs CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) & IRB per institutional guidelines.

Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol per institutional and regulatory guidelines.

8. CORRELATIVE/SPECIAL STUDIES

No plan for correlative studies.

9. STUDY CALENDAR

Schedules shown in the Study Calendar below.

	Pre-Study	Pre-tx Day -3 [d]	Pre-tx Day -2 [d]	Pre-tx Day -1 [d]	Tx Day 0	Tx Day 5-10[e]	Week 2 to 8	Post-tx Wk 2 to 6[g]	Post-tx Mo 3 to 4[g]
Informed consent	X								
Medical history	X								
Physical examination	X								
Performance status	X								
CBC w/differential	X		X	X	X	X	X [e]	X	X
Serum chemistry [b]	X								
Diagnostic Imaging (FDG-PET/CT)	X								
B-HCG	X[c]								
GCSF		X [a]	X [a]	X [a]					
Adverse Event [f]		X	X	X			X		
Treatment Planning (PET/CT)					X				
Chemo Radiation Treatment							X		
Adjuvant Chemo (if applicable)								X	

a: Investigational Agent: Dose as assigned; based on cohort (please refer to section 10.1).
b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.
c: Pregnancy test (women of childbearing potential) Prior to chemo radiation treatment
d: Prior to chemo radiation treatment
e. Occurs weekly during chemotherapy.
f. Adverse event monitoring will only be done starting from day after last GCSF injection and until 30 days post GCSF injection. The adverse event assessment can be done via a phone call by a research coordinator with PI oversight.
g. Post treatment windows will be based on end of brachytherapy,

10. MEASUREMENTS

Primary Outcome Measure Phase 1

Title: Determine the optimal dose of GCSF

Time Frame: 12-24 months

Safety Issue: no

Primary Outcome Measure Phase 2

Title: Determine the rate of grade 3 or greater neutropenia during treatment

Time Frame: 24-48 months

Safety Issue: no

10.1 Primary and Secondary Outcome measures

Phase 1 Endpoint: recommended phase 2 dose (RP2D).

Study schema

With the goal of administering the minimum GCSF dose for the shortest period of time required to stimulate marrow, the recommended phase 2 dose (RP2D) will be defined as the dose at which the standard uptake value (SUV) on D5 FDG-PET reaches target SUV_{mean} of 2.5 or higher with normalization of WBC and ANC prior to start of radiation.

Dosing will occur in cohorts of 3 patients and start at dose 780 mcg x 3 days. After the first cohort of patients, the trial design will de-escalate as follows:

If 3 out of 3 patients achieve the target SUV_{mean}, the next cohort of patients will de-escalate to 780 mcg/d x 2 days (Day -2, Day -3). If target SUV_{mean} is reached in 3 out of 3 patients for this cohort, the last cohort will receive 780 mcg/d x 1 day (Day -2). De-escalation continues until the target SUV_{mean} is no longer met in 3 out of 3 patients, and the previous dose achieving the target SUV_{mean} in 3 out of 3 patients will be declared RP2D.

The phase 1 study will require a minimum of 6 patients and a maximum of 9 patients to identify the RP2D. The RP2D is the dose that achieves the target SUV_{mean} in 3 out of 3 patients. Previous studies and our experience indicate the target SUV_{mean} will be achieved in the entire initial cohort at 780 mcg/d x 3 days; however, if it is not achieved, upon review the next cohort will escalate to 780 mcg/d for 4 days.

Phase 2 Primary Endpoints:

Primary: Rate of grade 3 or greater neutropenia (ANC < 1000/mm^3 at any point of therapy)

This will be monitored by the regular CBC with differential on the study schema and calendar, as per standard of care for patients receiving chemoradiation and/or chemotherapy.

Secondary Endpoints:

1. Rate of grade 3 or greater hematologic toxicity;
2. Median time to ANC nadir;
3. Proportion of prescribed chemotherapy cycles completed;
4. Correlation of GCSF-ABM volume sparred ($V_{10\ Gy}$ and $V_{20\ Gy}$) to time to ANC nadir;
5. Correlation of cumulative GCSF-ABM activity spared ($cSUV(10\ Gy)$ and $cSUV(20\ Gy)$, to time to ANC nadir.

These will be monitored by the regular CBC with differential on the study schema and calendar, as per standard of care for patients receiving chemoradiation and/or chemotherapy. The amount of GCS-ABM volume spared at dose levels of 10 Gy and 20 Gy will be measured on the treatment planning system.

11. REGULATORY CONSIDERATIONS

11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

11.2 Data and Safety Monitoring Plan

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for this study. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of the following types of documents participating in the study: regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

11.3 Data Management Plan

The Protocol Director, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the OnCore and RedCap database system and will be maintained by the study research coordinator. CRFs will be kept in a locked office, only accessible to the research team.

12. STATISTICAL CONSIDERATIONS

12.1 Statistical Design

This study is a sequential phase 1 GCSF dose finding study involving 6-9 patients, then phase 2 will expand to include a total of 24 patients (including 3-9 patients from phase I who achieve appropriate increase in BM SUV) at the optimal dose found in the phase 1 portion.

12.1.1 Randomization

No randomization

12.2 Interim analyses

No plan for interim analysis

12.3 Descriptive Statistics and Exploratory Data Analysis

Outcomes will be summarized using appropriate descriptive statistics.

12.4 Primary Analysis

The primary objective is to evaluate if the neutropenia toxicity rate is significantly less than 30%, which is the median neutropenia toxicity rate in the literature. In the primary analysis, the two disease groups will be combined given the limited number of patients and similar toxicity profiles between the two groups. However, each disease group will be evaluated separately in a secondary analysis. To evaluate the primary endpoint, a one-sample proportion test will be used at 5% significance level; similarly, we will evaluate if the hematologic toxicity rate is significantly less than 30% and compare the proportion of completed chemotherapy cycles with the historical completion rate of 70%. Lastly, linear regression will be used to estimate correlations and explore strength in degrees of association between each clinical variable and positive clinical outcomes (e.g. shorter time to ANC nadir)

12.5 Secondary Analysis

Linear regression will be used to estimate correlations and explore strength in degrees of association between each clinical variable and positive clinical outcomes (e.g. shorter time to ANC nadir)

12.6 Sample Size

12.6.1 Accrual estimates

At least 40 patients meeting eligibility criteria are seen each year.

12.6.2 Sample size justification

Sample Size:

Patients achieving the target SUV in phase 1 will be used for the phase 2 study. The phase 2 study has a sample size goal of 24 evaluable patients to evaluate a reduction in the neutropenia toxicity rate, where evaluable indicates the target SUV has been achieved. With this sample size, the trial has 80% power at a 5% significance level to test if the toxicity rate is significantly less than 30%. This calculation assumes an effect size of -0.52 using an arcsine transformation

(e.g. target toxicity rate is 10%) and was done in R version 3.5.3 using the function pwr.p.test() in the R package 'pwr'. Across both phase 1 and 2, the study will allow for 2 patients to be replaced due to drop-out. In phase 2, the study will allow for up to 9 additional patients to be enrolled if the target SUV is not met in the initial 24 patients.

12.7 Criteria for future studies

If the study meets the primary objective of showing grade 3 or greater neutropenia being significantly lower than historical we will plan to expand this study to a larger cooperative group study. Similarly, if participants are able to complete a greater proportion of chemotherapy and/or have delayed time to ANC nadir, we will also consider expanding to a larger cooperative group study.

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APPENDICES

APPENDIX A: Participant Eligibility Checklist

A Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration. The completed, signed, and dated checklist must be retained in the patient's study file and the study's Regulatory Binder.

The study coordinator, treating physician and an independent reviewer must verify that the participant's eligibility is accurate, complete, and legible in source records. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

The following is an **example** of a Participant Eligibility checklist template. Modify this checklist to fit your study and include it in the appendix section of your protocol document. The protocol-specific checklist is **required** by the SRC and must be approved by the IRB.

Protocol Title:	Phase 1-2 study of individualized bone marrow sparing image-guided radiotherapy incorporating novel use of granulocyte-colony stimulating factor and FDG-PET imaging
Protocol Number:	**IRB-56842** **GYN0007**
Principal Investigator:	Elizabeth Kidd MD

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB-approved protocol) (subject must have ALL of the following)	**Yes**	**No**	**Supporting Documentation***
1. Gynecologic cancer patients receiving pelvic radiation and chemotherapy, specifically: 1) Stage I-IIIC1 cervix cancer, 2) stage I-III primary vaginal cancer, 3) stage IIIA-IIIC1 endometrial cancer patients status post hysterectomy and lymph node assessment, and 4) recurrent endometrial cancer patients with pelvic confined disease	☐	☐	
2. No required para-aortic or extended field radiation	☐	☐	
3. Age > 18 years	☐	☐	
4. ECOG performance status 0-2	☐	☐	
5. Adequate kidney function (serum Cr <1.5 or creatinine clearance >50 mg/dl)	☐	☐	
6. Adequate bone marrow function (white blood cells > 3.0 X 109/L, platelets >100 x 10^9/L)	☐	☐	

Exclusion Criteria (From IRB approved protocol) (subject must not have ANY of the following)	**Yes**	**No**	**Supporting Documentation***
1. Treatment for other cancer in the past 2 years	☐	☐	
2. Previous pelvic radiation	☐	☐	
3. Medical condition that prevents receiving chemotherapy	☐	☐	

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	