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Using FLT PET to quantify and reduce hematologic toxicity due to chemoradiation therapy for pelvic cancer patients

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Abstract

Purpose—The purpose of this prospective clinical trial was to determine the efficacy of FLT identified active bone marrow sparing for pelvic cancer patients by correlating FLT uptake change during and after chemoradiation therapy to hematologic toxicity.

Methods—Simulation FLT PET images were used to spare pelvic bone marrow using IMRT (IMRT BMS) for 32 patients with pelvic cancer. FLT PET imaging taken after 1 and 2 weeks of chemoradiation therapy and 30 days and 1 year after chemoradiation therapy was complete were used to evaluate the acute and chronic dose response of pelvic bone marrow. CBCs were recorded at each imaging time point to correlate FLT uptake change to systemic hematologic toxicity.

Results—IMRT BMS plans significantly reduced dose to pelvic regions identified with FLT uptake when compared to control IMRT plans ($p < 0.001$ paired t-test). Radiation doses of 4Gy caused an ~50% FLT uptake decrease in pelvic bone marrow after either 1 or 2 weeks of chemoradiation therapy. Additionally, subjects with more FLT identified bone marrow exposed to 4 Gy after 1 week developed grade 2 leukopenia sooner ($p < 0.05$ Cox regression). Apparent bone marrow recovery at 30 days post therapy is not maintained 1 year after chemotherapy. FLT uptake in pelvic bone marrow regions that received > 35 Gy was 18.8 ± 1.8 % greater 30 days post therapy than 1-year post therapy. WBCs, platelets, lymphocytes, and neutrophils 1-year post therapy were all lower when compared to pretherapy levels ($p < 0.05$, paired t-test).

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Conclusions—IMRT BMS plans reduced dose to FLT identified pelvic bone marrow for pelvic cancer patients, but reduction in hematologic toxicity is challenging due to acute radiation sensitivity (~4 Gy), and chronic suppression of bone marrow activity that receives radiation doses > 35 Gy as measured by FLT uptake change correlated to CBCs.

Keywords

Pelvic cancer; IMRT; FLT PET; bone marrow

Introduction

Chemoradiation combinations improve outcomes for pelvic cancer patients, but at the cost of higher levels of hematologic toxicity. This may be more significant for pelvic cancer patients because approximately one third of adult bone marrow is located in the pelvic region [8,10,14]. Significant numbers of cervical cancer patients undergoing chemoradiation have increased hematologic toxicity (HT) compared to radiation therapy (RT) alone, leading to missed chemotherapy cycles and worse progression-free and overall survival [1,4,12,23–25]. Reducing hematologic toxicity may also benefit anal [9,11,20], and rectal cancer patients [6].

Limiting the radiation dose to proliferating pelvic bone marrow may reduce hematologic toxicities. Uptake of [^{18}F]fluorothymidine imaged with positron emission tomography (FLT PET) can identify active bone marrow [2]. This anatomically localized, active marrow distribution information can be incorporated as avoidance regions in RT plans and subsequently used to monitor the effects of chemoradiation on proliferative pelvic bone marrow [3,15,19]. This study examines the ability to reduce radiation dose to FLT identified active bone marrow for pelvic cancer patients and correlate FLT uptake change during and after chemoRT to hematologic toxicity, missed chemotherapy cycles, and chronic systemic effects.

Methods

Subjects with pelvic cancer requiring chemoRT were accrued prospectively to two IRB approved studies designed to quantify changes in pelvic bone marrow activity using FLT PET scans before, during, and after chemoRT (NCT01075412 or NCT01717391). FLT PET scans analyzed for this study were acquired during RT simulation, after 1 (5 RT fractions) and 2 weeks (10 RT fractions) of chemoRT, and 30 days and 1-year after chemoRT was completed. FLT PET images acquired during therapy were only scheduled according to the radiation therapy schedule. Week 1 images were acquired after the 5th fraction and before the 6th fraction and Week 2 images were acquired after the 10th fraction and before the 11th fraction. Most subjects received an FLT PET scan on the same day as the 5th and 10th fraction. Chemotherapy administration was not considered in scheduling FLT PET imaging. The most common regimens were for cervical (up to 6 cycles of weekly cisplatin started on the first day of RT), rectal (either continuous infusion of 5-FU or oral capecitabine BID), and anal (one or two four day infusions of 5-FU and mitomycin delivered during the first and fourth weeks of RT) cancers.

Subjects

Subject eligibility criteria included: understanding of informed consent, recommendation for pelvic irradiation with concurrent chemotherapy, 18 years of age, Karnofsky 60%, life expectancy > 6 months, normal organ and marrow function within 30 days of enrollment (leukocytes 3,000/ μ L, absolute neutrophil count 1,500/ μ L, platelets 100,000/ μ L, and creatinine 1.4 mg/dl), and negative pregnancy status. Subjects received chemotherapy as prescribed by the medical or gynecologic oncologist consistent with National Comprehensive Cancer Network guidelines [22]. The chemotherapy regimen varied based on the individual patient and disease site, but in all subjects consisted of cisplatin, 5-fluorouracil (5-FU), or a combination of mitomycin and 5-FU. 5-FU was administered intravenously or as the oral prodrug, Xeloda® (capecitabine).

FLT PET Imaging

FLT was produced based on the methods described by Machulla et al [13] and met all the requirements of the physician-sponsored FDA IND. Whole-body (WB) FLT PET images were obtained on a Siemens Biograph 40 PET/CT scanner (Siemens Medical Solutions USA, Inc, Malvern, PA) from the base of brain to the proximal thighs (3 minutes/bed position) at approximately 60–70 minutes post-administration of FLT (0.07 mCi/kg (2.6 MBq/kg), maximum dose = 5 mCi (185 MBq)).

The WB FLT PET image used to identify bone marrow during RT planning was acquired with the subject immobilized in the simulation position. After FLT PET image acquisition, the simulation RT planning CT was obtained. The simulation CT, the attenuation correction CT (AC CT), and FLT PET images were transferred to the Pinnacle Treatment Planning system v9.2 (Philips Medical Systems, Fitchburg, WI). The AC CT obtained for the PET image was registered to the simulation CT used as the primary planning data set. This registration matrix was then copied to the FLT PET image to register the FLT PET image to the simulation CT. FLT PET images used to quantify acute and chronic bone marrow response during and after chemoRT were also acquired with the subjects in the treatment position to aid registration. These FLT PET images were also registered to the simulation CT by transferring the transformation matrix from the AC CT to simulation CT registration in the treatment planning system.

Radiation Therapy Treatment Planning

RT plans were created for each subject based on tumor site. Contouring of the tumor and normal anatomy was performed on the simulation CT with the aid of secondary images as requested by the radiation oncologist. The target volumes for these patients were created according to the techniques appropriate for each disease site and determined by the radiation oncologist. Bone, bowel, rectal, bladder, and muscle tissues were removed from the Clinical Target Volume (CTV) appropriate for each site. A 5mm margin was added into bone and a 7mm margin was added in all other directions to the tumor and nodal CTVs to create the Planning Target Volume (PTV) for both the tumor and nodal volumes. Two sets of plans were created: a conventional IMRT plan (IMRT Plan), and a bone marrow sparing IMRT plan (IMRT BMS Plan) that incorporated the FLT PET images to create bone marrow avoidance regions of interest (ROIs) as previously described [17]. Bone marrow ROIs were

defined by thresholds of FLT standard uptake values (SUV) of 2, 3, and 4 within the bony pelvis as defined from the top of the L3 vertebral body to 5cm below the greater trochanter. Active bone marrow is defined for secondary analyses as the volume on FLT PET with SUV greater than 2.

IMRT Dose Objectives

Normal tissue and target volume dose objectives were specified for cervical and vaginal, rectal, and anal cancer subjects according to RTOG 0418, 0822, and 0529 protocols respectively, and at the discretion of the radiation oncologist for subjects with other pelvic cancers. The IMRT Plan was optimized to improve upon these guidelines for normal tissue sparing, if possible. The dose volume histograms (DVHs) created for the best balance between target coverage and normal tissue sparing was used as a baseline for the IMRT BMS Plan. An increase in percent volume at an organ-at-risk (OAR) objective dose point in the IMRT BMS Plan was to be less than 5% than that achieved in the IMRT Plan. The radiation oncologist determined if any changes from the conventional IMRT plan were clinically significant. No clinically significant compromises were made to target coverage or OAR sparing to spare bone marrow in IMRT BMS plans.

Bone Marrow Radiation Response Analysis

Dose reduction to FLT-defined bone marrow was analyzed using SUV 2, 3, and 4 threshold volumes created as described previously [17]. Briefly, FLT SUV thresholds of 2, 3, and 4 defined marrow volumes within the bony pelvis from the top of L3 to 5cm below the greater trochanter. No overlap between other SUV volumes or other structures was removed. The FLT uptake change between scans obtained at different time points during and after therapy was quantified by calculating the mean FLT SUV in 1 Gy/week dose bin volumes defined within this same bony pelvis region (top of L3 to 5cm below the greater trochanter). Figure 1 shows the dose bins for one subject. Isodose lines representing planned radiation dose after 5 fractions were converted to contours representing volumes of interest (VOIs) that received a 1 Gy range of radiation doses from 1 to 9 Gy or > 9 Gy. Change in uptake was calculated based on mean FLT SUV change within the dose bin relative to the mean FLT SUV in the same volume on the week 0 FLT PET image used as the baseline image. Complete blood counts (CBCs) were taken at the time of each FLT PET scan to correlate changes in pelvic FLT uptake to systemic hematologic toxicity. FLT uptake change was statistically analyzed using paired t-tests to assess associations with treatment plans and CBCs and using Cox regression to assess associations with time to CBC-related toxicities. All statistical tests were two-sided and assessed for significance at the 5% level.

Bone Marrow Chemotherapy Response Analysis

As seen in Figure 2, spinal marrow volumes-of-interest (VOIs) were defined in the sacrum, lumbar and the thoracic spine marrow using a 50% maximum pixel value threshold on the week 0 images (VIEW tool, PMOD Biomedical Image Quantification Software, PMOD Technologies, Ltd., Zurich, Switzerland, version 3.6). The sacral VOI represented proliferative tissue exposed to chemoradiation (within RT field) and the thoracic VOI represented tissue exposed to chemotherapy only (outside RT field). To facilitate comparisons, all individual's images were co-registered to their week 0 images (FUSION

tool, PMOD). The same VOIs were used at each therapeutic time point, with uptake measured as the standardized uptake value (SUV) at 60 minutes post-injection and as a percent of baseline uptake defined on the week 0 FLT PET image. Differences in FLT uptake change for subjects receiving different chemotherapy regimens was statistically analyzed using MANOVA.

Results

Simulation FLT PET scans were acquired for 27/35 accrued subjects (NCT01717391) with rectal (N = 9), cervical (N = 8), anal (N = 8), vaginal (N = 1), and bladder (N = 1) cancers. Subjects were accrued but not imaged due to infection (N = 1), unavailable FLT due to technical difficulties in production (N = 3), or subjects changing their minds (N = 4). Additionally, cervical cancer patients (N = 5) from our pilot study (NCT01075412) who had FLT PET scans acquired for simulation and after 1 and 2 weeks of therapy were included in the acute bone marrow response analysis. FLT PET scans from 32 subjects were used for the RT planning and acute bone marrow response analysis. FLT PET scans were acquired 30 days and 1-year post-therapy for 21 and 13 subjects, respectively, and 10 subjects that had both 30 day and 1-year post-therapy scans were used for the chronic bone marrow response analysis. Only subjects with a complete set of FLT PET scans were used to analyze the effect of chemotherapy (N = 11). A full series of FLT PET images for subjects accrued to NCT01717391 at week 0, week 1, week 2, 30 days and 1-year after therapy is shown in Figure 3. This image set is representative of the full set of images that were acquired of all that completed the series.

Bone Marrow Sparing Radiation Therapy Planning using FLT Guidance

IMRT BMS Plans were evaluated in comparison to the standard IMRT plan created without incorporating FLT PET information. Table 1 shows the average of FLT SUV 2, 3, and 4 volumes that received 5, 10, 20, and 30 Gy (V_5 , V_{10} , V_{20} , and V_{30}) and the average difference from the IMRT plan for all 32 subjects as well as the four sites with the largest subject numbers. The average change in PTV coverage was < 1%. The volume-weighted average dose received in the first week delivered with IMRT BMS plans (6.6 ± 0.5 Gy) was significantly lower than for IMRT plans (7.0 ± 0.6 Gy) ($p < 0.001$ paired t-test). Additionally, the volume of FLT identified as active pelvic bone (SUV > 2) receiving 4Gy in the first week was significantly larger for IMRT BMS plans (28.3 ± 4.2 %) when compared to IMRT plans (19.6 ± 4.8 %) ($p < 0.001$ paired t-test). Finally, the total volume of FLT identified active pelvic bone marrow (SUV > 2) exposed to radiation above 1 Gy/week was significantly smaller for IMRT BMS plans (651 ± 153 cc) when compared to IMRT plans (689 ± 163 cc) ($p < 0.001$ paired t-test).

Acute Bone Marrow Radiation Response

Week 1 and 2 mean FLT SUV normalized by week 0 mean FLT SUV is plotted as a function of the maximum bin dose in Figure 4 for all subjects. Both the week 1 and week 2 data indicate that a 50% decrease in FLT uptake takes place between 4 – 6 Gy for the majority of subjects. At week 1, FLT uptake averaged 49.9 ± 15.7 % of week 0 uptake in the 4 – 5 Gy/week dose bin. At week 2, FLT uptake averaged 62.6 ± 16.9 % in the 1 – 2 Gy/week dose

bin and $40.1 \pm 10.0\%$ in the 2 – 3 Gy/week dose bin of the week 0 uptake. This indicates that 4 – 5 Gy results in an approximately 50% decrease in FLT uptake when administered in either 1 or 2 weeks. Therefore, fractionation does not appear to improve acute bone marrow sparing.

The dose response of active bone marrow as measured by FLT uptake at week 1 was correlated to select CBC values measured at the time of each FLT PET scan. The SUV₂ volume in each dose bin created from the IMRT BMS plan was used as the active bone marrow metric and white blood cell (WBC), platelet, neutrophil, and lymphocyte counts were used as the systemic toxicity metric. Those subjects with a larger volume of FLT identified bone marrow receiving 4 Gy or higher in the first week developed grade 2 leukopenia sooner ($p < 0.05$ Cox regression).

Week 0 to week 1 percent change in mean FLT SUV (P_{SUV}) was modeled as an exponential function of maximum bin dose for each patient:

$$p_{SUV} = \exp(\alpha + \beta \times \text{Dose})$$

The resulting dose-response curves were then used to derive the dose at which a given percent change in FLT SUV occurred. Kaplan-Meier plots of cumulative grade 2 hematologic toxicity probabilities for WBCs, platelets, lymphocytes, and neutrophils are shown in Figure 5. Subjects were evenly stratified into high and low derived dose categories based on the median value (3.1 Gy). Derived dose was significant as a continuous predictor in the Cox regression analysis of time to WBC toxicity ($p < 0.005$) and likewise shows the most separation among the Kaplan-Meier plots. As depicted, those subjects that experienced a decrease in pelvic mean FLT SUV to 75% of week 0 mean FLT SUV from doses below 3.1 Gy were more likely to experience grade 2 leukopenia. This indicates that FLT change is associated with pelvic bone marrow sensitivity to radiation dose and its systemic effect.

Of the 32 subjects who received at least one FLT PET scan in the pelvic cancer study, 8 subjects had chemotherapy withheld due to low hematologic indices (25%) including two instances of neutropenic fever. Lymphocytopenia was the most common toxicity attributed to chemoRT with 72% and 19% of subjects experiencing grade 2 and 3 lymphocytopenia at least once during therapy. The next most clinically significant toxicity was grade 3 leukopenia, which 5 of 32 subjects (16%) experienced during therapy. Grade 2 leukopenia was experienced by 14 of 32 subjects (44%). Grade 2 and 3 thrombocytopenia rates were 22% and 9% while grade 2 and 3 neutropenia rates were 19% and 9%.

Chronic Bone Marrow Radiation Response

There is a high degree of variability in active bone marrow recovery at 30 days post therapy as shown in the plot of mean FLT SUV in each dose bin normalized by week 0 mean FLT SUV (Figure 6). For some subjects, there appears to be recovery to $> 50\%$ of week 0 mean FLT SUV with little dependence on radiation dose. The plot of mean FLT SUV in each dose bin normalized by week 0 mean FLT SUV at 1-year post therapy is also shown in Figure 6. Apparent recovery at 30 days post therapy appears to be lost at 1-year post therapy at higher

radiation doses (> 25 Gy). For 10 subjects who completed both the 30 day and 1-year post therapy scans, mean FLT SUV returned to 56.4 ± 17.7 % and 54.0 ± 15.5 % of week 0 mean FLT SUV at 30 days and 1-year post therapy, respectively, for regions that received total doses of 20 – 25 Gy. The mean FLT SUV recovery in bone marrow receiving 25 Gy or less at 1-year post therapy was within 3% of the recovery measured at 30-day post therapy. In regions that received > 35 Gy, FLT SUV recovery at 30 days post therapy was 18.8 ± 1.8 % greater than at 1-year. This pattern of temporary recovery followed by loss of function at doses > 35 Gy was also seen in CBC metrics. At 30 days post therapy, WBCs, platelets, and lymphocytes were statistically significantly lower than week 0 values ($p < 0.05$, paired t-test), but improved compared to levels measured during therapy. Whereas at 1-year post therapy, neutrophil values were also statistically significantly lower than week 0 values ($p < 0.05$, paired t-test). Plots of the mean WBCs, platelets, lymphocytes, and neutrophils normalized by the baseline values for all subjects at the week 0 imaging time point are shown in Figure 7. The lack of FLT uptake recovery illustrated in Figure 6 correlated to the reduced systemic hematologic metrics indicates that pelvic bone marrow activity is suppressed 1-year after therapy is complete.

Effect of Disease Site

Statistical analysis performed to identify the effect of disease site showed no difference in IMRT BMS plans when compared to the corresponding IMRT plans for the four largest groups: rectal, anus, cervix, and cervix with para aortic involvement (paired t-test, $p > 0.1$). The chemotherapy regimens differed between disease sites. Subjects with cervical cancer received weekly cisplatin ($N = 9$). Subjects with rectal cancer received either Xeloda® (capecitabine i.e., oral 5-FU prodrug) ($N = 5$) or a continuous infusion of 5-FU ($N = 4$). Subjects with anal cancer received two four- day infusions of 5-FU and mitomycin C ($N = 9$). Table 2 shows the specific chemotherapy regimens for each subject.

Marrow proliferative activity, as assessed by FLT uptake, was profoundly reduced in tissues exposed to both chemo- and radiation therapy (represented by sacral marrow, Figure 8) at all times compared to week 0. Type of chemotherapy was not a significant predictor of the degree of impairment from week 0 in a global statistical test of equality across capecitabine, 5-FU only, 5-FU + mitomycin, and cisplatin (MANOVA, $p = 0.7$) nor in a test of exposure to 5-FU or not (MANOVA, $p = 0.5$). Although the FLT uptake appears to begin to recover at 30 days post-therapy, especially for patients treated with cisplatin (orange line), at 1 year post-treatment, marrow uptake remained impaired, ranging from 7 – 38% of week 0 values. Whereas, marrow exposed only to chemotherapy (represented by thoracic marrow, Figure 9) exhibited enhanced mean uptake during early therapy and at 30 days post-therapy, except for patients treated with the combination of 5-FU and mitomycin. Neither imaging time point (MANOVA, $p = 0.1$) nor type of chemotherapy were significant whether expressed as the individual regimens (MANOVA, $p = 0.5$) or exposure to 5-FU or not (MANOVA, $p = 0.5$). Mean uptake returned to week 0 values by 1 year for all therapies in marrow exposed to chemotherapy only.

Discussion

FLT PET simulation images obtained from these prospective clinical studies demonstrated the ability to incorporate active bone marrow identified with FLT uptake to create IMRT plans that significantly spare pelvic bone marrow when compared to a conventional IMRT plan. However, the sparing was insufficient considering the extreme sensitivity (50% reduction in function at ~4 Gy). The rates of hematologic toxicity measured for the 32 subjects studied are at the lower end of the ranges reported in the literature. Instances of grade 3 or higher hematologic toxicity for patients undergoing chemoRT for cervical cancer have been reported at rates from 13 – 35 % with up to 40% of patients having one cycle of chemotherapy withheld [1,4,12,25]. Instances of grade 4 or higher hematologic toxicity for anal cancer subjects who receive a combination of 5-FU + mitomycin C, and RT were reported at rates of 18 – 38% with 2 of 17 (11%) patients having chemotherapy withheld (mitomycin C) [9,20]. The most common toxicity measured in this study was lymphocytopenia, which is not typically recorded in other studies. Typically, neutropenia or leukopenia was the limiting hematologic toxicity for cervical or anal cancer. The rate experienced in our study of grade 3 neutropenia (9%) and leukopenia (16%) are below or at the lower end of the reported rates of hematologic toxicity. The rate of missed chemotherapy cycles experienced due to hematologic toxicity by our subjects (26%) is lower than that reported for cervical cancer patients but greater than that reported for anal cancer patients, which may be expected for a mixed pool of subjects. Missed chemotherapy for rectal cancer subjects has been reported to be as high as 40%, but the reported chemotherapy regimen was more intense than that used in the current study [20]. Because of the small patient numbers and mixed population, the observed rates of toxicity resulting in missed chemotherapy cycles is consistent with the literature and no benefit from our current IMRT BMS plans can be concluded.

Most significant in our results, FLT PET images taken after 1 and 2 weeks of therapy indicated a very low dose threshold for bone marrow suppression of ~4Gy with no benefit from fractionation. Initial estimates reported a potential radiation dose threshold based on FLT uptake change of ~5Gy/week [15], which correlated with total dose levels of 20 and 30 Gy ($5\text{Gy} \times 5$ weeks of RT) established for cervical cancer patients by Mell *et al* [18]. However, in this study, the week 2 images taken for all subjects indicated little to no time benefit in fractionating radiation dose delivery and that the potential radiation dose threshold was ~4Gy *total* radiation dose. This creates a clinically challenging scenario for creating bone marrow sparing IMRT plans. IMRT provides superior normal tissue sparing at dose levels such as 20 – 30 Gy while increasing the total normal tissue volume that receives low doses (<10 Gy). Therefore, a RT strategy that is effective for reducing toxicity to normal tissues such as bladder, bowel, and rectum may not be as effective for reducing bone marrow toxicity due to its extreme sensitivity and the lack of fractionation benefit. Incorporating bone marrow specific avoidance volumes in the IMRT optimization process is effective at reducing dose to pelvic bone marrow for all pelvic cancer patients, but a more aggressive strategy such as using protons for RT delivery or pharmacologic radioprotection may be necessary to produce significant clinical benefit because of this acute bone marrow radiation dose sensitivity.

Another factor in effective pelvic bone marrow sparing during RT planning is the close proximity of the largest active pelvic bone marrow reservoir to the treatment volumes. The sacral and central portion of the pelvis has the greatest probability of containing active bone marrow [16]. Standard PTV margins extend into the richest bone marrow reservoir, and photon radiation dose fall off gradients are not steep enough to adequately cover target tissues and drop to levels as low as 4 Gy within the volume of the pelvic bones. Typically, the IMRT optimization process sacrifices the central active bone marrow close to the target volumes in favor of reducing dose to more peripheral regions because of these inherent limitations. These lower activity peripheral active bone marrow regions may not contribute as significantly to blood cell production. Therefore, IMRT plans that significantly spare FLT identified bone marrow may not be sufficiently sparing the reservoir that is most critical to reducing hematologic toxicity.

In addition to the acute effect of radiation therapy, FLT PET images taken 30 days and 1-year after completion of chemoRT indicate incomplete pelvic bone marrow recovery that appears to chronically suppress blood cell production as well. The dose dependence of bone marrow recovery 1-year after therapy suggests a secondary RT-planning threshold. Active marrow receiving more than 25–35 Gy appears to have no significant recovery. Decreasing the volume of active bone marrow that receives >25 Gy may reduce this apparently permanent bone marrow damage that is potentially partially responsible for significantly reducing CBC values from levels measured prior to therapy.

The complete series of pre-therapy, during therapy, and post-therapy FLT PET images (as seen in Figure 3), illustrate the difficulty in minimizing the total effect of chemoRT damage to pelvic bone marrow due to the extreme radiation sensitivity of active bone marrow during therapy and the lack of recovery after therapy. If RT plans are designed to reduce the volume of active bone marrow that receives low doses (5 and 10 Gy) at the expense of increasing the volume of active bone marrow that receives higher doses (>25 Gy), chronic hematologic suppression may be increased. This creates a difficult scenario especially for conventional photon-based RT planning techniques. A different beam arrangement or dividing FLT-identified bone marrow avoidance regions into groups based on location instead of SUV could preferentially direct dose away from the sacral and central portion of the pelvis at the expense of more peripheral regions of activity. Alternatively, proton delivery may offer an advantage due to the Bragg Peak and very low beam exit doses to reduce the active pelvic bone marrow volume that receives doses as low as 4 Gy without increasing the active pelvic bone marrow volume that receives higher doses [5,26] Further study is needed to determine the most robust proton plan design to limit radiation dose to either the total or central active pelvic bone marrow without compromising target coverage or other normal tissues. Finally, the role of radiation protective agents remains an area of relevant and active investigation.

The use of different chemotherapy agents in the treatment of our subjects has the potential to complicate the analysis of FLT PET imaging, but our intent was to demonstrate the utility of bone marrow sparing for a broad range of cancer patients. Previous studies have shown that the effect of cisplatin based chemotherapy on bone marrow as measured by FLT uptake change is much less than that of radiation (~12% vs. ~76% decrease) [19]. Dittmann *et al.* showed a 7 to 10 fold increase in FLT uptake in tumor cells 24 hours after treatment with 5-

FU [7]. However, the uptake curves for tumor cells and marrow cells are different as illustrated by Muzi *et al.*[21] No data has been reported showing the effect of mitomycin C on FLT uptake in tumor or bone marrow. We found no statistical difference in the analysis of bone marrow within or outside of the radiation therapy field between subjects receiving different chemotherapy agents or administrations. Therefore, we do not believe chemotherapy had a significant impact on the analysis of the effects of radiation on bone marrow.

FLT uptake may also be affected by S-phase arrest caused by chemoradiation therapy. Radiation is well known to activate cell cycle checkpoints primarily in the G1 and G2 phases resulting in delays in cell cycle progression. Such a delay in progression is anticipated to impact the percentage of S-phase cells and therefore, FLT uptake. In general, depending on the dose, radiation induced delays in cell cycle progression are transient. Prolonged delays in cell cycle progression are believed to activate the cell death pathways. Therefore, we hypothesize that the decrease in FLT uptake at 1 and 2 weeks of therapy is due to a radiation-induced toxicity of bone marrow cells as opposed to a cytostatic effect. This hypothesis is also supported by our observation of a significant decrease in FLT uptake at 30-days and 1-year after therapy. These results further suggest that FLT PET can be an effective tool to monitor bone marrow toxicity following chemoradiation therapy of pelvic cancers.

There are many potential sources of error in this study. Cone-beam CT was used to align the pelvic bones for treatment, but rotational corrections were not made. Therefore, the planned dose to the active bone marrow used in this study may vary from the dose actually delivered especially in the peripheral regions. Additionally, because the acquisition time of WB FLT PET scans is ~24 min, there was the potential for subject motion between the acquisition of the AC CT used for image registration and the FLT PET scan. The spatial resolution in this study was also limited by the resolution of the FLT PET images (~4mm³) and the use of the same ROI to analyze dose for each sequential scan. Because dose is increasing over time, the 1 Gy dose bin range at week 1 becomes 5 Gy after therapy. Even with these limitations, the ability to use noninvasive quantitative imaging to measure bone marrow proliferation during and after therapy has shown a consistent low dose radiation sensitivity that is independent of time.

Finally, the clinical significance of chronic bone marrow suppression is unknown. It may contribute to more long-lasting fatigue, increased susceptibility to infection, and limited options for potentially needed salvage therapy. Longer-term studies analyzing total bone marrow response will be needed to identify meaningful consequences of chronic bone marrow suppression, and how factors such as age, gender, pretherapy hematologic indices, and radiation delivery technique contribute to overall quality of life as patient long-term survival increases with improved therapies.

Conclusions

Bone marrow sparing RT plans using FLT-identified active bone marrow are possible for a variety of pelvic cancer patients and significantly reduce radiation dose to active bone

marrow when compared to the standard IMRT plan ($p < 0.001$). Additionally, FLT uptake change measured in pelvic bone marrow during chemoRT correlates significantly with systemic hematologic metrics ($p < 0.05$). However, the extreme radiation dose sensitivity of bone marrow measured by FLT uptake change, where as little as 4 Gy causes an ~50% decrease in 1 or 2 weeks, may make achieving clinically significant reductions in hematologic toxicity and improvements in chemotherapy compliance difficult with even the best IMRT planning techniques. However, reducing the bone marrow volume that receives 10 or 20 Gy in total dose (~2 Gy or ~4 Gy per week) may be beneficial to increasing the time before a hematologic toxicity event occurs during therapy resulting in the ability to deliver more chemotherapy. Moreover, long-term bone marrow recovery may be improved by reducing radiation doses > 35 Gy. Further study is needed to determine alternative treatment strategies to balance acute bone marrow radiation sensitivity and chronic bone marrow activity suppression to improve outcomes and quality of life for pelvic cancer patients.

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Summary

Bone marrow sparing RT plans using FLT-identified avoidance regions significantly reduce radiation dose to active bone marrow for pelvic cancer patients. However, bone marrow is acutely sensitive to radiation doses as low as 4 Gy, and fractionation does not appear to mitigate hematologic toxicity. Additionally, bone marrow recovery appears to be suppressed in regions that received > 35 Gy 1-year after therapy. This combination of acute and chronic radiation sensitivity makes reducing hematologic toxicity challenging.

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Figure 1.

1 Gy/week dose bin volumes created within the bony pelvis to analyze FLT uptake changes for one subject.

Figure 2.
Tumor and Marrow Volumes-of-Interest (VOIs).

Figure 3.

WB FLT PET images acquired for subject FLTPC004 at radiation therapy simulation (Week 0), 1 and 2 weeks after chemoradiation therapy (Week 1 and Week 2), and 30 days and 1-year post chemoradiation therapy (30 Days Post and 1-year).

Figure 4.

Mean FLT SUV within the dose bins created for each subject normalized by the week 0 mean FLT SUV measured 1 week and 2 weeks after chemoRT as a function of the maximum radiation dose within a dose bin.

Figure 5.

Kaplan-Meier plots of cumulative grade 2 hematologic toxicity probabilities. Subjects were evenly stratified into high and low derived dose categories based on the median value (3.1 G). Follow-up period extends to the time at which all patients experienced a grade 2 event or until the 1 – year follow-up imaging time point.

Figure 6.

Mean FLT SUV within the dose bins created for each subject normalized by the week 0 mean FLT SUV measured 30 days and 1-year after chemoradiation therapy was complete as a function of the maximum radiation dose within a dose bin.

Figure 7.

Mean WBCs, platelets, lymphocytes, and neutrophils during and after radiation therapy normalized by week 0 (baseline) values taken at the week 0 imaging time point.

Figure 8.

Sacral marrow (within RT field) FLT uptake expressed as mean percent of week 0 (baseline) values for subjects with complete data at week 1 and week 2 of therapy and 30 days and 1 year post-therapy by chemotherapy regimen (red = capecitabine, green = IV 5-FU, blue = 5-FU + mitomycin, orange = cisplatin).

Figure 9.

Thoracic marrow (outside RT field) FLT uptake expressed as mean percent of week 0 values (baseline) for subjects with complete data at week 1 and week 2 of therapy and 30 days and 1 year post-therapy by chemotherapy regimen (red = capecitabine, green = IV 5-FU, blue = 5-FU + mitomycin, orange = cisplatin).

Table 1

The average percent volume identified by FLT SUV 2, 3, and 4 within the boney pelvis that receives 5, 10, 20, and 30 Gy (V_5 , V_{10} , V_{20} , and V_{30}), the average difference from the standard IMRT plan, and the average PTV coverage change.

IMRT BMS Plans	Average SUV % Volume				% Difference from Baseline IMRT				Average PTV Change	
	V_5	V_{10}	V_{20}	V_{30}	V_5	V_{10}	V_{20}	V_{30}	V_{Rx}	
Average	81.1%	72.2%	60.9%	49.3%	-4.7%	-9.3%	-14.2%	-13.4%	-0.1%	
Standard Deviation	9.3%	10.0%	9.2%	9.0%	3.3%	4.2%	4.1%	7.6%	0.4%	
Average Cervix	82.7%	74.0%	62.4%	51.8%	-6.3%	-9.4%	-14.5%	-12.9%	-0.1%	
StDev	3.8%	5.5%	4.3%	5.7%	4.5%	5.5%	3.0%	10.6%	0.3%	
Average Rectum	80.6%	72.3%	60.4%	48.0%	-4.3%	-8.8%	-13.4%	-12.4%	-0.1%	
StDev	6.6%	8.7%	8.2%	8.3%	2.3%	4.6%	4.3%	5.5%	0.6%	
Average Anus	78.8%	69.4%	59.1%	46.3%	-2.8%	-8.7%	-14.3%	-13.8%	-0.1%	
StDev	10.2%	10.2%	8.9%	5.1%	1.3%	3.1%	6.3%	6.6%	0.5%	
Average Cervix+Para Aortics	93.3%	83.8%	72.4%	61.9%	-2.8%	-8.8%	-12.9%	-14.4%	0.0%	
St Dev	1.3%	2.1%	5.2%	8.5%	3.0%	3.7%	0.8%	3.4%	0.2%	

Table 2

The chemotherapy regimen for each subject.

Chemotherapy			
Subject	Site	Drug	Dose
FLTCC001	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTCC002	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTCC003	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTCC005	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTCC006	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTPC003	Rectum	Xeloda	1500 mg am and 2000 mg pm, hold for 7 days, then reduced to 1500 mg daily
FLTPC004	Rectum	Xeloda	850 mg/m ² /day (1300 mg) BID
FLTPC006	Rectum	Xeloda	850 mg/m ² /day (1650 mg) BID
FLTPC007	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days, mitomycin 10 mg/m ² (2/4); 5 mg/m ² (3/4)
FLTPC008	Vagina	Cisplatin	40 mg/m ² (65 mg)
FLTPC009	Cervix + PA	Cisplatin	40 mg/m ² (62 mg)
FLTPC011	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days, mitomycin 10 mg/m ² (4/1 and 4/29)
FLTPC012	Cervix	Cisplatin	40 mg/m ² (68 mg)
FLTPC014	Rectum	5-FU	225 mg/m ² /day continuous infusion
FLTPC015	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTPC016	Rectum	5-FU	225mg/m ² /day continuous infusion (389mg)
FLTPC020	Anus	Mitomycin/5-FU	mitomycin 10 mg/m ² (7/17 and 8/22), 5-FU: 1000 mg/m ² /day × 4 days (7/20)
FLTPC021	Rectum	5-FU	225 mg/m ² /day continuous infusion (405mg)
FLTPC022	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days, mitomycin 10 mg/m ² (8/12 and 9/9)
FLTPC023	Anus	Mitomycin/5-FU	5-FU: (1000 mg/m ² /day × 4 days on 8/26, 500 mg/m ² /day × 4 days on 9/30), mitomycin (10 mg/m ² on 8/26, 5 mg/m ² on 9/30)
FLTPC024	Cervix + PA	Cisplatin	40 mg/m ² (69 mg)
FLTPC025	Bladder	Mitomycin/5-FU	5-FU: 500 mg/m ² /day × 5 days (10/14 and 11/11), mitomycin 12 mg/m ² (10/14)
FLTPC026	Cervix	Cisplatin	40 mg/m ² (65 mg)
FLTPC027	Cervix	Cisplatin	40 mg/m ² (70 mg)
FLTPC028	Rectum	5-FU	1000 mg/m ² /day × 5 days
FLTPC029	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days (3/6), mitomycin 10 mg/m ² (3/6)
FLTPC030	Cervix	Cisplatin	40 mg/m ² (68 mg)
FLTPC031	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days (10/13, and 11/14), mitomycin 10 mg/m ² (10/13, and 11/14)
FLTPC032	Rectum	Xeloda	850 mg/m ² (1500 mg) BID
FLTPC033	Anus	Mitomycin/5-FU	5-FU: 1000 mg/m ² /day × 4 days (10/27, and 12/1), mitomycin 10 mg/m ² (10/27), and 5 mg/m ² on 12/1
FLTPC034	Rectum	Xeloda	825 mg/m ²
FLTPC035	Cervix + PA	Cisplatin	40 mg/m ² (70 mg)