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Optimization of the acute hematological toxicity profile in anal cancer patients undergoing concurrent chemo-radiation

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Chapter 1. Introduction and outline of the project

Concurrent chemo-radiotherapy (CT-RT) is presently considered as a standard of care in squamous cell carcinoma of the anal canal[1]. In this combined modality approach, radiation (RT) is combined with 5-fluorouracil (5-FU) and mytomicin C (MMC) following the seminal report by Nigro[2]. Clinical results in terms of both local control and survival are favorable as the rate of sphincter preservation[3,4]. Nevertheless, the acute toxicity profile is not negligible and major reactions can occur in the genitalia, skin or gastrointestinal tract, particularly if non-conformal techniques are used[5]. Hematologic toxicity (HT) can be a critical issue in this setting of patients leading to unplanned treatment breaks with a consequent increase in overall treatment time and a potential detrimental effect on treatment intensity or increasing the likelihood to develop bleeding, infections or asthenia that may impact on patient's compliance to therapy[6]. Hence, minimization of HT is cogent in anal cancer patients submitted to combination therapy. Chemotherapy (CT) is considered the most important trigger for HT because of its direct induction of myelosuppression[7]. Nevertheless, given the exquisite radiosensitivity of circulating blood cells and precursors within bone marrow (BM), RT has a consistent influence in the occurrence of HT[8]. This is particularly evident during combination therapy for pelvic malignancies, including anal cancer[9,10]. Interestingly, in the average adult population, pelvis and lumbar vertebrae comprise about half of the total hematopoietically BM[11]. Hence, selective sparing of pelvic bone structures may be a viable option to decrease HT during concomitant CT-RT in patients affected with pelvic malignancies[12,13] . The aim of the project developed within the present PhD program was the optimization of the acute hematologic toxicity profile in anal cancer patients undergoing concurrent CT-RT. First step was to gather all the available clinical data related to this topic to provide a background scenario with available results, open issues, ongoing studies and future perspectives. Secondly, we analyzed , on a retrospective basis, the whole cohort of anal cancer patients treated at the Department of Radiation Oncology of the University of Turin with concurrent CT-RT, focusing on both oncological

outcomes such as colostomy-free survival (CFS) and overall survival (OS) and acute and late toxicity profile, including HT. These data were obtained from patients affected with early stage disease or locally advanced anal cancer and treated with intensity-modulated radiotherapy (IMRT), employing either a static or volumetric approach with volumetric modulated arc therapy (VMAT). After evaluating HT in the aforementioned settings and establishing the magnitude of HT as a clinical issue, we tried to investigate the correlation between dosimetric parameters related to the dose received by osseous regions within pelvic bones during treatment and HT. The first approach we employed was based on the assumption that the whole bone could be considered as a surrogate structure for hematopoietically active BM. In this sense, we explored the potential correlation between dosimetric parameters and HT using the outer whole pelvic bone contour as a reference, finding out an interesting dose cut-off point for the lumbar-sacral bone marrow (LSBM), to be potentially used during the planning process. We were also able to provide a robust modeling for the relationship between mean dose to active BM and HT. Secondly, we employed functional imaging (^{18}FDG-PET) to characterize and define active BM. Using a similar approach as the one used for the whole outer bone contour, we investigated the potential correlation between active BM as defined using ^{18}FDG-PET and HT. We were hence able to describe a stronger correlation with HT for active BM located in the lumbar-sacral region compared to other pelvic sub-regions. As a third step, we performed a planning comparison study to investigate which approach could be more suitable to decrease the dose received by active BM during concurrent CT-RT for anal cancer treated with VMAT. We compared different planning options based on different optimizations processes addressed to BM as outlined according to different definitions. Those based on the outer whole bone contour or ^{18}FDG-PET-defined BM sub-regions were found to be the most promising, leading to a similar efficacy in terms of reduction to the dose delivered to BM. As a final step, we are implementing a prospective phase II trial employing dose-painted BM sparing IMRT, delivered with VMAT, to reduce the acute HT rate in anal cancer patients undergoing concurrent CT-RT

according to the Nigro's regimen. The definition of active BM chosen for the aforementioned trial was that based on ^{18}FDG-PET-driven delineation.

References

1. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015; 33: 259-66.
2. Nigro ND, Vaitkevicius VK, Considine B Jr. Combined therapy for cancer of the anal canal: a preliminary report. Dis Colon Rectum 1974; 17: 354-6.
3. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Early-stage node negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 1943-8.
4. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Locally advanced (T3-T4 or N+) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 2027-32.
5. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, Thomas CR Jr, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008; 299: 1914-21.
6. Julie DA, Oh JH, Apte AP, Deasy JO, Tom A, Wu AJ, Goodman KA. Predictors of acute toxicities during definitive chemoradiation using intensity-modulated radiotherapy for anal squamous cell carcinoma. Acta Oncol 2016; 55: 208-16.
7. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995; 31: 1319-39.
8. Filippi AR, Franco P, Ricardi U. Is clinical radiosensitivity a complex genetically controlled event? Tumori 2006;92: 87-91.
9. Lujan AE, Mundt AJ, Yamada SD, Rotmensch J, Roeske JC. Intensity-modulated radiotherapy as a means of reducing dose to bone marrow in gynecologic patients receiving whole pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2003;57:516-21.
10. Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2006; 66: 1356-65.
11. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol 1961;5:255-8.
12. Jianyang W, Yuan T, Yuan T, Ning L, Hua R, Hui F, et al. A prospective phase II study of magnetic resonance imaging guided hematopoietical bone marrow-sparing intensity-modulated radiotherapy with concurrent chemotherapy for rectal cancer. Radiol Med 2016; 121: 308-314.
13. Mell LK, Tiryaki H, Ahn KH, Mundt AJ, Roeske JC, Aydogan B. Dosimetric comparison of bone marrow-sparing intensity modulated radiotherapy versus conventional techniques for treatment of cervical cancer. Int J Radiat Oncol Biol Phys 2008; 71: 1504-10.

Chapter 2.

Hematologic toxicity in anal cancer patients during combined chemo-radiation: a clinical perspective

Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Hematologic toxicity in anal cancer patients during combined chemo-radiation: a radiation oncologist perspective.** *Expert Rev Anticancer Ther 2017;17:335-345*1.

Concurrent chemo-radiotherapy (CT-RT) is presently considered as a standard of care in squamous cell carcinoma of the anal canal[1]. In this combined modality approach, radiation (RT) is combined with 5-fluorouracil (5-FU) and mytomicin C (MMC) following the seminal report by Nigro[2]. Clinical results in terms of both local control and survival are favorable as the rate of sphincter preservation[3,4]. Nevertheless, the acute toxicity profile is not negligible and major reactions can occur in the genitalia, skin or gastrointestinal tract, particularly if non-conformal techniques are used[5]. Hematologic toxicity (HT) can be a critical issue in this setting of patients leading to unplanned treatment breaks with a consequent increase in overall treatment time and a potential detrimental effect on treatment intensity or increasing the likelihood to develop bleeding, infections or asthenia that may impact on patient's compliance to therapy[6]. Hence, minimization of HT is cogent in anal cancer patients submitted to combination therapy. Chemotherapy (CT) is considered the most important trigger for HT because of its direct induction of myelosuppression[7]. Nevertheless, given the exquisite radiosensitivity of circulating blood cells and precursors within bone marrow (BM), RT has a consistent influence in the occurrence of HT[8]. This is particularly evident during combination therapy for pelvic malignancies, including anal cancer[9,10]. Interestingly, in the average adult population, pelvis and lumbar vertebrae comprise about half of the total hematopoietically BM[11]. Hence, selective sparing of pelvic bone structures may be a viable option to decrease HT during concomitant CT-RT in patients affected with pelvic malignancies[12,13].

Bone marrow characteristics

The vast majority of the medullary cavity of osseous segments is made up of BM. In general, up to 50% of BM is active from a hematopoietic point of view (red marrow) and it is primarily located within the axial skeleton and proximal aspect of the limbs, while the remaining 50% is made up of inactive BM (yellow marrow) and can be mainly found in the appendicular skeleton[14]. Pathological studies showed that yellow BM is composed of approximately 95% of fat cells and 5% of nonfat

cells[15]. Conversely, red BM comprises 60% of hematopoietic cells and up to 40% fat cells[15]. The relative proportion of this 2 compartments is a strong influence on the magnetic resonance signal intensity during dedicated imaging procedures. Within red BM, 3 major components can be identified, namely progenitors of blood cells responsible for hematopoiesis, reticulo-endothelial cells and cells involved in the trabecular cellular pattern which act as a support tissue[15]. BM weight depends on gender and varies from 2600 and 3000 g[16]. Since one half of red marrow by weight is made of adipose tissue, up to 75% of total BM weight is made of adipose tissue in the adult population[16]. Inside active BM, hematopoietic stem cells are able to replicate and differentiate mature cells of myeloid, lymphoid and erythroid lineages, driven by a complex network of growth factors and cellular 'cross-talk'[17]. Bone marrow microenvironment, consisting of adipocytes, fibroblast, endothelial and adventitial cells and macrophages, also contributes in maintaining the hematopoietic function[17]. In children, the appendicular skeleton (humerus, femur) has hematopoietic activity. With age, active BM progressively retracts from peripheral to axial skeleton and from diaphyseal to metaphyseal long bones[17]. Moreover red marrow itself develops age-related changes with respect to distribution and composition, with an increase in the proportion of fat cells in the axial skeleton and a progressive conversion from red to yellow BM in the peripheral skeleton[15]. In the average adult population almost 60% of total BM is comprised within pelvic bones and lumbar spine[17]. This observation provides a causal relation between the dose received by these regions during CT-RT and the occurrence of HT.

Bone marrow and radiation

BM has a high intrinsic radiosensitivity which leads to some degree of damage for any dose received[17,18]. The sequence of histologic alterations has been clearly described by Sykes in humans[19]. Using fractionated RT, a moderate decrease in precursors of red blood cells and granulocyte can be observed after 4 Gy[19]. Dilatation of sinusoids with associated hemorrhage and vanishing of young hematopoietic precursors occurs after 10 Gy[19]. At 20 Gy radiation, cellularity of

nucleated cells has decreased down to 20%, while above 50 Gy a consistent hypoplasia can be seen with consequent fat accumulation[19]. Medium to long term effects may include partial recovery but also irreversible BM depression depending on several intrinsic and extrinsic factors[17]. Hence, a clear dose-response relationship can be pointed out. However, another parameter that should be taken into account is irradiated volume of BM, as clearly shown by data on acute response of the marrow organ after single total body exposure[17]. One week after total body RT up to 1.5-7.5 Gy, a rapid depletion of vital stem cells can be seen with a consequent prominent granulocytopenia and thrombocytopenia[20]. At those doses, the microvasculature survives allowing for eventual implantation and proliferation of infused stem cells, but the entity of BM damage is strictly correlated to the volume receiving RT[17]. Interestingly, when small field radiation is employed, exposing limited BM volumes (10-15%) to RT, unexposed BM is able to compensate for the hematopoietic demand increasing the progenitor cell population[17, 21]. Whenever larger fields are used, such as in the case of radiation treatments for anal cancer of other pelvic malignancies, HT may become an issue[17,21,22].

Bone marrow distribution in the body

The seminal work by Ellis derived an average active BM distribution in adult man using fractional regional estimates of BM weight compared to total bone weight as a surrogate for BM identification. Pelvic bone and sacrum accounted for 40% of the total BM amount, lumbar spine for 10% and thoracic vertebrae for 14% in that study[11]. Using the entire bone as a surrogate for BM is an option, but this method does not differentiate between active and inactive BM and does not provide any information on the correct localization of red marrow[9]. Functional imaging is a useful tool to selectively identify BM and potentially characterize red and yellow marrow[15]. Tc-99m sulfur colloid single-photon-emission computed tomography (SPECT) has been investigated in this setting, as Tc-99m sulfur colloids may be internalized and sequestrated by macrophages associated to the reticulo-endothelial compartment of BM, consequently providing a 3- dimensional map of

BM distribution[23]. With this method, Roeske et al were able to characterize BM mainly within lumbar vertebrae, sacrum and medial aspect of the iliac crests. However, the poor quantitative ability of this imaging modality should be taken into account[23]. Another option for BM functional imaging is 3'-deoxy-3'-^{18}F-fluorothymidine-labeled positron-emission tomography (^{18}F-FLT-PET), as a mean to identify cells with DNA synthesis[24]. ^{18}F-fluorothymidine (FLT) is a thymidine analogue able to be retained inside the cell through a thymidine kinase-mediated phosphorylation process which takes place mainly during the S phase of the cell cycle[25] . Even if FLT cannot be incorporated into DNA, its uptake is a marker of DNA replication and active cellular proliferation. A reduction in FLT uptake within bone regions is a sign for the loss of precursor cells in the proliferative compartment of BM[26]. Hayman et al investigated the relative distribution of active BM through the body, using ^{18}F-FLT-PET, in 13 patients affected with different types of cancer[25]. Interestingly, significant individual variations were noted among cases. The mean percentage of proliferating BM was 25.3% at the pelvis, 19.9% and 16.6% at the thoracic and lumbar spine, 9.2% at the sacrum and 8.8% at the ribs and clavicles. Less than 5% of active BM was found at the skull, proximal humeri, sternum, scapulas, cervical spine and proximal femurs[26]. Interestingly, a recent study by McGuire et al reported that, within the pelvis, regions located in the central part, such as the upper sacrum, the inner halves of iliac crests and the 5th lumbar vertebral body, have the ^{18}F-FLT highest uptake[27]. A larger cohort of 51 lung cancer patients was analyzed by Campbell et al with respect to BM distribution according to ^{18}F-FLT-PET[28]. The pelvic bones had the highest proportion of proliferating BM regardless of gender and age[28]. Interestingly, women had a higher proportion of functional BM in the pelvis, proximal femurs and skull, while men in the sternum and ribs, clavicles and scapulae[28]. Elderly patients (> 75 years) had a higher relative proportion of active BM in the ribs, clavicles and scapulae. The proximal long bones (femurs and humeri) had the largest variations in the mean proportion of functional BM with respect to age with a 20-30% increase according to sex and osseous segment taken into account[28]. Another potentially useful examination is ^{18}F-fluorodeoxyglucose-labeled positron-emission tomography (^{18}FDG-PET), which

has been demonstrated to be able to detect the volume of active BM with an uptake pattern corresponding to histologic distribution[15]. Franco et al described the relative distribution of active BM within the pelvic region using ^{18}FDG-PET[29]. Active BM was observed in 44% of the volume of pelvic bones with lumbar-sacral vertebrae (67%) and iliac bones (57%) having the highest percentages[29]. However, the ability of ^{18}FDG-PET to correctly discriminate between active and inactive BM is still a matter of debate[15,29].

References

1. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015; 33: 259-66.
2. Nigro ND, Vaitkevicius VK, Considine B Jr. Combined therapy for cancer of the anal canal: a preliminary report. Dis Colon Rectum 1974; 17: 354-6.
3. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Early-stage node negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 1943-8.
4. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Locally advanced (T3-T4 or N+) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 2027-32.
5. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, Thomas CR Jr, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008; 299: 1914-21.
6. Julie DA, Oh JH, Apte AP, Deasy JO, Tom A, Wu AJ, Goodman KA. Predictors of acute toxicities during definitive chemoradiation using intensity-modulated radiotherapy for anal squamous cell carcinoma. Acta Oncol 2016; 55: 208-16.
7. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995; 31: 1319-39.
8. Filippi AR, Franco P, Ricardi U. Is clinical radiosensitivity a complex genetically controlled event? Tumori 2006;92: 87-91.
9. Lujan AE, Mundt AJ, Yamada SD, Rotmensch J, Roeske JC. Intensity-modulated radiotherapy as a means of reducing dose to bone marrow in gynecologic patients receiving whole pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2003;57:516-21.
10. Mell LK, Schomas DA, Salama JK, Devisetty K, Aydogan B, Miller RC, et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in anal cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys 2008; 70: 1431-7.
11. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol 1961;5:255-8.
12. Jianyang W, Yuan T, Yuan T, Ning L, Hua R, Hui F, et al. A prospective phase II study of magnetic resonance imaging guided hematopoietical bone marrow-sparing intensity-modulated radiotherapy with concurrent chemotherapy for rectal cancer. Radiol Med 2016; 121: 308-314.
13. Mell LK, Tiryaki H, Ahn KH, Mundt AJ, Roeske JC, Aydogan B. Dosimetric comparison of bone marrow-sparing intensity modulated radiotherapy versus conventional techniques for treatment of cervical cancer. Int J Radiat Oncol Biol Phys 2008; 71: 1504-10.

14. Cristy M. Active bone marrow distribution as a function of age in humans. Phys Med Biol 1981; 26: 389-400.
15. Blebea JS, Houseni M, Torigian DA, Fan C, Mavi A, Zhuge Y, et al. Structural and functional imaging of normal bone marrow and evaluation of its age-related changes. Semin Nucl Med 2007; 37: 1129-33.
16. Vogler JB 3rd, Murphy WA. Bone marrow imaging. Radiology 1988; 168: 679-693.
17. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys 1995; 31: 1319-39.
18. Filippi AR, Franco P, Galliano M, Ricardi U. Peripheral blood complete remission after splenic irradiation in mantle-cell lymphoma with 11q22-23 deletion and ATM inactivation. Radiat Oncol 2006; 1:35.
19. Sykes M, Chu F, Savel H, Bonadonna G, Mathis H. The effects of varying dosages of irradiation upon sternal marrow regeneration. Radiology 1964; 83: 1563-70.
20. Tubiana M, Frindel E, Croizat H. Effects of radiation on bone marrow. Pathol Biol (Paris) 1979; 27: 326-34.
21. Rubin P, Scarantino C. The bone marrow organ: the critical structure in radiation-drug interaction. Int J Radiat Oncol Biol Phys 1978;4: 3-23.
22. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, et al. Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients. Br J Radiol 2016;89(1060): 20150832.
23. Roeske JC, Lujan A, Reba RC, Penney BC, Yamada SD, Mundt AJ. Incorporation of SPECT bone marrow imaging into intensity modulated whole-pelvic radiation therapy treatment planning for gynecologic malignancies. Radiother Oncol 2005;77:11-17.
24. Agool A, Schot BW, Jager PL, Vellenga E. 18F-FLT PET in hematologic disorders: a novel technique to analyze the bone marrow compartment. J Nucl Med 2006; 47: 1592-1598.
25. Hayman JA, Callahan JW, Herscjtal A, Everitt S, Binns DS, Hicks RJ, et al. Distribution of proliferating bone marrow adult cancer patients determined using FLT-PET imaging. Int J Radiat Oncol Biol Phys 2011;79:847-52.
26. McGuire SM, Menda Y, Boles Ponto LL, Gross B, Buatti J, Bayouth JE. 3'-deoxy-3'-[^{18}F]fluorothymidine PET quantification of bone marrow response to radiation dose. Int J Radiat Oncol Biol Phys 2011;81:888-893.
27. Campbell BA, Callahan J, Bressel M, Simoens N, Everitt S, Hofman MS, et al. Distribution atlas of proliferating bone marrow in non-small cell lung cancer patients measured by FLT-PET/CT imaging, with potential applicability in radiation therapy planning. Int J Radiat Oncol Biol Phys 2015;92:1035-43.
28. McGuire SM, Menda Y, Boles Ponto LL, Gross B, TenNapel M, Smith BJ, et al. Spatial mapping of functional pelvic bone marrow using FLT PET. J Appl Clin Med Phys 2014; 15: 4780.
29. Franco P, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, et al. Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemoradiation in anal cancer patients. Med Oncol 2016; 33: 72.

Chapter 3.

Hematologic toxicity in anal cancer patients: exploring its magnitude as a clinical issue

Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Hematologic toxicity in anal cancer patients during combined chemo-radiation: a radiation oncologist perspective.** *Expert Rev Anticancer Ther 2017;17:335-345*1.

Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M,Racca P, Ricardi U. **Early-stage node-negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy**. *Anticancer Res 2016;36;1943-1948.*

Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M, Racca P, Ricardi U. **Locally Advanced (T3-T4 or N^+) Anal Cancer Treated with Simultaneous Integrated Boost Radiotherapy and Concurrent Chemotherapy.** *Anticancer Res 2016;36;2027-2032.*

Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, Rondi N, Morino M, Racca P, Ricardi U. **Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients.** *Br J Radiol 2016;89(1060):20150832.*

Arcadipane F, **Franco P**, Ceccarelli M, Furfaro G, Rondi N, Trino E, Martini S, Iorio GC, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Image-guided IMRT with simultaneous integrated boost as per RTOG 0529 for the treatment of anal cancer.** *Asia Pac J Clin Oncol 2017 (in press).*

Hematologic toxicity in randomized phase III trial

HT is a clinically meaningful occurrence in anal cancer patients, potentially affecting patient compliance and treatment outcomes. This finding has been observed since the first randomized phase III trials exploring the role of concurrent CT-RT employing 5-FU and MMC in AC, namely the ACT I and EORTC 22861 trials (see Table 1)[30,31]. For example in the ACT I trial, patients were randomized to receive either exclusive RT (45 Gy in 20 or 25 fractions) over 4-5 weeks or the same regimen concomitant to 5-FU and MMC. Treatment response was assessed at 6 weeks and good responders were boosted with RT while poor responders were submitted to salvage surgery. Radiotherapy was delivered employing 2-dimensional approaches with supposedly large BM volumes within treatment fields (Table 1). These findings prompted clinicians to explore the withdrawal of MMC, as in the RTOG 8704/ECOG 1289 trial, were randomization consisted of treatment with either RT (45-50.4 Gy to the pelvic region) concurrent to 5-FU or 5-FU/MMC[31]. Removing MMC from treatment schedule lowered the rate of G4-G5 acute HT from 18% to 3%, but also the colostomy-free and disease free-survival rates, with an excess in definitive colostomies (15% vs 8%)[32]. More recent trials, such as RTOG 98-11 investigating the role of cisplatin (DDP) added to 5-FU and RT in decreasing the toxicity profile compared to standard RT + 5-FU/MMC continued showing high rates of HT [33]. Patients in the standard arm (5-FU/MMC) experienced a 61% rate of G3-G4 acute HT, while those in the experimental arm (5-FU/DDP) a 42% rate[33]. The use of DDP lowered the acute HT rate, which nevertheless remained consistent. Even better results were described in most the recent trials such as the ACT II and the ACCORD 3 trials, where, in the arms employing DDP, the rates of G3-G4 acute HT were 16% and 19%[34,35]. In these trials, DDP was also used as neoadjuvant or maintainance therapy combined to 5FU. All the aforementioned studies used standard RT techniques, such as 2-dimensional RT including anterior-posterior/ posterior-anterior (AP/PA) parallel opposed fields or AP/PA fields added to paired laterals fields or a 4-field BOX techniques or a 3-dimensional conformal RT approach based on a 4-field class

solution (Table 1). The boost dose to the macroscopic disease within the anal canal was delivered sequentially to the whole pelvis phase either with photons, electrons or ^{192}Ir implants. Pelvic bony segments containing BM were not taken into account to be selectively spared and thus, medium to high doses were received by these structures in all these studies.

Table 1. Acute hematologic toxicity profile in anal cancer patients within prospective phase III trials

Author	Year	Randomization	Pts	CT	RT technique	Boost strategy	HT scoring scale	≥ G3 HT
Flam et al	1996	RT-CT	145	5-FU	AP/PA	Sequential	NCI	G4-G5:
RTOG 8704/ECOG 1289		vs						3%
		RT-CT	146	5-FU/MMC				vs
								18%
UKCCCR	1996	RT alone	290	None	AP/PA	Sequential	NA	No grading available
ACT I		vs						*WBC < 1.000/ul*
		RT-CT	295	5-FU/MMC	Boost: electrons, photons,			0 (RT) vs 2% (RT-CT)
					^{192}Ir implants			*PLT < 25.000/ul*
								0 (RT) vs 2% (RT-CT)
Bartelink et al	1997	RT alone	52	None	AP/PA	Sequential	WHO	NA
EORTC 22861		vs						
		RT-CT	51	5-FU/MMC	Boost: electrons, photons,			
					^{192}Ir implants			
Ajani et al	2008	RT-CT	341	5-FU/MMC	AP/PA	Sequential	CTCAE v2.0	Overall:
RTOG 98-11		vs			AP/PA + paired laterals			61%
		RT-CT	341	5-FU/DDP	PA + laterals			vs
					Direct perineal boost:			42%
					electron, photons			
Peiffert et al	2012	ICT + RT-CT (standard boost)	75	5-FU/DDP	AP/PA	Sequential	CTCAE v3.0	Overall:
ACCORD 03		vs						
		ICT + RT-CT (intensified boost)	75	5-FU/DDP	4-field BOX technique			29% (ICT arms)
		vs						vs
		RT-CT (standard boost)	82	5-FU/DDP	Boost: electrons, photons,			19% (RT-CT arms)
		vs			^{192}Ir implants			
		RT-CT (intensified boost)	75	5-FU/DDP				
James et al	2013	RT-CT	246	5-FU/MMC	4-field BOX technique	Sequential	CTCAE v3.0	Overall:
ACT II		vs						
		RT-CT	246	5-FU/DDP	Boost: 3DCRT			MMC group: 26%
		vs						vs
		RT-CT + maintainance CT	226	5-FU/MMC + 5FU/DDP				DDP group: 16%
		vs						
		RT-CT + maintainance CT	222	5-FU/DDP + 5FU/DDP				

Legend: pts: patients; CT: chemotherapy; RT: radiotherapy; BM: bone marrow; opt: optimization; HT: hematologic toxicity; ICT: induction chemotherapy; 5-FU: 5-fluorouracil; MMC: mytomicin C; DDP: cisplatin; AP/PA: anterior-posterior/posterior-anterior; ^{192}Ir : iriudium 192; 3DCRT: 3-dimensional conformal radiotherapy; NCI: National Cancer Institute; WHO: World Health Organization; CTCAE: Common Terminology Criteria for Adverse Effects; WBC: white blood cells; PLT: platelets; ul: microliter.

Hematologic toxicity in IMRT series

Intensity-modulated radiotherapy (IMRT) is a RT approach able to deliver external beam radiation with robust conformality and modulation, abrupt dose falloff and reliable accuracy[1,2]. This technique has been implemented in several clinical context and is presently considered standard of care to deliver RT in anal cancer patients[3] . A large number of clinical series have been published in recent years (see Table 2) [4-14]. Compared to 2- or 3-dimensional approaches, IMRT is able to decrease medium to high dose to critical structures, conversely increasing volumes of normal tissues receiving low dose bath[50]. The contribution of this peculiar dose distribution to the occurrence, duration and characteristics of HT has yet to be determined. In this sense, the report by Robinson et al rises up some concerns on the significant increase in the dose received by pelvic bone marrow (PBM) during IMRT treatments compared to 3D-conformal approaches, with normal tissue control probability (NTCP) modeling suggesting an approximately doubling in the risk of occurrence of major HT. Early IMRT reports employed static techniques (either step and shoot or sliding window IMRT) and a sequential approach to deliver a boost dose to the primary tumor within the anal canal[4-6]. During the treatment planning process, optimization on BM as a critical structure was sporadically performed and, when present, was addressed only to iliac crests. For example, Salama et al reported on 53 patients treated with IMRT for anal cancer at 3 tertiary-care academic center. RT was delivered with a static approach mainly using 9 equally spaced fields with a planning priority set primarily to target coverage and secondarily to small bowel, bladder and genitalia avoidance. No specific dose constraints were applied to bony structures to decrease HT. Patient were given 45 Gy to the pelvic region and inguinal groins and a sequential boost dose to the macroscopic disease up to 50-54 Gy concurrent to 5-FU and MMC. A total of 39.6% of patients experienced G4 HT. The most common major events were acute G3-G4 leukopenia (53%), thrombocytopenia (28%) and anemia (9%)[15]. Most recent series used volumetric approaches such as volumetric-modulated arc therapy (VMAT) and tomotherapy, with a simultaneous integrated boost (SIB) strategy to boost the macroscopic disease and a plan optimization accounting for pelvic

BM[14,15]. Nevertheless the acute HT profile remains not negligible. In the multicentric series by Call et al, reporting on 152 anal cancer patients treated with IMRT and different combinations of concurrent drugs, the overall acute HT rate was 41%[15]. Franco et al observed in their cohort of patients treated with VMAT and concurrent 5-FU/MMC rates of leukopenia up to 36%, neutropenia 31% and thrombocytopenia 13%. Similar, findings come from the RTOG 0529 trial that investigated whether dose-painted IMRT could reduce by at least 15% the $\geq$ G2 gastrointestinal and genitourinary toxicity rates compared to conventional treatments as delivered in the RTOG 9811 trial. The primary end-point of the study was not reached. However, a significant reduction in acute G2 HT (73% vs 85 % for RTOG 98-11) was observed[11]. A better HT toxicity profile was seen with IMRT, but still with substantially high toxicity rates and substantial room for clinical improvement in this setting.

Table 2. Acute hematologic toxicity profile in anal cancer patients treated with IMRT

Author	Year	Pts	IMRT technique	Boost strategy	BM opt	CT	HT scoring scale	G3-G4 HT
Milano et a	2005	17	Stating angle	Sequential	Yes (iliac BM)	5-FU/MMC	RTOG	Overall: 53%
			7-field class solution			5-FU		Leukopenia: 47%
								Thromocytopenia: 18%
								Anemia: 12%
Salama et a	2007	53	Static angle	Sequential	No	5-FU/MMC	CTCAE v 3.0	Leukopenia: 53%
			9-field class solution	SIB		5-FU/DDP		Thromocytopenia: 28%
						5-FU		Anemia: 9%
Pepek et al	2010	47	NA	Sequential	Yes (iliac BM)	5-FU/MMC	CTCAE v 3.0	Overall: 24%
						Cape/MMC		Leukopenia: 24%
						Cape		Thromocytopenia: 3%
								Anemia: 3%
Bazan et al	2011	29	Static angle	Sequential	NA	5-FU/MMC	CTCAE v 3.0	Overall: 21%
						Cape/MMC		
						5-FU/DDP		
Vieillot et a	2012	72	Static angle	Sequential	Yes (iliac BM)	5-FU/MMC	CTCAE v 3.0	Overall: 25%
			5-7 field class solution			5-FU/DDP		Neutropenia: 21%
								Thromocytopenia: 9%
								Anemia: 6%
DeFoe et al	2012	78	Static angle	Sequential	es (pelvic bones	5-FU/MMC	CTCAE v 3.0	Overall: 43%
			5-9 field class solution			5-FU/DDP		Leukopenia: 36%
						Cape		Neutropenia: 39%
								Thromocytopenia: 12%
								Anemia: 4%
Kachnic et a	2012	43	Static angle	SIB	Yes (iliac BM)	5-FU/MMC	CTCAE v 3.0	Overall: 61%
			8-10 field class solution			5-FU/DDP		
						5-FU		
Kachnic et a	2013	52	NA	SIB	Yes (iliac BM)	5-FU/MMC	CTCAE v 3.0	Overall: 58%
RTOG 0529								
Chuong et a	2013	52	NA	Sequential	No	5-FU/MMC	CTCAE v 4.0	Leukopenia: 30%
				SIB		5-FU/DDP		Thromocytopenia: 21%
								Anemia: 13%
Belgioia et a	2015	41	Helical tomotherapy	SIB	es (pelvic bones	5-FU/MMC	CTCAE v 3.0	Overall: 7%
						Cape		
Franco et al	2016	39	VMAT	SIB	No	5-FU/MMC	CTCAE v 3.0	Leukopenia: 36%
								Neutropenia: 31%
								Thromocytopenia: 13%
								Anemia: 0%
Call et al	2016	152	Static angle	Sequential	Yes (iliac BM)	5-FU/MMC	RTOG	Overall: 41%
			7-9 field class solution	SIB		5-FU/DDP	CTCAE v 3.0	
						5-FU/MMC/DDP		
						5-FU/DDP/Cet		

Legend: pts: patients; IMRT: intensity-modulated radiotherapy; CT: chemotherapy; BM: bone marrow; opt: optimization; HT: hematologic toxicity; 5-FU: 5-fluorouracil; MMC: mytomicin C; DDP: cisplatin; Cape: capecitabine; Cet: cetuximab ; RTOG: Radiation Therapy Oncology Group; CTCAE: Common Terminology Criteria for Adverse Effects.

References

1. Merlotti A, Alterio D, Vigna-Taglianti R, Muraglia A, Lastrucci L, Manzo R, Gambaro G, Caspiani O, Miccichè F, Deodato F, Pergolizzi S, Franco P, Corvò R, Russi EG, Sanguineti G; Italian Association of Radiation Oncology. Technical guidelines for head and neck cancer IMRT on behalf of the Italian association of radiation oncology - head and neck working group. Radiat Oncol 2014;9:264.
2. Franco P, Zeverino M, Migliaccio F, Sciacero P, Cante D, Casanova Borca V, Torielli P, Arrichiello C, Girelli G, Numico G, La Porta MR, Tofani S, Ricardi U. Intensity-modulated adjuvant whole breast radiation delivered with static angle tomotherapy (TomoDirect): a prospective case series. J Cancer Res Clin Oncol 2013;139:1927-36.
3. Franco P, Zeverino M, Migliaccio F, Cante D, Sciacero P, Casanova Borca V, Torielli P, Arrichiello C, Girelli G, La Porta MR, Tofani S, Numico G, Ricardi U. Intensity-modulated and hypofractionated simultaneous integrated boost adjuvant breast radiation employing statics ports of tomotherapy (TomoDirect): a prospective phase II trial. J Cancer Res Clin Oncol 2014;140:167-77.
4. Milano MT, Jani AB, Farrey KJ, Rash C, Heimann R, Chmura SJ. Intensity-modulated radiation therapy (IMRT) in the treatment of anal cancer: toxicity and clinical outcome. Int J Radiat Oncol Biol Phys 2005;63:354-61.
5. Salama JK, Mell LK, Schomas DA, Miller RC, Devisetty K, Jani AB, et al. Concurrent chemotherapy and intensity-modulated radiation therapy for anal cancer patients: a multicenter experience. J Clin Oncol 2007; 25: 4581-6.
6. Pepek JM, Willett CG, Wu QJ, Yoo S, Clough RW, Czito BG. Intensity-modulated radiation therapy for anal malignancies: a preliminary toxicity and disease outcomes analysis. Int J Radiat Oncol Biol Phys 2010;78:1413-19.
7. Bazan JG, Hara W, Hsu A, Kunz PA, Ford J, Fisher GA, et al. Intensity-modulated radiation therapy versus conventional radiation therapy for squamous cell carcinoma of the anal canal. Cancer 2011; 117: 3342-51.
8. Vieillot S, Fenoglietto P, Lemanski C, Moscardo CL, Gourgou S, Dubois JB, Aillères N, Azria D. IMRT for locally advanced anal cancer: clinical experience of the Montpellier Cancer Center. Radiat Oncol 2012;7:45.
9. DeFoe SG, Beriwal S, Jones H, Rakfal S, Heron DE, Kabolizadeh P, et al. Concurrent chemotherapy and intensity-modulated radiation therapy for anal carcinoma--clinical outcomes in a large National Cancer Institute-designated integrated cancer centre network. Clin Oncol 2012; 24: 424-31.
10. Kachnic LA, Tsai HK, Coen JJ, Blaszkowsky LS, Hartshorn K, Kwak EL, et al. Dose-painted intensity-modulated radiation therapy for anal cancer: a multi-institutional report of acute toxicity and response to therapy. Int J Radiat Oncol Biol Phys 2012;82:153-8.
11. Kachnic LA, Winter K, Myerson RJ, Goodyear MD, Willins J, Esthappan J, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mitomycin-C for the reduction of acute morbidity in carcinoma of the anal canal. Int J Radiat Oncol Biol Phys 2013;86:27-33.
12. Chuong MD, Freilich JM, Hoffe SE, Fulp W, Weber JM, Almhanna K, et al. Intensity-Modulated Radiation Therapy vs. 3D Conformal Radiation Therapy for Squamous Cell Carcinoma of the Anal Canal. Gastrointest Cancer Res 2013; 6: 39-45.
13. Belgioia L, Vagge S, Agnese D, Garelli S, Murialdo R, Fornarini G, et al. Intensified intensity-modulated radiotherapy in anal cancer with prevalent HPV p16 positivity. World J Gastroenterol 2015; 21: 10688-96.

14. Call JA, Prendergast BM, Jensen LG, Ord CB, Goodman KA, Jacob R, et al. Intensity-modulated radiation therapy for anal cancer: results from a multi-institutional retrospective cohort study. Am J Clin Oncol 2016; 39: 8-12.
15. Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2006; 66: 1356-65.
16. Robinson M, Sabbagh A, Muirhead R, Durrant L, Van den Heuvel F, Hawkins M. Modeling early haematologic adverse events in conformal and intensity-modulated pelvic radiotherapy in anal cancer. Radiother Oncol 2015; 117: 246-51.

Chapter 4.

Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer

Franco P, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, Di Muzio J, Morino M, Racca P, Ricardi U. **Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer**. *Clin Transl Oncol 2017;19:67-75.*

The combination of radiotherapy and chemotherapy (CT) in a concurrent setting is presently considered as the standard therapeutic option for squamous cell anal cancer patients, providing high rates of loco-regional control (LC), overall survival (OS) and sphincter preservation[1]. Updated long-term results of the RTOG 98-11 trial which employed radiation and concomitant 5-fluorouracil (5-FU)/mitomicin-C (MMC) showed 5-year disease-free survival (DFS), colostomy-free survival (CFS) and OS rates of 67.8%, 71.9% and 78.3%, respectively, confirming consistent clinical results for this approach[2]. However, non-conformal techniques were used in RTOG 9811 (AP/PA parallel opposed fields or 4-field conformal approaches) leading to a high rate of major acute toxicities with respect to skin (G3-G4: 48%), gastrointestinal tract (G3-G4: 35%) and blood cells (G3-G4:61%). Intensity-modulated radiotherapy (IMRT) is able to improve conformality and increase dose fall-off within target volumes, thus reducing dose to organs at risk (OARs) in several clinical settings including anal cancer[3-5]. Hematological toxicity (HT) due to myelosuppression is still a major cause of treatment interruptions which may lead to an increase in overall treatment time with a consequent detrimental effect on clinical outcome[6]. The RTOG 0529 trial was able to show a decrease in the rate of $\geq$ G2 acute HT with the use of IMRT compared to standard approaches (73%-RTOG 0529 vs 85%-RTOG 9811)[7]. Nevertheless HT still remains a consistent issue in anal cancer patients, since a large volume of bone marrow (BM) is comprised within treatment fields during unconstrained IMRT to the pelvic region, where up to 40% of the total hematopoietically active BM is present on the average adult population[8]. IMRT planning can be optimized in order to selectively spare BM during treatment to improve acute HT profile. We herein defined dosimetric predictors of HT in anal cancer patients undergoing concurrent IMRT and CT.

Material and methods

We performed a retrospective review of the medical records of all patients affected with anal squamous cell carcinoma treated with IMRT and concurrent CT at the Department of Radiation Oncology of the University of Turin, Italy. Between April 2007 and March 2015 a total of 50

patients were treated employing combination therapy with definitive intent without CT dose reduction. This cohort formed the study sample whose data we employed for the present analysis.

Radiotherapy

All patients received IMRT. Technical details regarding set up, simulation and target volume selection and delineation have been previously described[1]. Dose prescriptions for target volumes were derived from Kachnic et al and adjusted according to clinical stage at presentation[9]. Patients having cT2N0 disease were prescribed 50.4 Gy/28 fractions (1.8 Gy daily) to the gross tumor PTV and 42 Gy/28 fractions (1.5 Gy daily) to the elective nodal PTV. Patients diagnosed with cT3-T4/N0-N3 disease were prescribed 54 Gy/30 fractions (1.8-2 Gy daily) to the anal gross tumor PTV, while gross nodal PTVs were prescribed 50.4 Gy/30 fr (1.68 Gy daily) if sized $\leq$ 3 cm or 54 Gy/30 fr (1.8 Gy daily) if > 3 cm; elective nodal PTV was prescribed 45 Gy/30 fractions (1.5 Gy daily) [9]. All patients were treated with IMRT employing a simultaneous integrated boost approach. Both static and volumetric techniques were used. Planning strategies exclusively included an unconstrained approach towards BM. Step and shoot IMRT plans were generated with different class solution, up to 7 modulated fields, depending on patients' anatomy and employing 6 MV photons. Volumetric-modulated arc-therapy (VMAT) plans were computed on Elekta Monaco treatment planning system (version 3.2), allowing for optimization with biological cost-functions for both PTV and OARs with 3 main functions (Poisson statistics cell-kill model, serial and parallel complication models), employing a single-arc of 360° (starting from 180°) or, more recently, the dual-arc approach after system upgrade. Radiotherapy delivery was performed under cone beam CT (CBCT) image guidance, with daily treatment couch repositioning performed after automatic matching of CBCT images and reference planning CT.

Bone marrow delineation

We outlined the external contour of pelvic bone marrow (PBM) on the planning CT employing bone windows as first described by Mell et al[10]. The PBM was delineated as a whole and then

divided into 3 subsites: a) the iliac BM (IBM), extending from the iliac crests to the upper border of femoral head; b) lower pelvis BM (LPBM), accounting for bilateral pube, ischia, acetabula and proximal femura, from the upper limit of the femoral heads to the lower limit of the ischial tuberosities and c) lumbosacral BM (LSBM), extending from the superior border of L5 somatic body[6,10]. Figure 1 shows an example of PBM delineation in the 3 different subsites.

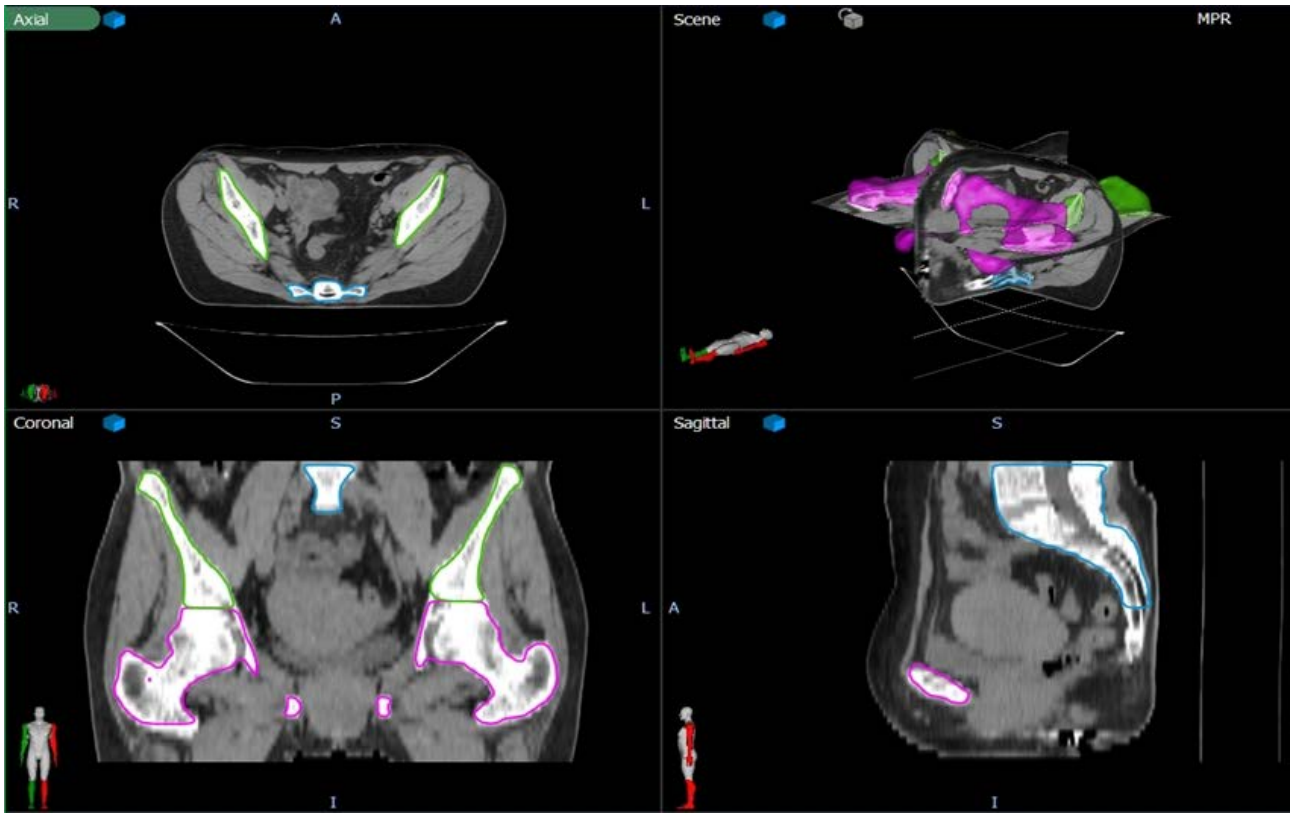


Figure 1. Delineation of iliac (green), lower pelvis (light violet), lumbar-sacral (light blue) bone marrow as seen on axial, coronal, sagittal and 3D view.

Chemotherapy

All patients received concurrent chemotherapy (CT), consisting of 5- fluorouracil (1000 mg/m^2/day) given as continuous infusion along 96 hours (days 1-5 and 29-33) associated with mitomycin C (10 mg/m^2) given as bolus (days 1 and 29). A total of 2 concurrent cycles were administered during EBRT. Blood cell counts were performed on a routine basis prior to each cycle. Prophylactic antiemetic medications were given as intravenous granisetron 3 mg and dexamethasone 8 mg. Granulocyte-monocyte colony stimulating factor (GCSF) and erythropoietin were allowed in case of major HT.

Hematologic toxicity evaluation

All patients underwent a weekly complete blood count. HT was graded according to the Radiation Therapy Oncology Group acute radiation-induced morbidity scoring system[11]. Endpoints evaluated in the present analysis were white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb) and platelet (Plt) count nadirs after each CT cycle and the highest-grade toxicity for all blood cells. HT was defined as each hematologic event having a grade $\geq$ 3.

Statistical analysis

Cumulative dose-volume histograms (DVHs) were created for PBM and all 3 BM subsites. Median doses and dosimetric parameters on the DVHs were then analyzed. WBC, ANC, Hb and PLT nadirs were correlated to age, mean dose and V_5-V_{10}-V_{15}-V_{20}-V_{30}-V_{40}-V_{45} for PBM, LSBM, LPBM and IBM as continuous variables and HIV status, grading, T and N stage as categoric variables . Generalized linear modeling was used to find correlations between clinical and dosimetric variables and blood cells nadirs. Shapiro-Wilk statistic was used to test for normality of variables. A log transform was used to eliminate skew in the dependent variables. Covariates found to be significant on univariate linear regression analysis were included in the multivariate linear regression model. R^2 and adjusted R^2 test were used to evaluate goodness of model fit. Logistic regression analysis was used to test correlation between HT events and dosimetric parameters. Covariates found to be

significant on univariate logistic regression analysis were included in the multivariate logistic regression model. Hosmer-Lemeshow test was used to evaluate goodness of model fit. The test of Holm was employed to check the false discovery rate of the variables found to be predictive for HT using a minimum uncorrected p-value threshold of 0.05. Fisher's exact and Wilcoxon's test were employed to test the difference in proportions and continuous variables on univariate analysis. Receiver Operating Characteristic (ROC) curves were used to evaluate the optimal cut-off points for predictive dosimetric variables. Youden method was used to find the optimal cut-off. Stata Statistical Software, version 13.1 (Stata Corporation, Texas) was employed for analysis.

Results

A total of 50 patients were included in the present study. Detailed patients characteristics are shown in Table 1. Mean age was 64 (range 39-79) and patients were mainly female (76%), HIV-negative (92%), with an anal canal primary (84%), T2-T3 stage (92%), N0 stage (70%), G2 (58%) and with no preventive colostomy (100%). Patients were mainly treated with a VMAT approach (70%). Mean doses to the PTVs volumes were 53 Gy, 50.4 Gy and 44 Gy for the gross tumor, gross nodal and elective nodal volumes, respectively. The mean interval between biopsy and radiation start was 80 days. Mean radiotherapy duration was 43 days. Patients undergoing a treatment break $\geq$ 3 days were 9%. All patients were submitted to 2 cycles of CT with no dose reduction during treatment. See Table 2 for details.

Table 1. Patients' characteristics

Variable	N (%)
Age	
Mean	64
Range	39-79
Sex	
Female	38 (76)
Male	12 (24)
HIV status	
Positive	4 (8)
Negative	46 (92)
Primary tumor site	
Anal canal	42 (84)
Anal margin	8 (16)
T stage	
T1	3 (6)
T2	33 (66)
T3	13 (26)
T4	1 (2)
N stage	
N0	35 (70)
N1	2 (4)
N2	12 (24)
N3	1 (2)
Global stage	
I	3 (6)
II	31 (62)
IIIA	3 (6)
IIIB	13 (26)
Grading	
G1	7 (14)
G2	29 (58)
G3	14 (28)
Prophylactic colostomy	
Yes	0 (0)
No	50 (100)

Table 2. Treatment characteristics

Variable	N (%)
IMRT approach	
S&S	16 (30)
VMAT	37 (70)
PTV dose-tumor (Gy)	
Mean	53
Range	50.4-54
PTV dose-positive nodes (Gy)	
Mean	50.4
Range	50.4-54
PTV dose-negative nodes (Gy)	
Mean	44
Range	42-45
5-FU + MMC cycles (full dose)	
2	50 (100)
Biopsy-RT interval (days)	
Mean	79
Range	25-161
RT duration (days)	
Mean	42
Range	37-59
RT breaks ≥ 3 days	
Yes	5 (10)
No	45 (90)

Legend: IMRT: intensity-modulated radiotherapy; S&S: step and shoot; VMAT: volumetric modulated arc-therapy; PTV: planning target volume; 5-FU: 5-fluorouracil; MMC: mytomicin C; RT: radiotherapy

Acute hematologic toxicity and dosimetric outcomes

The median nadir WBC, ANC, Hb and Plt counts were 2.7 k/μl (range: 0.7-6.2), 1.8 k/μl (range: 0.2-3.7), 11.4 g/dL (range: 7.9-14.7) and 121 k/μl (range: 41-253). Maximum detected acute HT comprised 38% of patients experiencing leukopenia ≥ G3 and 32% with neutropenia ≥ G3. Grade 2 anemia was observed in 4% of patients, while no G3-G4 events were seen. Up to 10% experienced ≥ G3 thrombocytopenia. See table 3 for baseline hematologic values and acute HT details. Dosimetric parameters to bony pelvic structures are shown in Table 4 with mean values and corresponding standard deviations. Figure 2 shows isodoses distribution in a case of locally advanced anal cancer.

Table 3. Acute hematologic toxicity

Hematologic parameters at baseline	
	Mean (range)
Hb	13.3 g/dL (9.1-16.2)
PLT	224 k/μl (96-380)
WBC	6.6 k/μl (3.8-11.3)
ANC	4.2 k/μl (1.6-9.6)

	N(%)				
Acute HT	*G0*	*G1*	*G2*	*G3*	*G4*
Leukopenia	5 (10)	11 (22)	15 (30)	17 (34)	2 (4)
Neutropenia	15 (30)	10 (20)	9 (18)	13 (26)	3 (6)
Anemia	31 (62)	17 (34)	2 (4)	0 (0)	0 (0)
Thrombocytopenia	29 (58)	10 (20)	6 (12)	5 (10)	0 (0)

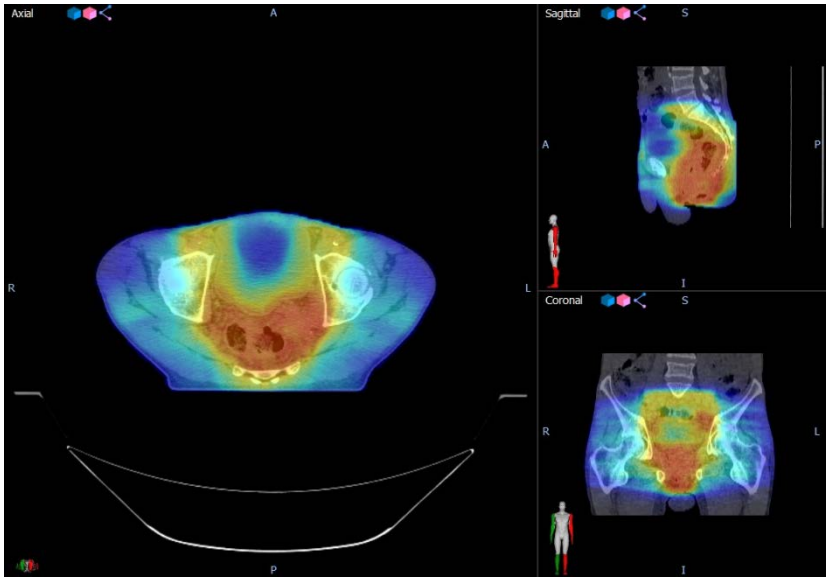
Legend: Hb: hemoglobin; PLT: platelets; WBC: white blood cells; ANC: absolute neutrophil count; g: grams; dL: deciliters; k: 10^3; μl: microliters; HT: hematologic toxicity.

Table 4. Dosimetric parameters

Parameter	Mean	SD
PBM		
Volume (cm^3)	1400	240
Mean dose (Gy)	29	5
V_5 (%)	94	6
V_{10} (%)	89	8
V_{15} (%)	83	9
V_{20} (%)	75	9
V_{30} (%)	52	11
V_{40} (%)	26	11
V_{45} (%)	9	9
IBM		
Volume (cm^3)	424	63
Mean dose (Gy)	25	6
V_5 (%)	90	9
V_{10} (%)	82	11
V_{15} (%)	74	10
V_{20} (%)	63	10
V_{30} (%)	36	11
V_{40} (%)	14	10
V_{45} (%)	4	7
LSBM		
Volume (cm^3)	392	71
Mean dose (Gy)	32	6
V_5 (%)	90	10
V_{10} (%)	86	13
V_{15} (%)	83	14
V_{20} (%)	79	15
V_{30} (%)	67	16
V_{40} (%)	41	15
V_{45} (%)	13	12
LPBM		
Volume (cm^3)	598	128
Mean dose (Gy)	30	4
V_5 (%)	99	3
V_{10} (%)	96	6
V_{15} (%)	89	9
V_{20} (%)	80	10
V_{30} (%)	54	13
V_{40} (%)	25	13
V_{45} (%)	9	10

Legend: HT: PBM: pelvic bone marrow; IBM: iliac bone marrow; LSBM: lumbar-sacral bone marrow; LPBM: lower pelvis bone marrow.

Figure 2. Isodoses visualization in a case of locally advanced anal cancer with dose spread to pelvic bones as seen on axial, coronal, sagittal view



Predictors of hematologic toxicity

The correlation between dosimetric parameters and WBC, ANC, Hb and Plt nadirs was investigated with the finding that PBM-V_{20} was significantly associated to WBC nadir on multivariate linear regression analysis (linear regression β coefficient: -0.035; SE: 0.017; p= 0.048; 95% CI:-0.069/-0.0003). The R^2 and adjusted R^2values of the multivariate model were 0.08 and 0.02, respectively, indicating a fair amount of unexplained variation in the regression model. Table 5 summarizes all selected covariates against WBC nadir after univariate linear regression analysis. Figure 3 shows a scatterplot of WBC nadir versus PBM-V_{20} with the trend line superimposed. A more likelihood to develop $\geq$ G3 HT was observed for increased LSBM-V_{40} (OR: 1.328; SE: 0.160; z: 2.35; p=0.019; 95% CI:1.048-1.682) on multivariate logistic regression analysis. Hosmer-Lemeshow X^2 was 10.5

(p=0.234) indicating that the model is a good fit. Holm method did not show any rejected p-value, with a false discovery rate controlled at 5%. Table 6 shows all selected covariates against ≥ G3 HT after univariate logistic regression analysis. In order to select optimal thresholds to be used during planning process, the ROC curve for ≥ G3 HT versus LSBM-V_{40} was analyzed. The optimal cut-off point according to Youden method was 41%, with a AUC= 0.614 (Figure 4). Patients with LSBM-V_{40} ≥ 41% were more likely to develop ≥ G3 HT (60.9% vs 39.1%; p=0.041). The sensitivity and specificity for this threshold were 61% and 67%, respectively. The positive and negative predictive values for LSBM-V_{40} ≥ 41% were 56.0% (95% CI: 46.1-67.9%) and 71.0% (95% CI:59.5-83.4%) with a relative risk of 1.56 (95% CI:1.17-2.41).

Table 5. Correlation between dosimetric parameters and WBC nadir on multivariate linear regression analysis

Variable	β	SE	t	p	95%CI
PBM-V_5(%)	0.305	0.163	1.87	0.070	-0.026-0.637
PBM-V_{10}(%)	-0.130	0.146	-0.89	0.378	-0.426-0.166
PBM-V_{15}(%)	-0.223	0.148	-1.50	0.141	-0.523-0.778
PBM-V_{20}(%)	**-0.035**	**0.017**	**-2.03**	**0.048**	**-0.069--0.0002**
IBM-V_5(%)	-0.085	0.089	-0.96	0.345	-0.267-0.095
IBM-V_{10}(%)	0.032	0.096	0.34	0.739	-0.162-0.227
IBM-V_{15}(%)	0.031	0.071	0.43	0.667	-0.114-0.176
LSBM-V_5(%)	-0.038	0.104	-0.37	0.715	-0.251-0.174
LSBM-V_{10}(%)	0.005	0.198	0.03	0.976	-0.396-0.408
LSBM-V_{15}(%)	-0.107	0.187	-0.57	0.573	-0.488-0.274
LSBM-V_{20}(%)	0.079	0.123	0.65	0.523	-0.170-0.329
LSBM-V_{30}(%)	0.009	0.599	0.15	0.881	-0.112-0.130
LSBM-V_{40}(%)	-0.036	0.024	-1.46	0.152	-0.085-0.139

Legend: HT: PBM: pelvic bone marrow; IBM: iliac bone marrow; LSBM: lumbar-sacral bone marrow; LPBM: lower pelvis bone marrow; β: linear regression coefficient; SE: standard error; t= t-statistic; p= associated p-value; CI: confidence interval.

Figure 3. Plot of white blood cell (WBC) count nadir and volume of pelvic bone marrow receiving 20 Gy

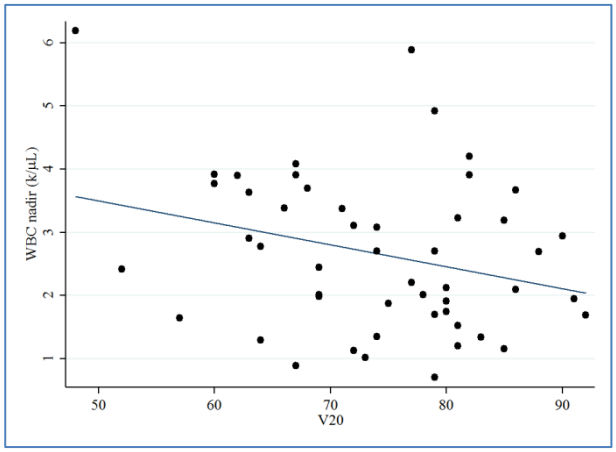
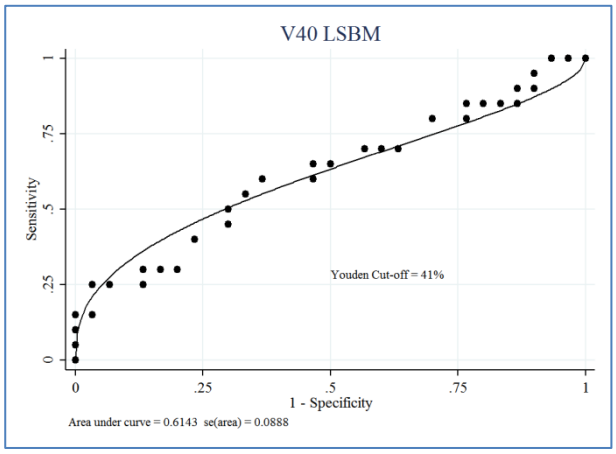


Table 6. Correlation between dosimetric parameters and ≥ G3 acute hematologic toxicity on multivariate logistic regression analysis

Variable	OR	SE	z	p	95%CI
PBM-V_{45}(%)	1.131	0.222	0.63	0.530	0.769-1.663
IBM-V_{40}(%)	0.812	0.093	-1.81	0.070	0.649-1.016
IBM-V_{45}(%)	1.242	0.249	1.08	0.278	0.838-1.841
LSBM-V_{10}(%)	1.154	0.216	0.77	0.442	0.800-1.666
LSBM-V_{20}(%)	1.187	0.272	0.75	0.454	0.757-1.861
LSBM-V_{30}(%)	0.946	0.140	-0.37	0.710	0.708-1.265
LSBM-V_{40}(%)	**1.327**	**0.160**	**2.35**	**0.019**	**1.047-1.682**
LSBM-V_{45}(%)	0.981	0.081	-0.23	0.820	0.833-1.155
LSBM-Mean dose	0.346	0.295	-1.24	0.215	0.065-1.846
LPBM-V_{45}(%)	0.788	0.121	-1.54	0.125	0.582-1.067

Legend: HT: PBM: pelvic bone marrow; IBM: iliac bone marrow; LSBM: lumbar-sacral bone marrow; LPBM: lower pelvis bone marrow; OR: odds ratio; z: z-statistic; p: associated p-value; SE: standard error; CI: confidence interval.

Figure 4. Receiver Operating Characteristic (ROC) curve of LSBM-V_{40} as predictor of $\geq$ G3 acute hematologic toxicity



Considerations

Concurrent chemo-radiation is the standard of care in patients affected with anal cancer. Combination therapy improves clinical outcomes over radiation alone as shown in the ACT-I and EORTC trials[12,13]. Moreover intensified CT regimens have been demonstrated to be superior to mono-chemotherapy as in the Intergroup trial[14]. However HT is a noteworthy issue for this subset of patients, with rates of $\geq$ G3 events up to 61% as reported in the RTOG 98-11 study which employed conventional techniques[15]. Even with the use of IMRT, high rates of HT have been reported, particularly if planning strategies with no specific constraints towards BM are adopted, as in Salama et al, with a 58% G3-G4 acute HT rate[16]. BM is an important dose-limiting cell renewal tissue for wide-field irradiation, such as in the case of anal cancer[17]. Since BM stem cells are extremely radiosensitive, radiation has a consistent myelosuppressive effect, causing BM stem cell apoptosis and stromal damage, with characteristics pathologic and radiographic changes[17]. The major functional sites for BM in the adult population are the pelvis and vertebrae that account for approximately 60% of the total amount. Pelvic bones may contain up to 40% of the total functional BM[18] . This is the reason why pelvic irradiation is a key factor in determining HT during combination therapy in anal cancer. The extent of radiation-induced bone marrow damage has been

demonstrated to be correlated with both radiation dose and BM volume receiving irradiation[18]. Few studies demonstrated the correlation between dosimetric parameters of pelvic osseous structures and blood cell count decrease and/or acute HT in patients undergoing chemo-radiation for pelvic malignancies[6,10]. In the context of anal cancer, Mell et al found on multiple regression analysis that and increased volume of PBM receiving doses between 5 Gy and 20 Gy is significantly associated to decreased WBC and ANC nadirs as the volume of LSBM receiving a dose range between 10 Gy and 20 Gy[6]. Conversely, no association was found between any dosimetric parameters and G3-G4 leukopenia or neutropenia, even if the volume of LSBM receiving 10 Gy (V_{10}-LSBM) had a non-statistically significant trend in increasing odds of G4 leukopenia (OR:1.06; 95%CI:0.99-1-12;p=0.051)[6]. In the context of cervical cancer, with patients treated with concurrent radiotherapy and weekly cisplatin 40 mg/m^2, Mell et al observed that PBM-$V_{10} \geq 90$ and PBM-$V_{20} \geq 75\%$ were associated with a lower WBC nadir and particularly LSBM-V_{10} and LSBM-V_{20}, while for ANC nadir LSBM-V_{10} was the only predictor[10]. Interestingly, the same study showed that an increased PBM-V_{10} and -V_{20} predicted for a higher likelihood to develop $\geq$ G2 leukopenia as the LSBM-V20, LPBM-V_{10} and –V_{20}. A higher PBM-V_{10} was also found to be a predictor of $\geq$ G2 neutropenia[10]. An association between PBM and LSBM V_{10} and V_{20} and WBC and ANC nadirs was found in both studies and with $\geq$ G2 leukopenia and neutropenia in cervical cancer, confirming the high radiosensitivity of BM stem cells, whose early destruction is thought to be responsible for acute myeolosuppression together with effects of peripheral blood stem cells and stromal tissue[17,19]. Our results further support the aforementioned findings since PBM-V_{20} was a significant predictor of WBC nadir (β coefficient: -0.035; SE: 0.017; p= 0.048). In our series mean PBM-V_{20} was 75 % (SD:$\pm$9%), consistently with threshold values found to be predictive for HT. For example, Albuquerque et al who found that PBM-$V_{20} \geq 80\%$ predicted for a 4.5 higher odds of developing $\geq$ G2 HT and Rose et al who demonstrated that patients with a PBM-$V_{10} \geq 95\%$ and PBM-$V_{20} > 76\%$ were more likely to experience $\geq$ G3 leukopenia in the context of cervical cancer treated with concurrent radiation and weekly cisplatin (40 mg/m^2)[20,21]. In anal cancer patients, Cheng et al

recently observed that several low-dose dosimetric parameters of either PBM and LSBM were associated with a higher chance to develop ≥ G3 HT, with volumes of LSBM receiving doses ranging from 5 to 20 Gy being the most consistent predictors[22]. Interestingly, in our study, we found out a correlation between the volume of specific sub-regions such as lumbar-sacral spine receiving medium-high dose and the odds of experiencing HT. Specifically in our series, LSBM-V_{40} correlated with a higher likelihood to develop ≥ G3 HT (OR: 1.328; SE: 0.160; p=0.019). This data suggest 2 different perspective on this subject. At first that specific BM sub-regions can be majorly responsible for hematopoiesis than the whole pelvis bone structure. Pathologic studies have shown that BM is composed of hematologically active 'red' marrow and inactive 'yellow marrow'[23]. Up to 50% of active BM is located within the pelvis and lumbar spine[23]. Nevertheless, studies employing morphological (MRI) and functional imaging (SPECT) identifies a high concentration of hematopoietically active regions in the lumbar-sacral spine, medial ilum and iliac crests[24]. Rose et al characterized active bone marrow in cervical cancer patients treated with concurrent radiotherapy and weekly cisplatin, observing that it was mainly located within the lumbar vertebrae, sacrum and pubic bones, while inactive BM was more frequently located in the ilia, ischia and proximal femura[23] . Our results seems to confirm these findings with dose received by lumbar-sacral spine playing a major role in the development of HT. Nevertheless, the definition of LSBM is crucial in this sense and may affect the strength of the relationship between bone marrow and dosimetric parameters. However, as demonstrated by Cheng et al, whole bone delineation is superior to marrow cavity contouring in predicting HT according to Lyman-Kutcher-Burman model[22] . Secondly, our data suggest the importance of doses up to 40 Gy received by BM subsites (LSBM in our study) in the occurrence of HT. First reports of Mell et al did not show correlation between doses higher than 30 Gy to BM and HT [6,10]. Conversely, Cheng et al found a borderline significance between PBM-V_{30} and LSS-V_{30} and ≥ G3 HT[22]. Moreover, Rose et al found, in their PET-based study, a significant correlation between V_{30} to active BM and WBC nadir and a trend for V_{40}[23]. A recent longitudinal study by Zhu et al in patients submitted to concurrent

chemoradiation for cervical cancer demonstrated that increased PBM V_{20}, V_{30} and V_{40} were significantly associated with a higher weekly reduction of WBC and ANC counts, estimating that every 1 Gy increase in mean PBM dose may lead to a 9.6/μl per week reduction in the natural logarithm of ANC count[25]. Interestingly, a recent paper by Wan et al found, in rectal cancer patients undergoing neoadjuvant EBRT and concurrent capecitabine, a significant correlation between LSBM-V_{40} and $\geq$ G2 HT with patients having LSBM-$V_{40} \geq 60\%$ more likely to develop HT[26]. In our series LSBM-V_{40} was a significant predictor of HT, with a cut-off value of 41% found using ROC curve analysis (AUC= 0.614). Patients with LSBM-$V_{40} \geq 41\%$ were more likely to develop $\geq$ G3 HT (55.3% vs 32.4%; $p<0.01$). This relative volume is more restrictive than the one reported by Wan et al (41% vs 60%), but this is a reasonable finding since more intense CT (5FU-MMC vs capecitabine) have an impact on the normal tissue complication probability of PBM and LSBM as observed by Bazan et al[27]. BM-sparing planning approaches to deliver IMRT in anal cancer should take into account low doses to PBM and specific sub-regions (LSBM), but also medium-high dose constraints can play a role, such as LSBM-V_{40}. The consideration of these constraints within IMRT planning strategies, generating a more abrupt and selective dose fall-off between target volumes and LSBM, particularly in the case of boosted nodal subvolumes, may mitigate acute HT profile[28] .

References

1. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015;33:259-66.
2. Gunderson LL, Winter KA, Ajani JA, Pedersen JE, Moughan J, Benson AB 3rd et al. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomicyn versus fluorouracil/cisplatin. J Clin Oncol. 2012;30:4344-51.
3. Franco P, Zeverino M, Migliaccio F, Sciacero P, Cante D, Casanova Borca V et al. Intensity-modulated adjuvant whole breast radiation delivered with static angle tomotherapy (TomoDirect): a prospective case series. J Cancer Res Clin Oncol. 2013;139:1927-36.

4. Merlotti A, Alterio D, Vigna-Taglianti R, Muraglia A, Lastrucci L, Manzo R et al. Technical guidelines for head and neck IMRT on behalf of the Italian association of radiation oncology – head and neck working group. Radiat Oncol. 2014;9:264.
5. Chuong MD, Freilich JM, Hoffe SE, Fulp W, Weber J, Almhanna K et al. Intensity-modulated radiation therapy vs 3d conformal radiation therapy for squamous cell carcinoma of the anal canal. Gastrointest Cancer Res. 2013;6:39-45.
6. Mell LK, Schomas DA, Salama JK, Devisetty K, Aydogan B et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in anal cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2008;70:1431-7.
7. Kachnic LA, Winter K, Myerson RJ, Goodyear MD, Willins J, Esthappan J et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mytomycin C for the reduction of acute morbidity in carcinoma of the anal canal. Int J Radiat Oncol Biol Phys. 2013;86:27-33.
8. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol. 1961;5:255-8.
9. Kachnic LA, Tsai HK, Coen JJ, Blaszkowsky LS, Hartshorn K, Kwak EL, et al. Dose-painted intensity-modulated radiation therapy for anal cancer: a multi-institutional report of acute toxicity and response to therapy. Int J Radiat Oncol Biol Phys. 2012;82:153-8.
10. Mell Lk, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys. 2006;66:1356-65.
11. Radiation Therapy Oncology group. Acute radiation morbidity scoring criteria. Available at: http://www.rtog.org . Accessed December, 15th 2015.
12. UKCCCR Anal cancer Trial Working Party. UK Co-ordination Committee on Cancer Research: Epidermoid anal cancer: results from the UKCCCR randomized trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. Lancet. 1996;348: 1049-54.
13. Bartelink H, Roelofsen F, Eschwege F, Rougier P, Bosset JF, Gonzalez DG, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Group. J Clin Oncol. 1997;15:2040-9.
14. Flam M, John M, Pajak TF, Petrelli N, Myerson R, Doggett S, et al. Role of mytomicin in combination with fluorouracil and radiotherapy, and salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. J Clin Oncol. 1996;14:2527-39.
15. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, Thomas CR Jr, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008;299:1914-21.
16. Salama J, Mell LK, Schomas DA, Miller RC, Devisetty K, Jani AB, et al. Concurrent chemotherapy and intensity modulated radiation therapy for anal cancer patients: a multicenter experience. J Clin Oncol. 2007;25:4581-6.
17. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995;31:1319-39.
18. Liang Y, Messer K, Rose BS, Lewis JH, Jiang SB, Yashar CM, et al. Impact of bone marrow radiation dose on acute hematologic toxicity in cervical cancer: principal component analysis on high dimensional data. Int J Radiat Oncol Biol Phys. 2010;78:912-9.
19. Sacks EL, Goris ML, Glatstein E, Gilbert E, Kaplan HS. Bone marrow regeneration following large field radiation: influence of volume, age, dose, and time. Cancer. 1978;42:1057-65.
20. Albuquerque K, Giangreco D, Morrison C, Siddiqui M, Sinacore J, Potkul R, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. Int J Radiat Oncol Biol Phys. 2011;79:1043-47.
21. Rose BS, Aydogan B, Liang Y, Yeginer M, Hassalle MD, Dandekar V, et al. Normal tissue complication probability modeling of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2011;79:800-7.

22. Cheng JC, Bazan JG, Wu JK, Koong AC, Chang DT. Lumbosacral spine and marrow cavity modeling of acute hematologic toxicity in patients treated with intensity modulated radiation therapy for squamous cell carcinoma of the anal canal. Pract Radiat Oncol. 2014;4:198-206.
23. Rose BS, Liang Y, Lau SK, Jensen LG, Yashar CM, Hoh CK, et al. Correlation between radiation dose to ^{18}FDG-PET defined active bone marrow subregions and acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2012;82:1185-91.
24. Roeske JC, Lujan A, Reba RC, Penney BC, Yamada DS, Mundt AJ. Incorporation of SPECT bone marrow imaging into intensity modulated whole-pelvic radiation therapy treatment planning for gynecologic malignancies. Radiother Oncol. 2005;77:11-7.
25. Zhu H, Zakeri K, Vaida F, Carmona R, Dadachanji KK, Bair R, et al. Longitudinal study of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. J Med Imaging Radiat Oncol. 2015;59:386-93.
26. Wan J, Liu K, Li K, Li G, Zhang Z. Can dosimetric parameters predict acute hematologic toxicity in rectal cancer patients treated with intensity-modulated pelvic radiotherapy? Radiat Oncol. 2015;10:162.
27. Bazan JG, Luxton G, Kozak MM, Anderson EM, Hancock SL, Kapp DS, et al. Impact of chemotherapy on normal tissue complication probability models of acute hematologic toxicity in patients receiving pelvic intensity modulated radiation therapy. Int J Radiat Oncol Biol Phys. 2012;87:983-91.
28. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, et al. Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients. Br J Radiol 2016;89(1060):20150832.

Chapter 5.

Lumbar-sacral bone marrow dose modeling for acute hematologic toxicity in anal cancer patients treated with concurrent chemo-radiation

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Concurrent chemo-radiation (CT-RT) is nowadays considered as the standard of care for anal cancer of squamous histology[1,2]. Updated long-term results of the RTOG 98-11 study in the concomitant radiation (RT) and 5-fluorouracil (5-FU)/mitomicin-C (MMC) arm demonstrated 5-year disease-free survival (DFS), colostomy-free survival (CFS) and OS rates of 67.8%, 71.9% and 78.3%, respectively, highlighting the clinical efficacy of this treatment strategy[3]. However, whenever non conformal radiotherapy techniques are employed, the toxicity profile might be not negligible. For example, the RTOG 98-11 trial, in which RT was delivered through AP/PA parallel opposed fields or 4-field conformal approaches, recorded substantial rates of major acute toxicities with respect to skin (G3-G4:48%), gastrointestinal tract (G3-G4:35%) and blood cells (G3-G4:61%)[4]. Intensity-modulated radiotherapy (IMRT) is able to provide robust conformality and abrupt dose fall-off within target volumes, reducing unintended dose to organs at risk (OARs) and has been largely employed in the context of anal cancer[5]. Hematological toxicity (HT) due to myelosuppression is a consistent cause of treatment interruption, with a potential increase in overall treatment time (OTT) and a consequent eventual detrimental effect on clinical outcomes[6]. Even if the RTOG 0529 trial showed a decrease rate of $\geq$G2 acute HT with the use of IMRT compared to standard approaches, HT still remains a consistent issue in anal cancer patients, given that extended volumes of bone marrow (BM) are usually comprised within treatment fields during unconstrained IMRT to the pelvic region, where up to 40% of the total hematopoietically active BM is present on average in adults[6,7]. Scant information is available on the tolerance of BM towards combination therapy since Emami table do not include it and Lyman-Kutcher-Burman method model has been rarely applied to BM[8,11]. Nevertheless, several dosimetric predictors have been found to correlate either with blood counts nadir and clinically significant or major HT in cervix, anal and rectal cancer[12,15]. Clinical data suggest that pelvic bone marrow (PBM) acts like a parallel organ, leading to the conclusion that mean dose to this osseous structure may consistently predict HT and may be used during IMRT planning in the optimization process to selectively spare BM during treatment to

improve toxicity profile. We herein present a retrospective analysis set up to evaluate NTCP with respect to acute HT employing the LKB model in anal cancer patients undergoing concurrent IMRT and chemotherapy based on 5-fluorouracil and mitomicin C.

Material and methods

We undertook a review of all medical records regarding patients treated with IMRT and concurrent CT for anal squamous cell carcinoma at the Department of Radiation Oncology of the University of Turin, Italy. From April 2007 to March 2015, a total of 53 patients were submitted to combination therapy with definitive intent. This cohort represented the study sample employed for the present analysis. The Institutional Review Board of the Department of Oncology of the University of Turin approved the present retrospective study. Patient records and information were anonymized and de-identifies prior to analysis.

Radiotherapy

All patients received simultaneous integrated boost (SIB) IMRT as previously described (ref cancer invest), employing both static and volumetric approaches. Planning strategies exclusively included an unconstrained approach towards BM. Dose prescriptions for target volumes are herein detailed in accordance to Kachnich et al[16]. Patients with cT2N0 disease were prescribed 50.4 Gy/28 fractions (1.8 Gy daily) to the gross tumor PTV and 42 Gy/28 fractions (1.5 Gy/daily) to the elective nodal PTV. Patients having cT3-T4/N0-N3 disease were given 54 Gy/30 fractions (1.8-2 Gy daily) to the anal gross tumor PTV, while gross nodal PTVs were prescribed 50.4 Gy/30 fr (1.68 Gy daily) if sized $\leq$3cm or 54 Gy/30 fr (1.8 Gy daily) if >3cm; elective nodal PTV was prescribed 45 Gy/30 fractions (1.5 Gy daily)[16].

Chemotherapy

All patients received concurrent chemotherapy (CT), consisting of 5- fluorouracil (1000 mg/m^2/day) given as continuous infusion along 96 hours (days 1-5 and 29-33) associated with mitomycin C (10

mg/m^2) given as bolus (days 1 and 29). A total of 2 concurrent cycles were planned at baseline for each patients. Blood cell counts were checked for each patient on a routine basis prior to each CT cycle.

Hematologic toxicity evaluation

All patients underwent a weekly complete blood count to grade HT according to the Radiation Therapy Oncology Group acute radiation-induced morbidity scoring system[17]. Endpoints evaluated in the present analysis were white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb) and platelet (Plt) count nadirs after each cycle of CT and the highest-grade toxicity for all blood cells.

Bone marrow delineation

The external contour of PBM was outlined on the planning CT using bone windows as first described by Mell et al[12]. The PBM was delineated as a whole and then divided into 3 subsites: a) the iliac BM (IBM), extending from the iliac crests to the upper border of femoral head; b) lower pelvis BM (LPBM), accounting for bilateral pube, ischia, acetabula and proximal femura, from the upper limit of the femoral heads to the lower limit of the ischial tuberosities and c) lumbosacral BM (LSBM), extending from the superior border of L5 somatic body[12,13].

Normal Tissue Control Probability (NTCP) modeling

Cumulative dose-volume histograms (DVHs) were created for PBM and all 3 BM subsites. Median doses and dosimetric point parameters on the DVHs were analyzed. In the present analysis, since no clear data are available in the medical literature regarding the most appropriate α/β value for BM with respect to acute HT, no dose per fraction DVH modification were introduced to take into account for different sensitivity to fraction size. A binary logistic regression analysis was performed to select the most predictive doses to the pelvic bone structures with respect to HT. Maximum likelihood estimation (MLE) analysis of logistic model was performed in order to correlate the observed toxicities ($\geq$G3 leukopenia) with the mean dose received by LSBM. The logistic dose-

response curve was parametrized in terms of D_{50} (dose leading to a 50% complication probability rate) and y_{50} (normalized slope of the sigmoid dose-response curve)[18]. Hence a logistic fit was performed in order to correlate the predicted toxicity probability, as indicated by NTCP modeling, and mean dose to LSBM. The *m* parameter (slope indicator of Lyman-Kutcher-Burman NTCP model) values were also calculated from the relation $m = 0.4/ y_{50}$[19]. BM was considered as a parallel organ and hence the volume parameter *n* was set to 1 for calculations[9]. The same analysis was performed after exclusion of HIV positive patients. A different logistic fit was also created for patients with no evidence of nodal involvement at diagnosis (N0) compared to those with positive lymphnodes (N+). DVH points of PBM were compared between N+ and N0 patients.

Statistical analysis

Univariate logistic regression analysis was performed to investigate the correlation between dosimetric and clinical factors and HT. The role of all dosimetric parameters on HT was analyzed by multivariate logistic regression analysis. Computations were performed with the STATA statistical package, release 13.0 (STATA Corp, College Station, TX). All p values were 2-sided, and $p < 0.5$ was considered statistically significant.

Results

Patients and treatment characteristics

Overall, we enrolled in the present study 53 patients whose characteristics are shown in Table 1. Mean age was 64 (range 39-79) and patients were mainly female (74%), HIV-negative (91%), with an anal canal primary (80%), T2-T3 stage (94%), N0 stage (72%), G2 (68%). Patients were mostly treated with a VMAT approach (70%). Mean doses to the PTVs volumes were 53 Gy, 50.4 Gy and 44 Gy for the gross tumor, gross nodal and elective nodal volumes, respectively. Mean EBRT duration was 43 days. Patients undergoing a treatment break $\geq$ 3 days were 9%.

Table 1.Patients' characteristics

Variable	N (%)
Age	
Mean	64
Range	39-79
Sex	
Female	39 (74)
Male	14 (26)
HIV status	
Positive	5 (9)
Negative	48 (91)
Primary tumor site	
Anal canal	45 (80)
Anal margin	8 (20)
T stage	
T1	2 (4)
T2	35 (66)
T3	15 (28)
T4	1 (2)
N stage	
N0	28 (72)
N1	2 (5)
N2	8 (20)
N3	1 (3)
Global stage	
I	39 (73)
II	2 (4)
IIIA	11 (21)
IIIB	1 (2)
Grading	
G1	4 (3)
G2	36 (68)
G3	13 (25)

Legend: HIV: human immunodeficency virus; T: tumor; N: nodal

Acute hematologic toxicity and dosimetric outcomes

The mean baseline WBC, ANC, Hb and Plt counts were 6.6k/μl (range:3.8-11.3), 4.2k/μl (range:1.6-9.6), 13.3g/dL (range:9.1-16.2) and 224 k/μl (range:96-380). The mean nadir WBC, ANC, Hb and Plt counts were 2.7k/μl (range:0.7-6.2), 1.8k/μl (range:0.2-3.7), 11.4g/dL (range:7.9-14.7) and 121k/μl (range:41-253). Maximum detected acute HT comprised 36% of patients experiencing leukopenia ≥G3 and 30% with neutropenia ≥G3. Grade 2 anemia was observed in 6% of patients and no G3-G4 events were observed, while 11% experienced ≥G2 thrombocytopenia (Table 2). Dosimetric parameters to bony pelvic structures are shown in Table 3 with mean values and corresponding standard deviations.

Table 2. Acute hematologic toxicity

	N(%)				
Acute HT	*G0*	*G1*	*G2*	*G3*	*G4*
Leukopenia	6 (11)	12 (23)	16 (30)	16 (30)	3 (6)
Neutropenia	17 (32)	10 (19)	10 (19)	11 (21)	5 (9)
Anemia	35 (66)	15 (28)	3 (6)	0 (0)	0 (0)
Thrombocytopenia	29 (55)	12 (23)	6 (11)	5 (9)	1 (2)

Legend: HT: hematologic toxicity; N: number

Table 3. Pelvic bone marrow dosimetric parameters

Parameter	Mean	SD
PBM		
Volume (cm^3)	1400	240
Mean dose (Gy)	29.8	4.9
V_5 (%)	94	6
V_{10} (%)	89	8
V_{15} (%)	83	9
V_{20} (%)	75	9
V_{30} (%)	52	11
V_{40} (%)	26	11
IBM		
Volume (cm^3)	424	63
Mean dose (Gy)	25.5	6.3
V_5 (%)	90	9
V_{10} (%)	82	11
V_{15} (%)	74	10
V_{20} (%)	63	10
V_{30} (%)	36	11
V_{40} (%)	14	10
LSBM		
Volume (cm^3)	392	71
Mean dose (Gy)	32.2	5.8
V_5 (%)	90	10
V_{10} (%)	86	13
V_{15} (%)	83	14
V_{20} (%)	79	15
V_{30} (%)	67	16
V_{40} (%)	41	15
LPBM		
Volume (cm^3)	598	128
Mean dose (Gy)	30.7	4.1
V_5 (%)	99	3
V_{10} (%)	96	6
V_{15} (%)	89	9
V_{20} (%)	80	10
V_{30} (%)	54	13
V_{40} (%)	25	13

Legend: PBM: pelvic bone marrow; IBM: iliac bone marrow; LSBM: lumbar-sacral bone marrow; LPBM: lower pelvis bone marrow; SD: standard deviation.

NTCP modeling

MLE analysis performed to find out a correlation between mean dose to pelvic osseous structures and HT showed a significant correlation between LSBM mean dose and ≥G3 leukopenia (β coefficient:0.122; p=0.030;95% CI:0.012-0.233). Table 4 shows MLE analysis results with respect to leukopenia and neutropenia. No correlation was found for anemia and thrombocytopenia. Thus we performed a logistic fit between LSBM mean dose and HT (≥G3 leukopenia) to predict toxicity probability according to NTCP modeling leading to the following calculated values: TD_{50}:37.5 Gy, γ_{50}:1.15, m:0.347, respectively (Figure 1a). We repeated the logistic fit between LSBM mean dose and HT excluding the 5 HIV+ patients and NTCP modeling resulted in TD_{50}:37.8 Gy, γ_{50}:1.25, m:0.320. The same analysis was then repeated for patients having nodal involvement at diagnosis (N+) compared to those without (N0). For N+ patients, predicted HT probability yielded to the following values: TD_{50}:35.2 Gy, γ_{50}:2.27, m:0.176 (Figure 1b) Comparison between N0 and N+ patients with respect to dosimetric parameters to PBM showed statistical significant difference in terms of V_{15} (Mean:81.1%;SD:±8.3 vs Mean:86.7%; SD:±4.9;p=0.04), V_{20} (Mean:72.7%;SD:±9.7 vs Mean:79.9%;SD:±5.7;p=0.01) and V_{30} (Mean:50.2%;SD:±10.7 vs Mean:57.3%;SD:±9.3;p=0.03) (Figure 2). Patients with a mean LSBM dose >32 Gy had a 1.81 (95%CI:0.81-4.0) relative risk to develop ≥G3 leukopenia. Node positive patients with mean LSBM dose >32 Gy had a 2.67 (95%CI:0.71-10) relative risk to develop the aforementioned event. To have a <5%, <10%,<20% risk to develop ≥G3 leukopenia, LSBM mean dose should be below 14 Gy, 20 Gy and 26 Gy, respectively. For node positive patients these thresholds were below 24 Gy, 27 Gy and 30 Gy (≥G3 leukopenia). On the whole cohort, within a dose range between 25 and 40 Gy, this probability rises from 17.5% to 57.1% for ≥G3 leukopenia. For node positive patients these ranges were 6.7%-77.6%.

Table 4. Maximum likelihood estimation of the correlation between mean dose to pelvic bone marrow considered either as a whole or divided in different sub-regions and neutropenia leukopenia.

	≥ G3 neutropenia				
	β coeff	Std err	z value	p value	95% CI
PBM-mean dose	-0.017	0.063	-0.28	0.781	-0.141-0.106
IBM-mean dose	-0.041	0.055	-0.76	0.048	-0.150-0.066
LPBM-mean dose	-0.007	0.073	-0.11	0.915	-0.151-0.135
LSBM-mean dose	0.065	0.053	1.23	0.220	-6.490-0.536
	≥ G3 leukopenia				
PBM-mean dose	0.042	0.057	0.74	0.461	-0.070-0.155
IBM-mean dose	0.017	0.043	0.40	0.688	-0.068-0.103
LPBM-mean dose	0.001	0.069	0.03	0.979	-0.134-0.138
LSBM-mean dose	**0.122**	**0.056**	**2.18**	**0.030**	**0.012-0.233**

Legend –Coeff: coefficient; Std err: standard error; CI: confidence interval; PBM: pelvic bone marrow; IBM: iliac bone marrow; LPBM: lower pelvis bone marrow; LSBM: lumbo-sacral bone marrow.

Figure 1.NTCP modeling of leukopenia on the whole cohort and on node positive patients

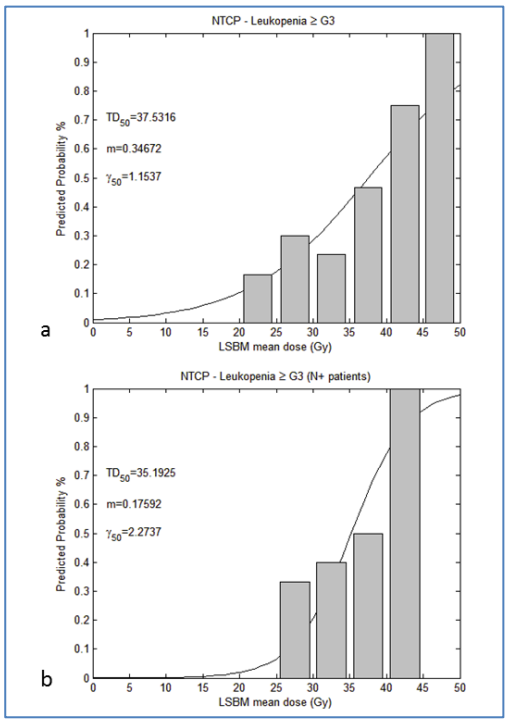
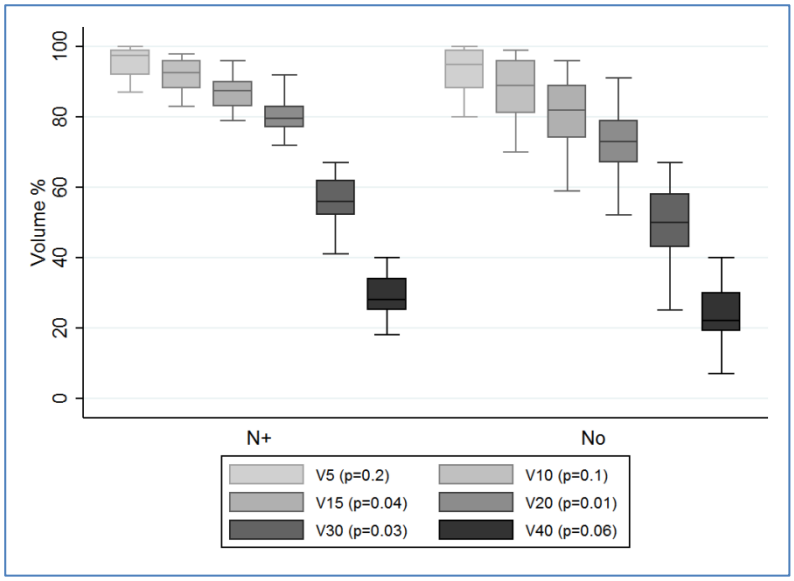


Figure 2. Pelvic bone marrow DVH points comparison between node positive and negative patients



Considerations

Concomitant chemo-radiation is the current standard therapeutic option for anal cancer patients. Combined modality treatment has better clinical outcomes than radiation alone and poly-chemotherapy has been shown to be superior to mono-chemotherapy in this setting[1,20].HT still remains a consistent issue for this patients, since a 61% rate of ≥G3 events was reported in the RTOG 98-11 study employing conventional techniques[4]. High rate of HT have been documented also for IMRT, whenever unconstrained planning strategies towards BM are employed, as in Salama et al who had shown acute G3-G4 rates as high as 58%. Anal cancer radiotherapy requires extended treatment volumes and BM is an important dose-limiting cell renewal tissue for wide-field irradiation[6]. As pointed out by Mauch et al, BM stem cells are very radiosensitive, hence radiotherapy has a consistent myelosuppressive effect, inducing BM stem cell apoptosis and stromal damage, with peculiar pathologic and radiographic modifications[6]. Functional BM of the adult population is mainly located within the pelvis and vertebrae (60% of the total amount) and pelvic bones, specifically, may contain up to 40%[22]. Thus, pelvic irradiation is a key factor for the determination of HT during combination therapy in anal cancer, since the extent of radiation-induced BM damage has been demonstrated to correlate with both radiation dose and irradiated osseous volume[23]. Several DVH points and mean PBM dose were associated with HT in these patients and other useful information come from cervical cancer studies[9-14]. In cervix cancer, Mell et al found that PBM-V_{10} was associated with ≥G2 leukopenia and neutropenia and patients with PBM-V_{10} ≥90% were more likely to develop HT and to have chemotherapy held[12]. Hence authors suggested a constraint of PBM-V_{10}<90% to reduce toxicity[12]. Moreover, Rose et al found that PBM-V_{10} ≥95% and PBM-V_{20}>76% were associated with increased ≥G3 leukopenia and suggested to keep dose below those levels, while Albuquerque et al demonstrated that PBM-V_{20}≥80% increases by 4.5-fold the odds to develop ≥G2 HT[14,24]. In anal cancer, Mell et al showed a

correlation between PBM-V_5,-V_{10},-V_{15},-V_{20} and WBC and ANC nadirs, but not with HT[13]. Conversely, Cheng et al found out that PBM-V_{10} and mean dose had a statistically significant relationship with ≥G3 HT, while Bazan et al found a correlation between PBM-V_{10},-V_{15},-V_{20} and the same events[9,10]. Interestingly, Bazan et al highlighted that patients with a PBM mean dose ≥30 Gy had a 14-fold increase in the odd of developing ≥G3 HT. Whenever mean PBM dose is kept below 22.5 Gy or 25 Gy the risk to develop ≥G3 HT can be reduced to < 5% or < 10%, respectively[9]. Those data suggest that PBM as a whole plays a role in the development of HT and that it acts like a parallel organ with a consistent volume effect. In cervix cancer, Zhu et al estimated that every 1 Gy increase in mean PBM dose may lead to a 9.6/μl per week reduction in the natural logarithm of ANC count[25]. Nevertheless, data coming from functional imaging suggest that BM may be asymmetrically represented within osseous structures[26,27]. Rose et al characterized with ^{18}FDG-PET scan active BM subregions in cervical cancer patients treated with concurrent radiotherapy and weekly cisplatin, identifying lumbar vertebrae, sacrum and pubic bones as those most represented[27]. This is in line with our study, where MLE analysis showed a significant correlation between LSBM mean dose and ≥G3 leukopenia (β coefficient:0.122; p=0.030;95%CI:0.012-0.233). Accordingly, Cheng et al showed that LSBM mean dose and DVH points better predicted for ≥G3 HT compared to same PBM parameters and estimated that mean LSBM doses of 21 Gy and 23.5 Gy result in a 5% and 10% risk of ≥G3 hematologic events[10]. Moreover, Bazan et al found a significant correlation between LSBM mean dose and V_5-V_{20} and ≥G3 HT suggesting to keep V_{10}<85% and mean dose<28 Gy to minimize toxicity profile[9]. Our NTCP model of LSBM mean dose lead to an estimation of TD_{50}:37.5 Gy, γ_{50}:1.15, m:0.347 for ≥G3 leukopenia. Bazan et al reported a lower TD_{50}:32Gy for the whole PBM and ≥G3 HT with a steeper correlation (m:0.175), which may reflect the contribution of volume effect to the clinical outcome probability rate (PBM vs LSBM in our study). Our NTCP model seems to highlight a different contribution of the dose received by LSBM to the occurrence of HT between node positive and negative patients. TD_{50} is slightly lower for N+ patients (35.2 Gy vs 37.5 Gy) but interestingly

γ_{50} is higher than in the cohort taken as a whole (2.21 vs 1.15) (Figure 1). Hence, our NTCP model predicts that for node positive patients the risk to develop ≥G3 leukopenia rises from 6.7% to 77.6% by increasing LSBM mean dose from 25 Gy to 40 Gy. The inclusion in the NTCP model of node negative patients consistently decreases this effect, with a probability rise from 17.5% to 57.1% for the whole cohort within the same LSBM mean dose range. Node positive patients have significantly higher PBM volumes receiving doses ranging from 15 to 30 Gy with borderline significance for 40 Gy (Figure 2). That suggests that PBM node positive patients, given the higher dose received to macroscopic nodal disease, may have a lower hematopoietic activity to compensate for CT-RT damage. With this as a background, each adjunctive Gy received by LSBM as mean dose, above a threshold of 25 Gy, may lead to a very consistent and rapid increase in the probability to develop HT. The other observation is a potential lower sensitivity of LSBM within a low dose range. Our NTCP model predicts that, in order to have a <5%, <10%,<20% risk to develop ≥G3 leukopenia, LSBM mean dose should be kept below 14 Gy, 20 Gy and 26 Gy on the whole cohort. For node positive patients these thresholds rises to 24 Gy, 27 Gy and 30 Gy (≥G3 leukopenia). These findings suggest a different saturation level for node positive patients, conditioning a different threshold of appearance of the steep relationship between LSBM mean dose and HT. Since node positive patients have a higher PBM volumes receiving doses in the range of 15-30, the contribution of LSBM mean dose to the development of ≥ ≥G3 leukopenia requires a higher threshold to be observed. Other intrinsic different characteristics for node positive patients may influence this finding, but these dosimetric consideration may play and important role. The other interpretation of this finding is that we had a low number of patients receiving LSBM mean dose < 25 Gy, conditioning a low probability of observation of HT in this dose range. Our data have been obtained within a cohort of patients treated with IMRT, employing a simultaneous integrated boost approach, with different dose level according to initial staging both for prophylactic and definitive volumes[28-30]. Thus our results may be affected by the treatment solution employed. Our results shows that a LSBM mean dose > 32 Gy exposes node positive patients to a progressively

higher risk to develop HT, with a steeper correlation compared to node negative patients. Thus particular attention should be paid to spare LSBM in this subset pf patients[31,32].

References

1. UKCCCR Anal cancer Trial Working Party. UK Co-ordination Committee on Cancer Research: Epidermoid anal cancer: results from the UKCCCR randomized trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. Lancet.1996; 348:1049-54.
2. Bartelink H, Roelofsen F, Eschwege F, Rougier P, Bosset JF, Gonzalez DG, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Group. J Clin Oncol.1997;15:2040-9.
3. Gunderson LL, Winter KA, Ajani JA, Pedersen JE, Moughan J, Benson AB 3rd, et al. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomicyn versus fluorouracil/cisplatin. J Clin Oncol.2012;30:4344-51.
4. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008; 299:1914-21.
5. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015;33:259-66.
6. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995;31:1319-39.
7. Kachnic LA, Winter K, Myerson RJ, Goodyear MD, Willins J, Esthappan J, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mytomycin C for the reduction of acute morbidity in carcinoma of the anal canal. Int J Radiat Oncol Biol Phys. 2013;86:27-33.
8. Emami B, Lyman J, Borwn A, Coia L, Goitein M, Munzenrider JE, et al. Tolerance of normal tissue to therapeutic irradiation. Int J Radiat Oncol Biol Phys. 1991;21:109-22.
9. Bazan JG, Luxton G, Mok EC, Koong AC, Chang DT. Normal tissue complication probability modeling of acute hematological toxicity in patients treated with intensity-modulated radiation therapy for squamous cell carcinoma of the anal canal. Int J Radiat Oncol Biol Phys. 2012;84:700-6.
10. Cheng JC, Bazan JG, Wu JK, Koong AC, Chang DT. Lumbosacral spine and marrow cavity modeling of acute hematologic toxicity in patients treated with intensity modulated radiation therapy for squamous cell carcinoma of the anal canal. Pract Radiat Oncol. 2014;4:198-206.
11. Bazan JG, Luxton G, Kozak MM, Anderson EM, Hancock SL, Kapp DS, et al. Impact of chemotherapy on normal tissue complication probability models of acute hematologic toxicity in patients receiving pelvic intensity modulated radiation therapy. Int J Radiat Oncol Biol Phys 2012;87:983-91.

12. Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys. 2006;66:1356-65.
13. Mell LK, Schomas DA, Salama JK, Devisetty K, Aydogan B, Miller RC, et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in anal cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2008;70:1431-1437.
14. Albuquerque K, Giangreco D, Morrison C, Siddiqui M, Sinacore J, Potkul R, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. Int J Radiat Oncol Biol Phys. 2011;79:1043-1047.
15. Wan J, Liu K, Li K, Li G, Zhang Z. Can dosimetric parameters predict acute hematologic toxicity in rectal cancer patients treated with intensity-modulated pelvic radiotherapy? Radiat Oncol. 2015;10**:**162.
16. Kachnic LA, Tsai HK, Coen JJ, Blaszkowsky LS, Hartshorn K, Kwak EL, et al. Dose-painted intensity-modulated radiation therapy for anal cancer: a multi-institutional report of acute toxicity and response to therapy. Int J Radiat Oncol Biol Phys. 2012; 82:153-8.
17. Radiation Therapy Oncology group. Acute radiation morbidity scoring criteria. Available at: http://www.rtog.org . Accessed September, 16th 2015.
18. Bentzen SM, Tucker SL. Quantifying the position and steepness of radiation dose-response curves. Int J Radiat Biol 1997;71:531-42.
19. Dasu A, Toma-Dasu I, Fowler JF. Should single or distributed parameters be used to explain the steepness of tumor control probability curves? Phys Med Biol. 2003;48:387-97.
20. Flam M, John M, Pajak TF, et al. Role of mytomicin in combination with fluorouracil and radiotherapy, and salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. J Clin Oncol. 1996;14:2527-39.
21. Salama J, Mell LK, Schomas DA, Miller RC, Devisetty K, Jani AB, et al. Concurrent chemotherapy and intensity modulated radiation therapy for anal cancer patients: a multicenter experience. J Clin Oncol. 2007; 25: 4581-6.
22. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol. 1961; 5: 255-8.
23. Liang Y, Messer K, Rose BS, Rose BS, Lewis JH, Jiang SB, et al. Impact of bone marrow radiation dose on acute hematologic toxicity in cervical cancer: principal component analysis on high dimensional data. Int J Radiat Oncol Biol Phys. 2010;78:912-9.
24. Rose BS, Aydogan B, Liang Y, Liang Y, Yeginer M, Hasselle MD, et al. Normal tissue complication probability modeling of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2011; 79: 800-7.
25. Zhu H, Zakeri K, Vaida F, Carmona R, Dadachanji KK, Bair R, et al. Longitudinal study of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. J Med Imaging Radiat Oncol.2015;59:386-93.
26. Roeske JC, Lujan A, Reba RC, Penney BC, Yamada DS, Mundt AJ. Incorporation of SPECT bone marrow imaging into intensity modulated whole-pelvic radiation therapy treatment planning for gynecologic malignancies. Radiother Oncol. 2005;77:11-7.
27. Rose BS, Liang Y, Lau SK, et al. Correlation between radiation dose to ^{18}FDG-PET defined active bone marrow subregions and acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2012;83:1185-91.
28. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Early-stage node negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res. 2016;36:1943-48.

29. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Locally advanced (T3-T4 or N+) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res. 2016;36:2027-32.
30. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, et al. Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients. Br J Radiol. 2016;89(1060):2015832.
31. Franco P, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, et al. Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer. Clin Transl Oncol. 2016 (in press).
32. Franco P, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, et al. Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemoradiation in anal cancer patients. Med Oncol. 2016;33:72.

Chapter 6.

Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemo-radiation in anal cancer patients

Franco P, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, Cassoni P, Arena V, Bustreo S, Faletti R, Rondi N, Morino M, Ricardi U. **Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemo-radiation in anal cancer patients.** *Med Oncol 2016;33:72.*

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Concomitant chemo-radiation (CH-RT) is standard of care in epidermoid cancer of the anal canal[1]. Local control, survival and anal sphincter preservation rates are favorable in patients treated with combined modality therapy[2]. However, toxicity rates are not negligible and severe reactions can be observed to gastrointestinal tract, genitalia and skin, especially whenever non conformal techniques are employed[3]. In this setting, hematologic toxicity (HT) may limit and decrease treatment intensity, with patients potentially experiencing unplanned treatment breaks with a consequent increased in overall treatment time or infections, bleeding or asthenia[4]. Even if intensity-modulated radiotherapy (IMRT) is employed, major HT rates may be up to 60%[5,6]. Chemotherapy is the most important trigger of HT because it directly induces myelosuppression[7]. In the average adult population, about half of the total hematopoietically active bone marrow (BM) is comprised within the pelvis and the lumbar vertebral tract[8]. Hence, selective sparing of pelvic osseous structures may be a viable option to decrease HT during concomitant CH-RT in anal cancer patients[9]. In several studies, a significant correlation between different dose-volume metrics and blood cell nadirs and/or HT was found[10-15]. Other than anal cancer, different clinical context were investigated such as cervical, rectal, lung and prostate cancer. Avoidance of the whole pelvic region may be challenging because of the large volume involved. Nevertheless, different imaging modalities can be employed to identify specific subsites within bony structures with a high concentration of hematopoietically active BM, such as magnetic resonance imaging (MRI), single-positron emission tomography (SPECT), ^{18}fluorodeoxyglucose (FDG)-labeled or^{18}fluorothymidine (FLT)-labeled positron emission tomography (PET)[16-18]. ^{18}FDG-PET imaging is able to identify active BM according to the real pathologic distribution[19]. The present study tested the hypothesis that active bone marrow (^{ACT}BM) as detected through ^{18}FDG-PET has a correlation with nadir values of blood cells in anal cancer patient submitted to CH-RT. Furthermore, we evaluated the strength of association according to different specific subsites to better tailor BM-sparing radiotherapy approaches and to perform robust modeling of HT enhanced by functional imaging.

Material and methods

We retrospectively reviewed all medical records within the Department of Radiation Oncology at the University of Turin, Italy to select anal cancer patients treated with concomitant CH-RT. Between April 2007 and March 2015 a total of 44 patients were submitted to definitive treatment, after having undergone a baseline ^{18}FDG-PET for staging purposes prior to therapy. This population was used as the sample of the present study. Written informed consent from all patients was collected. The Review Board of our Institution Department approved the present study.

Radiotherapy

IMRT was delivered to all patients, as previously described[1]. Dose fractionation was derived from Kachnic et al.[20]. Patients having stage cT2N0 anal cancer were given 50.4 Gy/28 fractions (1.8 Gy daily) to the gross tumor PTV and 42 Gy/28 fractions (1.5 Gy daily) to the prophylatic nodal PTV. Patients with stage cT3-T4/N0-N3 disease were treated with 54 Gy/30 fractions (1.8-2 Gy daily) to the anal gross tumor PTV and 50.4 Gy/30 fr (1.68 Gy daily) to the gross nodal PTV having a maximum dimension $\leq$ 3 cm or 54 Gy/30 fr (1.8 Gy daily) in case of nodes > 3 cm. Elective nodal PTV was given 45 Gy/30 fractions (1.5 Gy daily)[19]. A simultaneous integrated boost (SIB) approach was used in all patients, either with static (step and shoot) or volumetric modulated arc therapy (VMAT) as previously described[21]. Treatment plan optimization was made on Oncentra Masterplan or Monaco (Elekta, Stockholm, Sweden) plaftorms. Cone beam computed tomography (CBCT) was used for daily image guidance.

Bone marrow delineation on planning CT

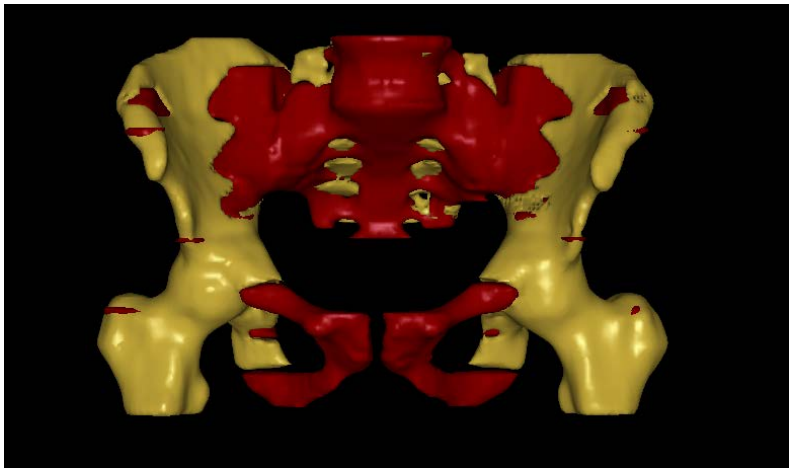
The whole pelvic bone marrow (PBM) was outlined following outer contours on the planning CT scan employing a bone window, as described by Mell et al[10]. Three subregions were identified: a) the iliac BM (IBM), which comprises the area between the iliac crests and the upper border of femoral head; b) lower pelvis BM (LPBM), accounting for bilateral pube, ischia, acetabula and

proximal femura, from the upper border of the femoral heads to the lower aspect of the ischial tuberosities and c) lumbosacral BM (LSBM), including the area between the superior border of L5 somatic body to the lower aspect of the coccyx[10].

Active bone marrow delineation on FDG-PET

All images derived from planning CT were exported on the Velocity platform (Varian Medical Systems, Palo Alto, CA) together with treatment volumes, organs at risk (OARs) and dose references. Given that FDG- PET images were acquired separately, we perfomed a deformable co-registration between CT and PET images.[18]FDG-PET standardized uptake values (SUVs) were calculated for PBM volumes, after correcting for body weight. To standardize SUVs among all patients, we normalized BM and liver SUVs. We defined as active bone marrow (^{ACT}BM), the volume having higher SUV values than the SUV_{mean} for each patient, rather than the whole cohort, as proposed by Rose et al[18,22]. The areas identified with the method described above were outlined within PBM as a whole and named ^{ACT}BM and within each of the 3 subregions identified on planning CT (LSBM, IBM, LPBM) and named ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM, respectively. Inactive BM (^{INACT}BM) was identified as the difference between BM volumes as defined on planning CT (PBM) and ^{ACT}BM. The same procedure was done for all 3 subregions to identify inactive BM within all of them. The 3 volumes were hence called ^{INACT}LSBM, ^{INACT}IBM, ^{INACT}LPBM. Figure 1 highlights ^{ACT}BM (red) and ^{INACT}BM as identified with the use of ^{18}FDG-PET in a specific patient.

Figure 1. Three-dimensional view of the pelvic region with active (red) and inactive bone marrow (yellow) subregions characterized.



Chemotherapy

All patients underwent concomitant chemotherapy following the Nigro's regimen with 5-fluorouracil (1000 mg/m^2/day) administered as continuous infusion along 96 hours (days 1-5 and 29-33) and mitomycin C (10 mg/m^2) given as bolus (days 1 and 29). Up to 2 cycles were given concurrently during radiotherapy.

Hematologic toxicity evaluation

Weekly blood tests with complete blood count were taken for all patients. HT was scored according to the Radiation Therapy Oncology Group acute radiation-induced morbidity system[23]. Endpoints analyzed were white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hb) and platelet (Plt) count lowest nadir experienced during treatment or up to 2 weeks after the completion of CH-RT.

Statistical analysis

Cumulative dose-volume histograms (DVHs) were analyzed for PBM, ^{ACT}BM, ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM and ^{INACT}BM, ^{INACT}LSBM, ^{INACT}IBM, ^{INACT}LPBM. We collected median doses and selected dosimetric parameters on DVH curves for the aforementioned structures. WBC, ANC, Hb and Plt nadirs were correlated as continuous variables

to mean dose and V_{10}-V_{15}-V_{20}-V_{30}-V_{40}-V_{45}-V_{50} for all the aforementioned structures. Generalized linear modeling was used to investigate the eventual correlation between dosimetric variables and blood cells nadirs. We used Shapiro-Wilk statistic to test for normality of variables. A log transform was also used to eliminate skew in the dependent variables. Significant covariates on univariate linear regression analysis were included in the multivariate linear regression model. R^2 and adjusted R^2 test were employed to evaluate goodness of model fit. Stata Statistical Software, version 13.1 (Stata Corporation, Texas) was employed for analysis.

Results

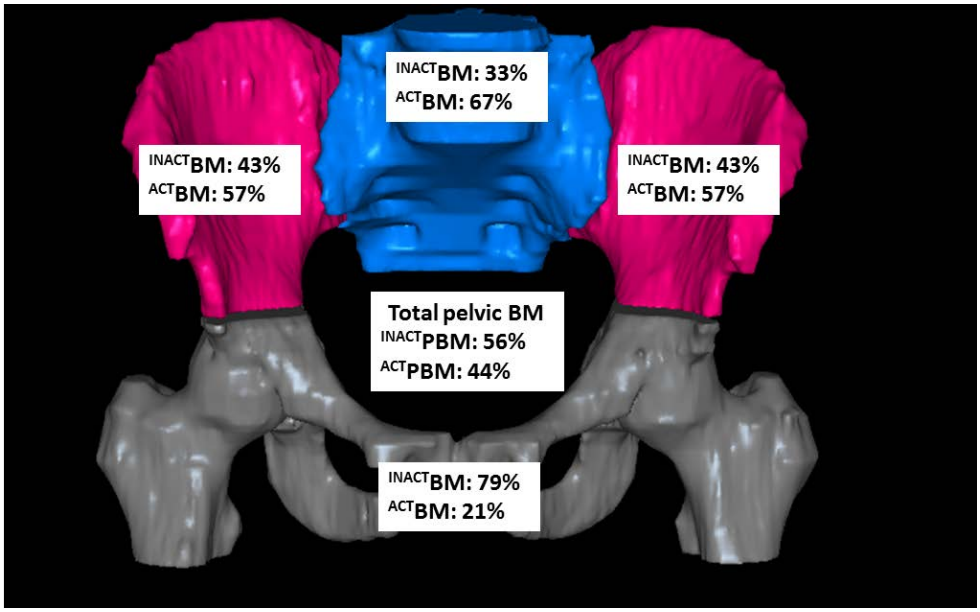
Overall, 44 patients were selected and investigated. Patients characteristics are shown in Table 1. Mean age was 64 (range 45-79) and patients were mainly female (73%), with an anal canal primary (89%), T3 stage (68%), N0 stage (71%). Patients were more frequently submitted to VMAT (83%) and were given 54 Gy to the primary tumor (66%) and 45 Gy in 30 fractions to the prophylactic nodal volumes (61%). All macroscopic nodal areas received 50.4 Gy in 30 fractions. ^{ACT}BM identified within the pelvic region was 44% while ^{INACT}BM was 57%. LSBM is the subsite where active BM was most represented (67%) compared to inactive BM (33%). Within IBM, 57% of the volume comprised hematopoietically active BM and 43% of inactive BM. LPBM had 21% of active BM (mainly in the pubic bones) and 79% of inactive. Differential distribution of ^{ACT}BM and ^{INACT}BM according to different subregions is shown in Figure 2.

Table 1. Patients' and treatment characteristics

Variable	N (%)
Sex	
Female	32 (73)
Male	12 (27)
Age	
Mean	64
Range	45-79
Primary tumor site	
Anal margin	5 (11)
Anal canal	39 (89)
T stage	
T1	1 (2)
T2	12 (27)
T3	30 (68)
T4	1 (2)
N stage	
N0	31 (71)
N1	1 (2)
N2	10 (23)
N3	2 (4)
Global stage	
I	1 (2)
II	29 (67)
IIIA	2 (4)
IIIB	12 (27)
IMRT approach	
Step and Shoot	12 (27)
VMAT	36 (83)
PTV dose-tumor (Gy)	
54 Gy/30 fractions	29 (66)
50.4 Gy/28 fractions	15 (34)
PTV dose-positive nodes (Gy) (*13 pts***)**	
50.4 Gy/30 fractions	13 (100)
PTV dose-negative nodes (Gy)	
45 Gy/30 fractions	27 (61)
42 Gy/28 fractions	17 (39)

Legend: N: number; IMRT: intensity-modulated radiotherapy; PTV: planning target volume; VMAT: volumetric modulated arc-therapy.

Figure 2. Three-dimensional view of the pelvic region with distribution of active and inactive bone marrow subdivided according to subsites: lumbar-sacral (light blue), iliac (green) and lower-pelvic (pink) bone marrow.



Acute hematologic toxicity and dosimetric outcomes

In Table 2, baseline hematologic parameters are shown together with the lowest nadir measuredduring treatment and up to 2 weeks after the completion of CH-RT. Absolute volumes and dose/volume parameters to bony structures with respect to the whole pelvic region and the 3 specific subregions can be seen in Table 3.

Table 2. Blood cells values

Hematologic parameters	Baseline	Lowest nadir
	Mean (range)	*Mean (range)*
Hb	13.3 g/dL (9.1-16.2)	11.6 g/dL (9.1-14.5)
PLT	224 k/µl (96-380)	115 k/µl (18-253)
WBC	6.6 k/µl (3.8-11.3)	2.7 k/µl (0.7-7.1)
ANC	4.2 k/µl (1.6-9.6)	1.7 k/µl (0.2-5.9)

Legend: WBC: white blood cells; ANC; absolute neutrophil count; PLT: platelets; Hb: hemoglobin; g: grams; k: 10^3; ul: microliters.

Table 3. Dose parameters of the pelvic bones according to bone marrow activity

			Active BM		Inactive BM	
Parameter	Mean	SD	Mean	SD	Mean	SD
PBM						
Volume (cm^3)	1412	231	623 (44%)	151	789 (56%)	129
Mean dose (Gy)	30	5	31	4	30	3
V_5 (%)	94	5	94	7	95	5
V_{10} (%)	89	7	89	9	89	7
V_{15} (%)	83	9	85	10	81	9
V_{20} (%)	75	9	79	11	71	10
V_{30} (%)	52	11	58	12	47	11
V_{40} (%)	26	12	30	12	21	8
V_{45} (%)	8	10	9	10	7	7
IBM						
Volume (cm^3)	429	59	245 (57%)	76	184 (43%)	58
Mean dose (Gy)	26	7	26	5	22	4
V_5 (%)	91	8	95	8	87	11
V_{10} (%)	82	10	89	11	75	13
V_{15} (%)	74	10	82	13	66	13
V_{20} (%)	63	10	71	14	55	12
V_{30} (%)	35	12	40	14	30	10
V_{40} (%)	14	11	14	10	13	6
V_{45} (%)	4	8	4	8	3	5

LSBM						
Volume (cm^3)	397	71	265 (67%)	52	132 (33%)	41
Mean dose (Gy)	33	6	33	5	31	6
V_5 (%)	90	11	90	10	90	13
V_{10} (%)	86	13	86	12	85	15
V_{15} (%)	83	13	84	13	81	16
V_{20} (%)	80	14	81	13	77	16
V_{30} (%)	68	15	71	15	60	17
V_{40} (%)	41	16	43	16	34	14
V_{45} (%)	12	12	12	13	11	12
LPBM						
Volume (cm^3)	590	124	125 (21%)	65	465 (79%)	95
Mean dose (Gy)	30	4	34	4	29	4
V_5 (%)	99	4	100	1	99	2
V_{10} (%)	95	7	99	3	95	7
V_{15} (%)	89	10	97	6	87	10
V_{20} (%)	79	12	91	10	76	12
V_{30} (%)	53	13	66	18	48	13
V_{40} (%)	24	13	32	16	20	10
V_{45} (%)	8	10	11	11	7	8

Legend: PBM: pelvic bone marrow; LSBM: lumbar-sacral bone marrow; IBM: iliac bone marrow; LPBM:lower pelvis bone marrow; ^{ACT}BM: active bone marrow; ^{INACT}BM: inactive bone marrow; SD: standard deviation

Predictors of hematologic toxicity

The correlation between mean doses to ^{ACT}BM and ^{INACT}BM and WBC, ANC, Hb and Plt nadirs was investigated and is shown in Table 4. ^{ACT}BM was found to be significantly correlated to WBC (β=-1.338; 95%CI: -2.455/-0.221; p=0.020), ANC (β=-1.651; 95%CI: -3.284/-0.183; p=0.048) and Plt (β=-0.031; 95%CI: -0.057/-0.004; p=0.024) nadirs. Conversely, no correlation was seen

between ^{INACT}BM and blood cell nadirs. Table 5 and 6 summarizes all dosimetric variables pertinent to each of the 3 subregions within pelvic bones tested on multivariate analysis against blood cell nadirs. WBC nadir was significantly correlated to LSBM mean dose (β=-1.852; 95%CI: -3.205/-0.500; p=0.009), V_{10} (β=-2.153; 95%CI: -4.263/-0.721; p=0.002), V_{20} (β=-2.081; 95%CI: -4.880/-0.112; p=0.003), V_{30} (β=-1.971; 95%CI: -4.748/-0.090; p=0.023) and to IBM V_{10} (β=-0.073; 95%CI: -0.106/-0.023; p=0.016). ANC nadir found to be significantly associated to LSBM V_{10} (β=-1.878; 95%CI: -4.799/-0.643; p=0.025), V_{20} (β=-1.765; 95%CI: -4.050/-0.613; p=0.030) and to IBM V_{10} (β=-0.039; 95%CI: -0.066/-0.010; p=0.027). Borderline significance was found for correlation between Plt nadir and LSBM V_{30} (β=-0.056; 95%CI: -2.748/-0.187; p=0.060) and V_{40} (β=-0.059; 95%CI: -3.112/-0.150; p=0.060) and IBM V_{30} (β=-0.028; 95%CI: -0.074/-0.023; p=0.056). No inactive BM subsites were found to be significantly correlated to any blood cell nadir. The R^2 and adjusted R^2values of the multivariate analysis are shown in Table 4-6, with a fair amount of unexplained variation in the regression model.

Table 4. Correlation between mean dose to active/inactive subregions of pelvic bone marrow and blood cells nadirs

	^{ACT}BM - Mean Dose (Gy)					^{INACT}BM - Mean Dose (Gy)				
Nadirs	β	R^2	95% CI	t	p	β	R^2	95% CI	t	p
WBC (k/μL)	-1.338	0.141	-2.455/-0.221	-2.43	**0.020**	-0.718	0.064	-1.645/0.209	-1.57	0.125
ANC (k/μL)	-1.651	0.105	-3.284/-0.183	-2.05	**0.048**	-0.708	0.030	-2.060/0.643	-1.06	0.295
PLT (k/μL)	-0.031	0.133	-0.057/-0.004	-2.35	**0.024**	-0.004	0.003	-0.026/0.019	-0.32	0.751
Hb (g/dL)	-0.719	0.042	-1.878/0.439	-1.26	0.216	-0.587	0.044	-1.508-0.333	-1.29	0.204

Legend: ^{ACT}BM: active bone marrow; ^{INACT}BM: inactive bone marrow; WBC: white blood cells; ANC; absolute neutrophil count; PLT: platelets; Hb: hemoglobin; k: 10^3; ul: microliters;g: grams; β: β-coefficient; $R^{2:}$ R-squared; CI: confidence interval; t: t-value; p: p-value.

Table 5. Correlation between points on the dose-volume histograms of active/inactive subregions of pelvic bone marrow and white blood cell and absolute neutrophil count nadirs

	WBC nadir					ANC nadir				
Parameter	β	R^2	95%CI	t	p	β	R^2	95%CI	t	p
^{ACT}LSBM										
Mean dose	-1.852	0.177	-3.205/-0.500	-2.78	**0.009**	-1.944	0.095	-3.974/0.085	-1.94	0.060
V_{10} (%)	-2.153	0.230	-4.263/-0.721	-2.63	**0.002**	-1.878	0.130	-4.799/0.643	-2.33	**0.025**
V_{20} (%)	-2.081	0.220	-4.880/-0.112	-1.97	**0.003**	-1.765	0.120	-4.050/0.613	-1.52	**0.030**
V_{30} (%)	-1.971	0.130	-4.748/-0.090	-1.91	**0.023**	-1.546	0.070	-3.156/1.695	-1.37	0.110
V_{40} (%)	-1.560	0.080	-3.912/0.750	-1.41	0.080	-1.321	0.030	-5.678/1.871	-1.32	0.300
V_{45} (%)	-1.271	0.001	-3.204/1.351	-0.78	0.840	-1.123	0.003	-4.989/2.267	-0.66	0.740
V_{50} (%)	-0.745	0.001	-2.138/0.677	-1.12	0.410	-0.617	0.002	-3.181/1.177	-1.18	0.420
^{ACT}IBM										
Mean dose	-1.051	0.079	-2.265/0.162	-1.76	0.087	-1.415	0.070	-3.162/0.331	-1.61	0.109
V_{10} (%)	-0.073	0.150	-0.106/-0.023	-2.95	**0.016**	-0.039	0.130	-0.066/-0.010	-2.49	**0.027**
V_{20} (%)	-0.042	0.045	-0.084/-0.009	-2.39	0.200	-0.025	0.030	-0.051/0.120	-2.09	0.280
V_{30} (%)	-0.029	0.030	-0.074/-0.013	-2.03	0.270	-0.017	0.030	-0.042/0.021	-1.75	0.300
V_{40} (%)	-0.025	0.030	-0.059/-0.006	-1.54	0.290	-0.012	0.020	-0.037/0.019	-1.10	0.360
V_{45} (%)	-0.012	0.010	-0.056/0.037	-0.37	0.560	-0.010	0.010	-0.041/0.027	-0.59	0.530
V_{50} (%)	-0.028	0.010	-0.119/0.046	-0.86	0.470	-0.021	0.010	-0.086/0.039	-0.89	0.520
^{ACT}LPBM										
Mean dose	-0.481	0.023	-1.530/0.567	-0.93	0.358	-0.668	0.018	-1.230/0.984	-0.82	0.418
V_{10} (%)	-0.065	0.066	-0.129/0.012	-2.38	0.160	-1.944	0.100	-4.811/0.657	-2.33	0.057
V_{20} (%)	-0.027	0.051	-0.068/0.010	-1.43	0.170	-2.082	0.060	-4.123/0.769	-1.52	0.140
V_{30} (%)	-0.021	0.043	-0.063/0.024	-1.16	0.200	-1.866	0.030	-3.176/1.678	-1.37	0.330
V_{40} (%)	-0.019	0.021	-0.072/0.033	-0.77	0.400	-1.320	0.010	-6.725/1.870	-1.32	0.590
V_{45} (%)	-0.001	0.001	-0.058/0.063	-0.03	0.380	-1.111	0.020	-5.322/2.245	-0.66	0.360
V_{50} (%)	-0.036	0.012	-0.138/0.079	-0.58	0.470	-0.643	0.010	-3.179/1.188	-1.18	0.520

Legend: PBM: pelvic bone marrow; ^{ACT}BM: active bone marrow; ^{INACT}BM: inactive bone marrow; WBC: white blood cells; ANC; absolute neutrophil count; k: 10^3; ul: microliters; g: grams; β: β-coefficient; $R^{2:}$ R-squared; CI: confidence interval; t: t-value; p: p-value.

Table 6 Correlation between points on the dose-volume histograms of active/inactive subregions of pelvic bone marrow and platelets and hemoglobin count nadirs

	PLT nadir					Hb nadir				
Parameter	β	R^2	95%CI	t	p	β	R^2	95%CI	t	p
^{ACT}LSBM										
Mean dose	-0.031	0.094	-0.065/0.001	-1.76	0.061	-0.661	0.023	-2.108/0.786	-0.89	0.361
V_{10} (%)	-0.045	0.060	-2.152/0.543	-2.12	0.150	-0.023	0.040	-3.222/0.651	-1.21	0.220
V_{20} (%)	-0.067	0.090	-2.249/0.654	-1.98	0.075	-0.189	0.020	-3.050/0.698	-1.89	0.340
V_{30} (%)	-0.056	0.090	-2.748/0.187	-1.90	0.060	-0.098	0.010	-3.178/1.567	-1.35	0.550
V_{40} (%)	-0.059	0.090	-3.112/0.150	-1.39	0.060	-0.101	0.010	-4.778/1.850	-1.24	0.580
V_{45} (%)	-0.026	0.003	-2.804/1.349	-0.64	0.710	-0.031	0.003	-4.264/2.183	-0.78	0.750
V_{50} (%)	-0.012	0.030	-2.165/0.774	-1.08	0.260	-0.015	0.001	-3.873/1.296	-1.12	0.810
^{ACT}IBM										
Mean dose	-0.006	0.006	-0.030/0.018	-0.48	0.634	-1.004	0.074	-2.345/0.189	-1.50	0.097
V_{10} (%)	-0.071	0.030	-0.106/0.023	-2.34	0.290	-0.051	0.060	-0.066/0.045	-2.56	0.140
V_{20} (%)	-0.041	0.060	-0.084/0.011	-2.12	0.150	-0.073	0.040	-0.056/0.145	-2.11	0.230
V_{30} (%)	-0.028	0.100	-0.074/0.023	-2.01	0.056	-0.012	0.090	-0.061/0.089	-1.67	0.006
V_{40} (%)	-0.023	0.060	-0.059/0.033	-1.49	0.140	-0.028	0.070	-0.057/0.023	-1.18	0.110
V_{45} (%)	-0.010	0.020	-0.056/0.038	-0.31	0.330	-0.011	0.060	-0.056/0.031	-0.56	0.630
V_{50} (%)	-0.019	0.030	-0.119/0.049	-0.82	0.330	-0.039	0.004	-0.078/0.041	-0.91	0.910
^{ACT}LPBM										
Mean dose	-0.023	0.078	-1.049/0.004	-1.69	0.089	-0.843	0.062	-1.952/0.264	-1.51	0.131
V_{10} (%)	-0.064	0.001	-0.127/0.014	-2.17	0.820	-0.944	0.060	-2.456/0.768	-2.24	0.130
V_{20} (%)	-0.026	0.002	-0.069/0.012	-1.36	0.930	-0.082	0.040	-3.134/0.867	-1.43	0.200
V_{30} (%)	-0.020	0.130	-0.065/0.026	-1.19	0.078	-0.866	0.010	-3.041/1.778	-1.26	0.880
V_{40} (%)	-0.017	0.070	-0.074/0.035	-0.88	0.100	-0.320	0.010	-4.767/1.761	-1.12	0.580
V_{45} (%)	-0.001	0.020	-0.060/0.065	-0.05	0.430	-1.001	0.010	-4.322/2.267	-0.59	0.540
V_{50} (%)	-0.034	0.040	-0.135/0.080	-0.61	0.220	-0.643	0.002	-2.134/1.791	-101	0.800

Legend: PBM: pelvic bone marrow; ^{ACT}BM: active bone marrow; ^{INACT}BM: inactive bone marrow; PLT: platelets; Hb: hemoglobin; k: 10^3; ul: microliters; g: grams; β: β-coefficient; $R^{2:}$ R-squared; CI: confidence interval; t: t-value; p: p-value.

Considerations

Concurrent CH-RT is presently considered as standard of cancer in anal cancer patients, as it provides positive data for both early- and advanced-stage disease[24,25]. Adding chemotherapy may enhance radiotherapy results in this context[26,27]. However, HT is still a consistent problem during CH-RT with major toxicity rates (≥G3) around 60%, regardless of technique employed, as shown in the RTOG 98-11 trial and multicentric studies[3,28]. BM stem cells are extremely radiosensitive. Their irradiation leads to apoptosis and stromal damage, with peculiar pathologic and radiographic changes, with a final overall myelosuppressive effect[7]. Consequently, BM represents a crucial dose-limiting cell renewal tissue for extended-field radiotherapy, particularly when associated with myeloablative chemotherapy regimens[7]. The pelvic region and vertrebrae account for approximately 60% of the total BM functional sites in the average adult population[6]. Up to 40% is present within the pelvis itself[6]. Therefore, its tailored avoidance during CH-RT may be an option to decrease HT profile and to enhance treatment tolerance. The level of radiation-induced damage to BM correlates with both radiation dose and BM volume receiving radiation[29]. Moreover, the influence of low dose to pelvic osseous structures in determining a decrease in blood cell nadirs and the occurrence of acute HT has been documented during CH-RT for pelvic malignancies[9,10]. In anal cancer patients, Mell et al demonstrated that a higher V_5 to V_{20} to PBM significantly predicted for lower WBC and ANC nadirs as V_{10} to V_{20} to LSBM[9]. In cervical cancer patients, the same group showed that PBM $V_{10} \geq 90$ and PBM $V_{20} \geq 75\%$ were correlated with a lower WBC nadir as LSBM V_{10} and V_{20}[10]. Notably, patients with an increased PBM V_{10} and V_{20} were most likely to develop ≥ G2 leukopenia as those with higher LSBM V_{20} and LPBM V_{10} and V_{20}[10]. In anal cancer, Cheng et al recently showed that several low-dose dosimetric endpoints coming either from PBM and LSBM

were associated with a higher likelihood to experience ≥ G3 HT and LSBM volumes receiving doses in the range of 5-20 Gy were pointed out as the strongest predictors[12]. This is a proof of principle that BM stem cells have high radiation sensitivity of BM. Their early cell-killing is believed to drive acute myelosuppression together with effects on peripheral blood stem cells and surrounding stromal tissue[7]. Pelvic bony structures can be selectively avoided during IMRT delivery to reduce HT. This would require their inclusion in the calculation algorithm during planning process and the selection of proper dose-constraints to be assigned to them. However if the aforementioned regions are defined on the planning CT by contouring the marrow cavity or the whole bony structures, their sparing can be challenging, maintaining at the same time an adequate target coverage and avoiding other organs at risk such as bladder, external genitalia or bowel. Hence, a more precise and selective definition of active BM might be helpful. Pathologic studies have shown that BM comprises an active part which is involved in the hematopoietic process ('red marrow') and an inactive portion which is mostly made of fat tissue ('yellow marrow')[7,8]. ^{18}FDG-PET has been demonstrated to be able to detect and quantify the volume of ^{ACT}BM with an uptake pattern specular to its histologic distribution[19]. In our study we investigated the hypothesis that ^{18}FDG-PET could be a useful tool to detect regions within pelvic osseous structures containing hematologically active BM, in anal cancer patients submitted to CH-RT. ^{18}FDG-PET was chosen because it is a widespread examination available in most hospitals and because it is usually included in the diagnostic and staging work up of patients affected with cancer of the anal canal. Moreover a previous study by Rose et al tested the correlation between BM subregions as identified with ^{18}FDG-PET and HT in 26 women affected with cervical cancer and treated with concurrent weekly cisplatin (40 mg/m2) and IMRT. Interestingly, mean dose received by ^{ACT}BM was found to be correlated to log nadirs of WBC, ANC, Plt and Hb with the strongest correlation for Plt (β=-6.16; 95%CI: -9.37/-2.96; p<0.001)[18]. Conversely, no correlation was observed between ^{INACT}BM and blood cells nadirs[18]. Our findings seems to confirm these findings as ^{ACT}BM defined by ^{18}FDG-PET was found to be significantly correlated to WBC, ANC, and Plt nadirs as was (Table 4).

Conversely, ^{INACT}BM was not found to be correlated. This seems to confirm the assumption that the contribution of pelvic bone structures (PBM) to global hematopoiesis is mainly driven by ^{ACT}BM and that ^{18}FDG-PET could be, in general, a reasonably reliable instrument for its detection. LSBM was the pelvic subregion most frequently correlated to blood cell nadirs. LSBM V_{10} and V_{20} had significant correlation to WBC and ANC nadir. LSBM V_{30} was significantly correlated to WBC nadir and had borderline correlation to Plt nadir. LSBM V_{40} had borderline correlation to Plt nadir. IBM V_{10} had significant correlation to WBC and ANC nadir, while V_{30} had borderline significance to Plt nadir. Among the aforementioned parameters, LSBM V_{10} and V_{20} models were found to be the more robust as shown by R^2 and adjusted R^2values. This seems to be reasonable as LSBM has a consistent absolute volume (397 cm^3 $\pm$ 71) and comprises a high percentage of active BM (67%). This is in line with previous studies performed delineating BM volumes on planning CT[12,21]. These information may be useful in determining proper cut-off points for dosimetric parameters to decrease HT profile. Several considerations should be made on the reliability of our findings. Some parts of inactive BM may play a role in hematopoiesis even if they have a low SUV value and may potentially be taken into account. Moreover, ^{18}FDG-PET may not be accurate enough to precisely distinguish between active and inactive BM because of its poor spatial resolution. As in Rose et al, we employed the SUV_{mean} calculated within BM for each patient to define and contour the ^{ACT}BM subregions[18]. The use of a patient specific BM SUV threshold instead of a population-based modality is able to act as a control tool for eventual differences in terms of imaging process across different platforms and in terms of BM SUV values according to age categories[18,19]. This seems to be a reliable and reproducible way to define PBM subregions[18]. As in Rose et al, pelvic bones SUV_{mean} was selected because it is a reasonable compromise between the chance to have a volume sufficiently large for contouring purposes, but restrained to be avoided during IMRT delivery. However, this choice could have a potential influence on our results. The optimal threshold to define ^{ACT}BM has yet to be established. Rose et al recently investigated different threshold levels in the definition of ^{ACT}BM, specifically the highest 25%, 50% and 75% of SUV values, to be

eventually correlated to HT. None of them was able to improve the performance of the model compared to total PBM[22]. Another consideration should be made on the optimal tracer for BM contouring. Since BM cells usually do not show a high metabolic activity, ^{18}FDG uptake may not be sufficient to provide an accurate distinction between ^{ACT}BM and ^{INACT}BM. Other traces such as fluorothymidine may be more suitable to track actively proliferating BM[17]. ^{18}FDG however is a widespread tracer and ^{18}FDG-PET is a common exam in the staging process of anal cancer patients and, given the retrospective nature of our study, was the only functional imaging examination available. In adjunct, as pointed out by Rose et al, intensive chemotherapy regimens, such as 5-FU and MMC as used in our study, may induce recruitment of different BM areas into hematopoiesis, compared to the ones active on baseline. This phenomenon may also influence the correlation between dose to BM and HT. In conclusion, our study suggests that ^{18}FDG-PET-driven selection of active BM within pelvic bones may be a useful tool to selectively identify BM subregions responsible for blood cells decrease during concurrent CH-RT with 5-FU and MMC in anal cancer patients. LSBM seems to be a crucial subregion able to drive HT. Further investigation is needed to determine the best modality to identify ^{ACT}BM (tracers, thresholds), the influence of different chemotherapy regimens and the real clinical significance of out hypothesis.

References

1. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015;33:259-66.
2. Gunderson LL, Winter KA, Ajani JA, Pedersen JE, Moughan J, Benson AB 3rd, et al. Long-term update of US GI intergroup RTOG 98-11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomicyn versus fluorouracil/cisplatin. J Clin Oncol. 2012;30:4344-51.
3. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, Thomas CR Jr, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008;299:1914-21.
4. Albuquerque K, Giangreco D, Morrison C, Siddiqui M, Sinacore J, Potkul R, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. Int J Radiat Oncol Biol Phys. 2011;79:1043-47.
5. Kachnic LA, Winter K, Myerson RJ, Goodyear MD, Willins J, Esthappan J, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mytomycin C for the reduction of acute morbidity in carcinoma of the anal canal Int J Radiat Oncol Biol Phys.2013,86:27-33.

6. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, et al. Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients. Br J Radiol. 2016;89(1060):2015832.
7. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995;31:1319-39.
8. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol 1961;5:255-8.
9. Mell LK, Schomas DA, Salama JK, Devisetty K, Aydogan B, Miller RC, et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in anal cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys. 2008;70:1431-7.
10. Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys. 2006;66:1356-65.
11. Rose BS, Aydogan B, Liang Y, Yeginer M, Hassalle MD, Dandekar V, et al. Normal tissue complication probability modeling of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2011;79:800-7.
12. Cheng JC, Bazan JG, Wu JK, Koong AC, Chang DT. Lumbosacral spine and marrow cavity modeling of acute hematologic toxicity in patients treated with intensity modulated radiation therapy for squamous cell carcinoma of the anal canal. Pract Radiat Oncol. 2014;4:198-206.
13. Wan J, Liu K, Li K, Li G, Zhang Z. Can dosimetric parameters predict acute hematologic toxicity in rectal cancer patients treated with intensity-modulated pelvic radiotherapy? Radiat Oncol. 2015;10:162.
14. Deek MP, Benenati B, Kim S, Chen T, Ahmed I, Zou W, et al. Thoracic vertebral body irradiation contributes to acute hematologic toxicity during chemoradiation therapy for non-small cell lung cancer. Int J Radiat Oncol Biol Phys.2016;94:147-54.
15. Sini C, Fiorino C, Perna L, Noris Chiorda B, Deantoni CL, Bianchi M, et al. Dose-volume effects for pelvic bone marrow in predicting hematological toxicity in prostate cancer radiotherapy with pelvic node irradiation. Radiother Oncol. 2016;118:79-84.
16. Roeske JC, Lujan A, Reba RC, Penney BC, Yamada DS, Mundt AJ. Incorporation of SPECT bone marrow imaging into intensity modulated whole-pelvic radiation therapy treatment planning for gynecologic malignancies. Radiother Oncol. 2005;77:11-7.
17. McGuire SM, Menda Y, Boles Ponto LL, Gross B, TenNapel M, Smith BJ, et al. Spatial mapping of functional pelvic bone marrow using FLT PET. J Appl Clin Med Phys. 2014;15:4780.
18. Rose BS, Liang Y, Lau SK, Jensen LG, Yashar CM, Hoh CK, et al. Correlation between radiation dose to ^{18}FDG-PET defined active bone marrow subregions and acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys. 2012;83:1185-91.
19. Blebea JS, Houseni M, Torigian DA, Fan C, Mavi A, Zhuge Y, et al. Structural and functional imaging of normal bone marrow and evaluation of its age-related changes. Semin Nucl Med. 2007;37:185-94.
20. Kachnic LA, Tsai HK, Coen JJ, Blaszkowsky LS, Hartshorn K, Kwak EL, et al. Dose-painted intensity-modulated radiation therapy for anal cancer: a multi-institutional report of acute toxicity and response to therapy. Int J Radiat Oncol Biol Phys. 2012;82:153-8.
21. Franco P, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, et al. Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer. Clin Transl Oncol. 2016 (in press).
22. Rose BS, Jee KW, Niemierko A, Murphy JE, Blaszkowsky LS, Allen JN, et al. Irradiation of FDG-PET-defined active bone marrow subregions and acute hematologic toxicity in anal cancer patients undergoing chemoradiation. Int J Radiat Oncol Biol Phys. 2016;94:747-54.
23. Radiation Therapy Oncology group. Acute radiation morbidity scoring criteria. Available at: http://www.rtog.org . Accessed April, 12th 2016.
24. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Early-stage node negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res. 2016;36:1943-48.

25. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Locally advanced (T3-T4 or N+) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res. 2016;36:2027-32.
26. UKCCCR Anal cancer Trial Working Party. UK Co-ordination Committee on Cancer Research: Epidermoid anal cancer: results from the UKCCCR randomized trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. Lancet. 1996;348: 1049-54.
27. Bartelink H, Roelofsen F, Eschwege F, Rougier P, Bosset JF, Gonzalez DG, et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Group. J Clin Oncol. 1997;15:2040-9.
28. Salama J, Mell LK, Schomas DA, Miller RC, Devisetty K, Jani AB, et al. Concurrent chemotherapy and intensity modulated radiation therapy for anal cancer patients: a multicenter experience. J Clin Oncol. 2007;25:4581-6.
29. Liang Y, Messer K, Rose BS, Lewis JH, Jiang SB, Yashar CM, et al. Impact of bone marrow radiation dose on acute hematologic toxicity in cervical cancer: principal component analysis on high dimensional data. Int J Radiat Oncol Biol Phys. 2010;78:912-9.

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Chapter 7.

Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation

Franco P, Fiandra C, Arcadipane F, Trino E, Giglioli FR, Ragona R, Ricardi U**. Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation**. *BMC Cancer 2017;17:110.*

At present, concurrent chemo-radiation (CT-RT) is a standard therapeutic option in patients with squamous cell carcinoma of the anal canal[1,2]. Given the high repopulation rate of this type of tumor, treatment compliance is crucial to avoid unintended interruptions potentially extending overall treatment time[3]. In adjunct, maintaining a proper package of chemotherapy (CT) administration in terms of number of cycles and dose is important to achieve adequate tumor control. Hence, decreasing the toxicity profile associated to CT-RT is crucial. If non-conformal techniques are used, as in the RTOG 98-11 trial, crude rates of major acute toxicities can be as high as 48% for skin and 35% for the gastrointestinal district[4]. Intensity-modulated radiotherapy (IMRT) provides robust conformality and modulation, abrupt dose fall-off and reliable consistency and may reduce the dose to organs at risk such as bladder, bowel, perineal skin, genitalia and bone marrow, potentially lowering toxicity[5]. However, even with this approach, acute toxicity is not negligible, as seen in the RTOG 05-29 trial[6]. In this subset of patients, another key endpoint for treatment tolerance is hematologic toxicity (HT) that can affect compliance to therapy, increasing the likelihood to develop bleeding, infections or asthenia[7]. The most important trigger for HT is CHT that induces myelosuppression[8]. Nevertheless, since bone marrow (BM) is highly radiosensitive and, in the average adult population, is comprised for half of its extension within pelvic bones and lumbar vertebrae, the radiation dose received by this compartment may be critical[9,10]. Several retrospective studies correlated different dose parameters of pelvic osseous structures to HT in different oncological scenarios[11-13]. Thus, selective sparing of pelvic bones is thought to be a suitable option to decrease HT during concomitant CT-RT in patients affected with pelvic malignancies including anal cancer[10]. The correct identification of BM within bony structures is the starting point to avoid it during RT. Several approaches have been used. Contouring the whole bone is the method with the highest chance to be inclusive with respect to BM[11]. Delineating the marrow cavity identified as the trabecular bone with lower density on computed tomography is another option[14]. The identification of hematopoietically active bone marrow using either magnetic resonance (MR), single-photon-emission positron tomography (SPECT), ^{18}F-fluorodeoxyglucose-labeled positron-emission

tomography (^{18}FDG-PET) or 3'-deoxy-3'-^{18}F-fluorothymidine-labeled positron-emission tomography (^{18}FLT-PET), gives the potential opportunity to selectively avoid the portion of BM responsible for blood cells generation[15-18]. Aim of the present planning comparison study is to test the hypothesis that the use of ^{18}FDG-PET to identify pelvic active BM to be employed during automatic optimization process might enhance the chance to reduce the dose to the same structures compared to a planning process based on the whole-bone delineation of pelvic bones. This preliminary study aims at finding the most appropriate planning approach to be integrated within a prospective phase II trial in preparation at our Institute to decrease the hematologic toxicity profile in anal cancer patients undergoing CT-RT, employing dose-painted image-guided IMRT.

Methods

Ten patients affected with locally advanced squamous cell carcinoma of the anal canal and/or margin were retrieved from our Institutional databased and employed for the present study. In our center, ^{18}FDG-PET-CT exam is prescribed to all anal cancer patients prior to treatment in order to complete the diagnostic and staging work-up. These examinations were employed for our analysis. Hence, it was not necessary to submit any patient to an extra diagnostic procedure for the present study. Written informed consent was obtained from all patients, for ^{18}FDG-PET-CT examination, radiotherapy treatment and clinical data utilization. The Review Board of the Department of Oncology at the University of Turin approved the present study. Overall patient and tumor characteristics are shown in Table 2. Tumors were staged according to the 7th edition of the TNM classification (2010).

Delineation of target volumes and organs at risk

Patients had a virtual simulation procedure in supine position with both an indexed shaped knee rest and ankle support (CIVCO Medical Solutions, Kalona, IO, USA), without custom immobilization. A CT scan was performed with 3 millimeters slice thickness axial images acquired

from the top of L1 vertebral body to the mid-femural bones. The gross tumor volume (GTV) comprised all primary and nodal macroscopic disease and was defined based on diagnostic MR and PET-CT images. Primary and nodal GTVs were expanded isotropically with 20 mm and 10 mm respectively to generate the corresponding clinical target volumes (CTVs) and then modified to exclude osseous and muscular tissues. The elective CTV encompassed the whole mesorectum and draining lymphatic regions, namely inguinal, external and internal iliac, obturator and perirectal nodes. For locally advanced cases (cT4 and/or N2/N3), presacral nodes were also included within the CTV. Lymphatic areas were contoured as a 10 mm isotropic expansion surrounding regional vessels and then modified to exclude bones and muscles. Thereafter a 10 mm isotropic margin was added for the corresponding planning target volume (PTV) to account for organ motion and set up errors. Bladder, small and large bowel, external genitalia, femoral heads were defined as organs at risk (OARs).

Radiotherapy dose prescription

Dose prescriptions for target volumes were derived from Kachnic et al and adjusted according to clinical stage at presentation[6]. Patients diagnosed with cT3-T4/N0-N3 disease were prescribed 54 Gy/30 fractions (1.8-2 Gy daily) to the anal gross tumor PTV, while gross nodal PTVs were prescribed 50.4 Gy/30 fr (1.68 Gy daily) if sized $\leq$ 3 cm or 54 Gy/30 fr (1.8 Gy daily) if > 3 cm; elective nodal PTV was prescribed 45 Gy/30 fractions (1.5 Gy daily)[6]. This is a frequently used fractionation to deliver IMRT treatments in this setting and it is a standard approach in our Institution[1-3,5]. This is the reason why it was chosen for the present study.

Chemotherapy

All patients received concurrent CT, consisting of 5- fluorouracil (5-FU) (1000 mg/m^2/day) given as continuous infusion along 96 hours (days 1-5 and 29-33) associated with mitomycin C (MMC) (10

mg/m^2, capped at maximum 20 mg single dose) given as bolus (days 1 and 29). A total of 2 concurrent cycles were administered.

Bone marrow delineation

The external contour of pelvic bone marrow (PBM) was outlined on the planning CT using bone windows as first described by Mell et al[11]. The PBM was delineated as a whole and then divided into 3 subsites: a) the iliac BM (IBM), extending from the iliac crests to the upper border of femoral head; b) lower pelvis BM (LPBM), accounting for bilateral pube, ischia, acetabula and proximal femura, from the upper limit of the femoral heads to the lower limit of the ischial tuberosities and c) lumbosacral BM (LSBM), extending from the superior border of L5 somatic body [11].

Active bone marrow delineation on FDG-PET

All images derived from planning CT were exported on the Velocity platform (Varian Medical Systems, Palo Alto, CA) together with treatment volumes, OARs and dose references. Given that FDG-PET-CT images were acquired separately, we performed a rigid co-registration between planning CT and PET-CT images. Patients were set up in treatment position during the acquisition of FDG-PET-CT. The ^{18}FDG-PET standardized uptake values (SUVs) were calculated for PBM volumes, after correcting for body weight. To standardize SUVs among all patients, we normalized BM and liver SUVs. We defined as active bone marrow BM the volume having higher SUV values than the SUV_{mean} for each patient, rather than the whole cohort, as proposed by Rose et al[19,20]. The areas identified with the method described above were outlined within PBM as a whole and named ^{ACT}PBM and within each of the 3 subregions identified on planning CT (LSBM, IBM, LPBM) and named ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM, respectively. Inactive BM (1-^{ACT}PBM) was identified as the difference between BM volumes as defined on planning CT and active BM. The same procedure

was done for all 3 subregions to identify inactive BM within all of them. The 3 volumes were hence called 1^{-ACT}LSBM, 1^{-ACT}IBM, 1^{-ACT}LPBM.

Planning process

All treatment plans were generated using the Pinnacle3 ver. 9.1 platform (Philips, Eindhoven, The Netherlands), including the Auto-planning (AP) module. The AP engine is a progressive region of interest (ROI)-based optimization tool that creates all the required contours iteratively in order to optimize the dose distribution and takes PTV/OARs overlaps into account during the optimization process. Moreover, AP is able to adjust the priority of clinical goals based on the probability to be achieved. Besides clinical objectives and priorities, AP has a compromise setting to allow for sparing of serial organs such as the spinal cord over targets, and advanced settings to allow for setting global parameters such as priorities between targets and OARs, dose fall-off, maximum dose and cold spot management. Therefore the main input data required by AP to drive optimization are: target optimization goal, i.e. prescription dose to the PTVs, engine type (biological or non biological), OARs optimization goals (max dose, max DVH or mean dose), priority (high, medium or low) and compromise (yes or no depending on the strength of the constraint). The standard OARs considered in the optimization process were: bladder (D_{max},D_{mean},V_{35},V_{40},V_{50} as relative volumes), femural heads (D_{max},D_{mean},V_{30},V_{40}, as relative volumes), external genitalia (D_{max},D_{mean},V_{20},V_{30},V_{40} as relative volumes), large and small bowel (D_{max},D_{mean},V_{30},V_{45}, as absolute volumes), iliac crests (V_{30},V_{40},V_{50} as relative volumes) and pelvic BM defined either as whole bone contour or using ^{18}FDG-PET (lowest dose as possible) (Table 1) . Four type of plans were created accounting for the various BM delineation approaches. Each of the four trials was optimized considering as additional OAR (Figure 1):

Plan A. IBM (reference plan; accounting only for iliac crest as per RTOG 05-29 trial)

Plan B. IBM, LSBM, PBM and LPBM (accounting for all the pelvic BM as outlined by the outer surface of external osseous structures)

Plan C. ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM, 1^{-ACT}LSBM, 1^{-ACT}IBM, 1^{-ACT}LPBM (accounting for both the active BM subregions as defined by ^{18}FDG-PET but also for the remaining parts of bony structures, to address a possible uncertainty in the SUV based delineation process. Higher priority was assigned to active BM regions)

Plan D. ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM (accounting only for the active BM subregions as defined by ^{18}FDG-PET)

See Figure 1 for visual description of the 4 planning solutions with respect to the considered BM structures. A similar PTV coverage and avoidance of "standard" OARs were required among the plans. A comparison of the dose received by active pelvic BM (^{ACT}PBM, ^{ACT}LSBM, ^{ACT}IBM, ^{ACT}LPBM) with the 4 different approaches was done in terms of DVH parameters such as Dmax , Dmean and Vx where x was varied from 5 to 45 Gy with 5 Gy steps of progressive increase.

Figure 1. Visual representation of the 4 planning approaches. Bone marrow is represented in red. Optimization was addressed to iliac crest in Plan A (Figure 1A), the whole pelvic bones defined as external osseous contour in Plan B (Figure 1B), active (red) and inactive (yellow) bone marrow as defined with ^{18}FDG-PET (Figure 1C) with a higher priority for active and a lower for inactive, active bone marrow only as defined with ^{18}FDG-PET (Figure 1D).

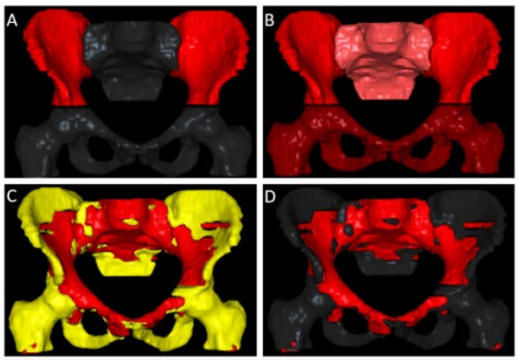

Table 1. Dose constraints to target volume and organs at risk employed during optimization

	Parameter	Goal
PTV	$D_{95\%}$	≥95%
	D_{max}	≤107%
Bladder	V_{30}	<50%
	V_{40}	<35%
	V_{50}	<5%
Large bowel	V_{30}	<200cc
	V_{35}	<150cc
	V_{40}	<20cc
	D_{max}	<50Gy
Small bowel	V_{30}	<200cc
	V_{35}	<150cc
	V_{40}	<20cc
	D_{max}	<50Gy
External genitalia	V_{20}	<50%
	V_{30}	<35%
	V_{40}	<5%
Femural heads	V_{30}	<50%
	V_{40}	<35%
	V_{50}	<5%
Iliac crests	V_{30}	<50%
	V_{40}	<35%
	V_{50}	<5%

Legend: PTV: planning target volume; $V_{20,30,35,40,50}$: volumes receiving 20,30,35,40,50 Gy; cc: cubic centimeters

Statistical analysis

All the results are reported as the sample mean and standard deviation (SD) of all 98 dosimetric parameters subdivided in four groups. Multiple comparisons were performed using univariate analysis of variance (ANOVA). ANOVA provides a statistical test of whether or not the means of several groups are all equal, and therefore generalizes the Student t-test to more than two groups. The difference between multiple subsets of data is considered statistically significant if ANOVA gives a significance level P (P value) less than 0.05, otherwise was reported as not significant (NS). In cases where the ANOVA resulted as statistically significant we evaluated the probability that the means of two populations were equal using Fisher-Hayter pairwise comparisons. This post-test approach is used in statistics when one needs to address pairs comparison in multiple groups after running ANOVA. The STATA software package (Stata Statistical Software: Release 13.1. Stata Corporation, College Station, TX, 2013) was used for all statistical analysis.

Results

Detailed characteristics of the 10 selected patients are shown in Table 2. Mean age at diagnosis was 65. Sex was equally distributed. Most of the patients had a locally advanced disease presentation (Stage IIIB: 80%), with monolateral involvement of pelvic lymphnodes (external and internal iliac nodes), which was deemed more challenging to be tested in the planning process. The mean absolute overlap volume between ^{ACT}PBM and elective nodal PTV (the more sized volume containing also macroscopic nodal and tumor volumes) was 95.4 cm^3 (SD: $\pm$ 37.5 cm^3). Mean ^{ACT}PBM absolute volume was 799.9 cm^3 (SD: $\pm$ 100.8 cm^3). The mean relative overlap volume was 12.2% (SD: $\pm$ 5.2%). No differences were observed among the 4 planning solutions in terms of target coverage and dose to OARs (bladder, bowel, genitalia and femoral heads. With respect to the dose received by BM delineated as the whole osseous contour of pelvic bones, no

significant differences were found in terms of D_{max} and D_{mean} to PBM, LPBM and IBM and in terms of V_{30},V_{40} and V_{45} for IBM between Plan A, B,C and D. The only significant difference (p=0.038) was found in terms of D_{mean} to LSBM between Plan A (D_{mean}=30.88; SD=3.68) and Plan B (D_{mean}=26.44; SD=3.85) or Plan C (D_{mean}=26.52; SD=3.97) (see Table 3). With respect to the dose received by active BM within the whole pelvic bones, as outlined using ^{18}FDG-PET , a significant difference was found in terms of D_{mean} to ^{ACT}PBM (p=0.014) between Plan A (D_{mean}=29.33; SD=2.38) vs Plan C (D_{mean}=25.76; SD: 2.74) and Plan D (D_{mean}=26.02; SD= 2.69) (Table 4). Several other dosimetric parameters were significantly different for ^{ACT}PBM such as V_{20} (p=0.015) between Plan A (Mean=74.26%; SD=7.13) vs Plan C (Mean=63.50%; SD=8.59) and Plan D (Mean=64.24%; SD=8.43), V_{25} (p=0.030) between Plan A (Mean=63.49%; SD=7.48) vs Plan C (Mean=51.49%; SD=7.52) and Plan D (Mean=52.18%; SD=7.97), V_{30} (p=0.020) between Plan A (Mean=52.63%; SD=7.17) vs Plan C (Mean=40.27%; SD=7.12) and Plan D (Mean= 41.31%; SD=7.71), V_{35} (p=0.010) between Plan A (Mean=41.72%; SD=6.78) vs Plan B (Mean= 33.35%; SD=6.13), Plan C (Mean=30.06%; SD=6.43) and Plan D (Mean=31.14%; SD=6.73), V_{40} (p=0.020) between Plan A (Mean=28.82%; SD=5.67) vs Plan B (Mean=21.54%; SD=5.10), Plan C (Mean=19.94%; SD=7.27) and Plan D (Mean=20.67%; SD=5.24) (Table 4). Focusing on different subsites, a significant difference was found for ^{ACT}LSBM in terms of V_{30} (p=0.020) between Plan A (Mean=66.53%; SD=11.19) vs Plan B (Mean=52.06%; SD=13.20), Plan C (Mean=50.07%; SD= 13.19) and Plan D (Mean=51.46%; SD=12.97), V_{35} (p=0.010) between Plan A (Mean=56.95%; SD=12.73) vs Plan B (Mean=42.15%; SD=12.79), Plan C (Mean=40.19%; SD=11.90) and Plan D (Mean=41.42%; SD=12.30), V_{40} (p=0.050) between Plan A (Mean=41.04%; SD=14.37) vs Plan C (Mean=28.17%; SD=9.40). No significant difference was found in terms of any dosimetric parameter for ^{ACT}LSPBM and ^{ACT}IBM between any plan solution (Table 5). Again, no statistically significant difference was found for every dose metric analyzed between 1-^{ACT}PBM, 1^{-ACT}LSBM, 1^{-ACT}IBM, 1^{-ACT}LPBM among all planning approaches (Table 3).

Table 2. Patient and treatment characteristics

Variable	N (%)
Age	
Mean	65
Rage	50-78
Sex	
Female	5 (50%)
Male	5 (50%)
T stage	
T2	5 (50%)
T3	5 (50%)
N stage	
N0-1	2 (20%)
N2	6 (60%)
N3	2 (20%)
Global stage	
II	2 (20%)
IIIB	8 (80%)
PTV dose-tumor (Gy)	
54 Gy	10 (100%)
PTV dose-positive nodes (Gy)	
54 Gy	2 (20%)
50.4 Gy	5 (50%)
PTV dose-elective volumes (Gy)	
45 Gy	10 (10%)

Legend: T: tumor; N: nodal; N°: number; PTV: planned target volume.

Table 3. Comparison of doses to pelvic bone marrow and its subsistes (defined with outer bone contours) and to inactive bone marrow and its subsites (defined with ^{18}FDG-PET) among the 4 plans

		Plan A		Plan B		Plan C		Plan D		p≤ 0.05 ANOVA	Fisher-Hayter test
Structure	Parameter	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)		
PBM	D_{max}	53.50	2.30	53.57	2.20	53.55	2.13	53.73	2.09	NS	
	D_{mean}	25.72	2.44	23.30	2.38	23.25	2.81	23.58	2.74	NS	
LSBM	D_{max}	48.56	2.17	48.83	1.79	49.21	1.88	49.27	1.95	NS	
	D_{mean}	30.88	3.68	26.44	3.85	26.52	3.97	26.97	3.80	0.038	A vs B and C
IBM	D_{max}	48.64	3.04	48.89	3.17	49.35	2.99	49.16	3.21	NS	
	D_{mean}	22.16	1.59	21.57	1.48	20.48	2.31	20.84	2.38	NS	
	V_{30}	24.58	4.74	24.65	3.76	21.14	6.60	22.06	7.36	NS	
	V_{40}	7.13	3.03	6.34	2.75	6.65	2.42	7.18	2.70	NS	
	V_{45}	1.15	1.90	1.09	2.15	1.49	1.53	1.45	1.81	NS	
LPBM	D_{max}	53.60	2.45	53.76	2.33	53.89	2.33	54.01	2.27	NS	
	D_{mean}	25.99	4.12	23.76	4.36	24.46	4.62	24.60	4.48	NS	
1-^{ACT}PBM	D_{max}	53.38	2.25	53.38	2.12	53.36	2.05	53.55	2.09	NS	
	D_{mean}	22.70	3.58	20.21	3.40	21.18	3.92	21.56	3.79	NS	
1-^{ACT}LSBM	D_{max}	48.32	2.17	48.60	1.85	48.94	1.90	48.95	2.00	NS	
	D_{mean}	28.95	4.43	24.00	4.88	25.12	3.93	25.83	3.74	NS	
1-^{ACT}IBM	D_{max}	48.60	3.06	48.70	3.32	49.24	3.07	49.06	3.17	NS	
	D_{mean}	19.98	3.65	18.96	3.59	19.05	3.71	19.43	3.77	NS	
1-^{ACT}LPBM	D_{max}	53.28	2.20	53.27	2.04	53.36	2.05	53.38	2.01	NS	
	D_{mean}	21.85	4.16	19.60	3.88	20.77	4.82	21.06	4.60	NS	

Legend: D_{max}: maximal dose; D_{mean}: mean dose; SD: standard deviation $V_{30,40,45}$: relative volume receiving 30,40,45 Gy; PBM: pelvic bone marrow; LSBM: lumbar-sacral bone marrow; IBM: iliac bone marrow; LPBM: lower pelvis bone marrow; ^{ACT}: active; A, B, C: plan A,B,C; NS: not significant.

Table 4. Comparison of doses to active whole pelvic and lumbar-sacral bone marrow (defined with ^{18}FDG-PET) among the 4 plans

Table 4.

		Plan A		Plan B		Plan C		Plan D		p≤ 0.05 ANOVA	Fisher-Hayter test
Structure	Parameter	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)		
^{ACT}PBM	D_{max}	52.67	2.72	52.93	2.82	53.03	2.79	53.18	2.63	NS	
	D_{mean}	29.33	2.38	26.99	2.38	25.76	2.74	26.02	2.69	0.014	A vs C and D
	V_5	94.59	4.23	92.85	5.05	92.57	5.32	92.71	5.11	NS	
	V_{10}	87.84	6.04	85.10	7.10	84.05	7.73	84.35	7.53	NS	
	V_{15}	82.82	7.06	78.54	7.52	75.17	9.14	75.82	8.44	NS	
	V_{20}	74.26	7.13	68.58	6.94	63.50	8.59	64.24	8.43	0.015	A vs C and D
	V_{25}	63.49	7.48	56.35	6.90	51.49	7.52	52.18	7.97	0.030	A vs C and D
	V_{30}	52.63	7.17	44.87	6.71	40.27	7.12	41.31	7.71	0.020	A vs C and D
	V_{35}	41.72	6.78	33.35	6.13	30.06	6.43	31.14	6.73	0.010	A vs B,C and D
	V_{40}	28.82	5.67	21.54	5.10	19.94	7.27	20.67	5.24	0.020	A vs B,C and D
	V_{45}	9.16	3.51	7.64	2.75	7.20	2.83	6.91	2.21	NS	
^{ACT}LSBM	D_{max}	48.46	2.01	48.66	1.51	49.08	1.70	49.13	1.54	NS	
	D_{mean}	37.86	18.56	27.87	4.38	27.35	4.65	27.65	4.40	NS	
	V_5	89.89	8.72	87.71	9.16	87.24	9.48	87.43	9.30	NS	
	V_{10}	83.87	8.66	79.88	9.49	79.18	10.58	79.32	10.03	NS	
	V_{15}	79.94	9.09	74.87	9.99	74.19	11.68	74.46	10.64	NS	
	V_{20}	77.23	9.44	68.80	11.31	67.97	13.53	68.45	11.75	NS	
	V_{25}	73.13	10.15	60.88	12.60	59.46	14.15	60.71	12.54	NS	
	V_{30}	66.53	11.19	52.06	13.20	50.07	13.19	51.46	12.97	0.020	A vs B,C and D
	V_{35}	56.95	12.73	42.15	12.79	40.19	11.90	41.42	12.30	0.010	A vs B,C and D
	V_{40}	41.04	14.37	29.81	10.52	28.17	9.40	29.29	9.72	0.050	A vs C
	V_{45}	16.11	12.75	10.53	5.13	9.48	3.92	9.23	3.74	NS	

Legend: D_{max}: maximal dose; D_{mean}: mean dose; SD: standard deviation $V_{5,10,15,20,25,30,35,40,45}$: relative volume receiving 5,10,15,20,25,30,35,40,45 Gy; PBM: pelvic bone marrow; LSBM: lumbar-sacral bone marrow; ^{ACT}: active; A, B, C, D: plan A,B,C, D; NS: not significant.

Table 5. Comparison of doses to iliac and lower pelvic bone marrow (defined with ^{18}FDG-PET) among the 4 plans

		Plan A		Plan B		Plan C		Plan D		p≤ 0.05 ANOVA
Structure	Parameter	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)	Mean	SD(+/-)	
^{ACT}IBM	D_{max}	48.19	3.11	48.34	3.22	48.77	3.21	48.68	3.37	NS
	D_{mean}	24.44	6.63	24.22	2.56	22.28	3.65	22.63	3.62	NS
	V_5	96.74	4.70	96.20	6.16	95.82	6.27	95.52	6.66	NS
	V_{10}	90.99	8.49	89.74	9.65	87.22	12.22	87.89	11.38	NS
	V_{15}	84.31	10.64	81.75	12.04	74.75	17.13	76.28	16.98	NS
	V_{20}	66.15	9.73	65.45	11.85	55.52	15.35	57.14	15.83	NS
	V_{25}	45.84	8.97	46.28	9.29	37.87	11.85	37.80	12.04	NS
	V_{30}	29.15	8.09	30.23	5.82	23.82	8.82	24.89	9.31	NS
	V_{35}	16.43	6.62	16.82	4.14	13.33	5.82	14.75	5.98	NS
	V_{40}	7.48	4.37	6.61	3.19	5.95	3.24	6.67	3.13	NS
	V_{45}	0.94	1.57	0.93	1.86	1.02	1.24	1.05	1.46	NS
^{ACT}LPBM	D_{max}	52.66	2.71	52.93	2.82	53.00	2.86	53.14	2.70	NS
	D_{mean}	33.09	4.61	30.63	4.70	29.34	4.80	29.54	4.63	NS
	V_5	98.30	3.37	96.93	5.11	97.15	5.66	98.01	4.14	NS
	V_{10}	90.09	9.01	88.32	9.85	89.50	9.28	88.95	10.05	NS
	V_{15}	86.12	10.76	82.13	12.69	80.73	14.49	81.07	12.61	NS
	V_{20}	82.07	12.89	76.19	13.85	71.53	15.41	72.34	14.82	NS
	V_{25}	75.68	14.62	67.33	14.57	61.89	15.33	63.45	15.46	NS
	V_{30}	66.78	15.38	57.91	14.86	51.34	13.93	52.29	14.28	NS
	V_{35}	55.63	15.81	46.22	13.29	40.63	12.42	41.33	12.55	NS
	V_{40}	38.94	12.30	32.28	10.88	29.06	10.86	29.37	10.56	NS
	V_{45}	16.98	7.53	14.15	6.52	13.54	7.12	12.89	8.85	NS

Legend: D_{max}: maximal dose; D_{mean}: mean dose; SD: standard deviation $V_{5,10,15,20,25,30,35,40,45}$: relative volume receiving 5,10,15,20,25,30,35,40,45 Gy; IBM: iliac bone marrow; LPBM: lower pelvis bone marrow; ^{ACT}: active; A, B, C: plan A,B,C; NS: not significant.

Considerations

Please see Chapter 8.

Chapter 8. Discussion, conclusions and future perspectives

Correlation between dose to pelvic bones and HT has been explored in several studies in the context of anal cancer[14-20]. The first report is by Mell et al who observed on multiple regression analysis that an increased volume of pelvic bone marrow (PBM) receiving doses between 5 and 20 Gy was significantly associated to decreased white blood cells (WBC) and absolute neutrophil count (ANC) nadirs as was the volume of lumbar-sacral bone marrow (LSBM) receiving a dose range between 10 and 20 Gy. On the contrary, Authors could not find any association between dosimetric parameters and G3-G4 leukopenia or neutropenia, even if the volume of LSBM receiving 10 Gy (V_{10}-LSBM) had a non-statistically significant trend in increasing the likelihood of experiencing G4 leukopenia (OR:1.06; 95%CI:0.99-1-12;p=0.051)[18]. This finding shows the high sensitivity of BM stem cells towards radiation. Their early destruction is thought to be responsible for acute myelosuppression together with effects on peripheral blood stem cells and stromal tissue[7]. These data are supported by Franco et al who described PBM-V_{20} as a significant predictor of WBC nadir (β coefficient: -0.035; SE: 0.017; p= 0.048)[20]. In that cohort of anal cancer patients, mean PBM-V_{20} was 75 % (SD:$\pm$9%), consistently with threshold values found to be predictive for HT in other clinical contexts, such as the data reported by Rose et al in cervical cancer patients[21]. Hence, PBM dose metrics have been shown to be predictive of blood cell nadirs, even at low doses, especially in terms of leukopenia, neutropenia and thrombocytopenia. In a small retrospective study of anal cancer patients treated within the UK ACT II trial, Robinson et al performed a tailored analysis of patients treated with 3D-conformal radiation vs patients submitted to IMRT[15]. In general, an IMRT treatment strategy significantly increased irradiation of PBM, with a potential suppressive effect on WBC and neutrophilic cells corresponding to a higher risk of developing major HT[15]. Surprisingly, the observed rates of major HT were similar between the 2 groups, highlighting the fact that the correlation between PBM dose and blood cells nadirs found in linear regressions analyses not always corresponds to a correlation with a major grade toxicity event in logistic regression analyses.

Even more difficult is to demonstrate the clinical meaningfulness of toxic events based on a dedicated scoring scale. Nevertheless, some informative studies reporting on graded HT toxicity are present. Cheng et al recently observed that several low-dose dosimetric parameters of either PBM and LSBM were associated with a higher chance to develop ≥ G3 HT. Of notice, volumes of LSBM receiving doses ranging from 5 to 20 Gy were found to be the most consistent predictors[16]. That points out the hypothesis that dose to specific osseous segments may have a strong correlation to HT, depending on the relative percentage of active bone marrow that they may comprise. In this sense, LSBM has a consistent relative proportion of active BM[14]. In the study by Franco et al, authors showed a significant correlation between LSBM-V_{40} and a higher likelihood to develop ≥ G3 HT (OR: 1.328; SE: 0.160; p=0.019)[21]. The optimal cut-off point for LSBM-V_{40} was found to be 41%. Patients with LSBM-V_{40} ≥ 41% were more likely to develop ≥ G3 HT (60.9% vs 39.1%; p=0.041)[21]. This findings seems to be confirmed also when BM is defined according to ^{18}FDG-PET imaging to delineate its active portion. Franco et al showed that volume of LSBM receiving doses in the range of 10 to 30 Gy were significantly correlated to WBC and ANC nadirs[14]. Other subsites within pelvic bones, such as IBM and LPBM, do have a role in the occurrence of HT[14]. However, it has to be noted that the role of ^{18}FDG-PET in the precise identification of active BM has been recently debated. Rose et al investigated the ability of ^{18}FDG-PET-defined active BM to predict ANC nadir during or within 2 weeks of completion of treatment in anal cancer patients[17]. The model performance of Equivalent Uniform Dose (EUD) to active BM was equivalent to that of inactive and total BM, suggesting that ^{18}FDG may not be the ideal tracer to provide accurate discrimination between hematopoietic elements and background non-hematologic cells[17].

Several authors investigated the correlation between dose to pelvic osseous structures and HT in oncological scenarios other than anal cancer[10,17,21-29]. In cervical cancer, with patients treated with concurrent RT and weekly DDP 40 mg/m^2, Mell et al observed that PBM-V_{10} ≥ 90 and PBM-V_{20} ≥ 75% were associated with a lower WBC nadir. Moreover, an increased PBM-V_{10} and -V_{20}

predicted for a higher likelihood to develop ≥ G2 leukopenia as the LSBM-V20, lower-pelvis bone marrow (LPBM)-V_{10} and $-V_{20}$. A higher PBM-V_{10} was also found to be a predictor of ≥ G2 neutropenia[10]. In line with this findings are the reports by Rose et al and Albuquerque et al, again in cervical cancer patients[21,22]. Rose et al observed that PBM-V_{10} > 95% and PBM-V_{20} > 76% increased the likelihood to experience ≥ G3 leukopenia, while Albuquerque et al showed that PBM-V_{20} > 80% increased the risk to develop ≥ G2 overall HT[21,22]. These studies do stress the importance of volumes of PBM receiving low doses in the occurrence of HT, when myelosuppressive CT regimens (such as DDP) are used. A recent longitudinal study by Zhu et al, in a similar setting of patients, demonstrated that increased PBM-V_{20}, -V_{30} and -V_{40} were significantly associated with a higher weekly reduction of WBC and ANC counts, estimating that every 1 Gy increase in mean PBM dose could lead to a 9.6/μl per week reduction in the natural logarithm of ANC count[29].The regimen of CT employed strongly affects the correlation between dose to pelvic bony structures and the occurrence of HT. This has been elegantly shown by Bazan et al, in patients submitted to different combination of RT and CT for different malignancies[30]. Patients undergoing whole pelvis RT and 5-FU had a higher BM tolerance towards radiation compared to those receiving DDP or MMC. Patients incorporating MMC in their combined modality treatment program had a lower maximum tolerated dose-50% (TD_{50}) and a steeper NTCP curve. Overall, the dose tolerance of PBM and LSBM resulted to be lower for patients receiving MMC compared to dose treated with DDP[30]. Interesting data come from Sini et al in the context of prostate cancer patients undergoing post-prostatectomy whole pelvic RT[27]. Data on these patients are very intriguing, given their 'chemo-naïve' profile. The absence of any confounding effect due to CT may provide the chance to explore a 'pure' dose-volume effect for irradiated BM. Authors observed that higher PBM-V_{40} were significantly associated to a higher likelihood to develop acute G3 (OR=1.018) and late G2 (OR=1.005) lymphopenia. Moreover, IBM-V_{40} was found to be correlated to the probability risk for 1-year G2 lymphopenia, with a dichotomizing cut-off point at 94.6 cc absolute IBM volume[27].The finding of the role of higher doses to the whole pelvic BM,

such as PBM-$V_{40,}$ and to specific subregions, such as LSBM-$V_{40,}$ is in line with data coming from rectal and anal cancer[20,23,24]. For example, Wan et al showed, in rectal cancer patients undergoing pre-operative CT-RT with concomitant capecitabine, a significant correlation between LSBM-V_{40} and ≥ G2 HT with patients having LSBM-V_{40} ≥ 60% more likely to develop HT[26]. As previously described, the same dose-volume parameter (LSBM-$V_{40)}$ was found by Franco et al, but with a more restrictive cut-off point at 41%, which seems reasonable taking into account the different CT regimens used (capecitabine vs 5FU-MMC).

The observation that dose to pelvic structures plays a role in the occurrence of HT is particularly important in the setting of pelvic malignancies, since pelvic bones harvest a high relative proportion of active BM[31]. Hayman et al investigated the relative distribution of active BM through the body, using ^{18}FLT-PET, in 13 patients affected with different types of cancer, observing that 25.3% was at the pelvis, 16.6% at lumbar spine and 9.2% at the sacrum[32]. In adjunct, in a recent study, McGuire et al demonstrated that regions located in the central part of the pelvis (upper sacrum, inner halves of iliac crests and the 5th lumbar vertebral body), have the highest uptake of ^{18}FLT[33]. Similar results were obtained by Franco et al using ^{18}FDG with the evidence of up to 67% of active bone marrow comprised within the sacrum relative to the whole sacral bone volume[14]. Hence, from a radiation oncology perspective, a potential strategy to decrease the HT profile in this subset of patients, is to selectively spare osseous structures within the pelvis during the radiotherapy planning and delivery process[34]. That means that areas containing hematopoietically active bone marrow needs to be properly outlined on the planning CT and taken into account during the planning process with appropriate dose-constraints to drive isodose line distribution. An ideal BM-sparing approach must come without compromising coverage of target volumes and avoidance of other organs at risk, such as bladder, bowel, genitalia and femoral heads. The ideal strategy to selectively spare pelvic BM has yet to be established. In the planning comparison study we performed for the present project, we compared 4 different approaches. The basic approach (Plan A) was taken from

the RTOG 05-29 trial and optimization on BM was limited to the iliac crests (IBM), as outlined on planning CT using the external surface of bones as reference. This strategy did not take into account for the part of BM comprised within sacrum and ischiatic bones. Plan B included in the planning algorithm the whole pelvis [all 3 subsites: iliac bone marrow (IBM), LSBM, LPBM] delineated using the outer surface on CT. This approach, based on Mell et al contouring protocol, took into account the whole BM comprised within pelvic bones[10]. Conversely, Plan C and D employed functional imaging for active BM identification within pelvic bones, as previously described. In Plan C, the highest priority was given to active BM defined with ^{18}FDG-PET, but inactive BM was also taken into account in the planning process with a lower priority score. This approach was chosen considering the observation by Rose et al, who showed that both active and inactive BM as defined using ^{18}FDG-PET may be associated to neutrophilic cell nadir[17]. In plan D, we accounted only for active BM within the pelvis as a structure to be spared. In general, no significant differences were found in terms of target coverage and organs at risk (other than BM) avoidance among all plan solutions, highlighting the fact that neither of these approaches negatively affected those treatment objectives. The inclusion in the optimization process of pelvic subsites other than iliac crests (IBM) such as LSBM and LPBM, lead to a significant decrease in the mean dose to LSBM (not to IBM, LPBM or PBM as a whole).For IBM this is due to the fact that this region was included as OAR in all 4 planning strategies. For LPBM, a possible explanation could be the low dose to the structure obtained with all 4 methods and for PBM, which is the summation of all 3 subregions, the insufficient contribution of LSBM mean dose reduction to the whole pelvis dose. This finding means that, compared to the RTOG 05-29 planning strategy of addressing iliac crest only in the optimization process, a more comprehensive approach may further spare BM comprised in the lumbar-sacral region (Plan A - D_{mean}=30.88 vs Plan B - D_{mean}=26.44 and Plan C - D_{mean}=26.52; p= 0.038). This may be important since LSBM may contain a higher proportion of hematopoietically active BM and the RT dose received by this subsite has been demonstrated to be highly involved in the occurrence of acute HT[14]. Using the external surface of LSBM (Plan B)

or ^{18}FDG-PET-defined ^{ACT}LSBM seems not play a role in the chance to reduce LSBM mean dose. This can be partially due to the relative overlap volume between PTV and ^{ACT}PBM, which was, on average, as high as 12.2% in our set of patients. Focusing on the dose received by active bone marrow outlined with ^{18}FDG-PET within pelvic bones employing the 4 different planning strategies, several interesting findings can be pointed out. The mean dose received by the active BM within the whole pelvis (^{ACT}PBM) could be significantly reduced by including other subsites than iliac crest in the optimization process (Plan A - D_{mean}=29.33 vs Plan C - D_{mean}=25.76 and Plan D - D_{mean}=26.02; p= 0.014)[34]. This reduction in the mean dose is mainly driven by a reduction in the ^{ACT}PBM volumes receiving doses ranging from 20 Gy to 40 Gy (significant difference in terms of V_{20},V_{25},V_{30},V_{35} and V_{40} between Plan A and others). The subsite the mostly contributes to the reduction of ^{ACT}PBM dose is ^{ACT}LSBM whose volume receiving doses ranging from 30 Gy to 40 Gy was significantly different between Plan A and other solutions (V_{30},V_{35},V_{40})[34]. The chance to reduce ^{ACT}LSBM and consequently ^{ACT}PBM doses addressing all pelvic subsites during the planning process seems to be similar with all modalities employed (Plan B,C and D). Dosimetric data were generally lower than those reported to have clinical meaningfulness in patients affected with pelvic malignancies. For example in cervical cancer patients, Mell et al showed that patients having PBM-V_{10} $\geq$ 90% and PBM V_{20} $\geq$ 75% were most likely to develop $\geq$ G2 leukopenia and to have chemotherapy held[10].Accordingly, Rose et al found that PBM- V_{10} $\geq$ 95% and PBM V_{20} $\geq$ 76% were associated to a higher chance to develop $\geq$ G3 leukopenia in a similar cohort[21]. In the planning study, we were able to be consistently below these thresholds with all the 4 strategies, but those employing functional imaging (Plan C and D) seemed to be the most promising, particularly with respect to ^{ACT}PBM-V_{20}, which was 63.5% and 64.2% with these 2 solutions[34]. In anal cancer patients, Bazan et al showed that patients with PBM mean dose $\geq$ 30 Gy had a 14-fold increase in the odds of developing $\geq$ G3 HT[19]. Moreover, according to Lyman-Kutcher-Burman modeling, Franco et al outlined that LSBM mean dose should be kept < 32 Gy to minimize >G3 HT rates in a similar population[35]. In our planning comparison study, ^{ACT}PBM mean dose was below 27 Gy with

plan B,C and D approaches with (non significantly) lower values for the strategies employing ^{18}FDG-PET. In adjunct ^{ACT}LSBM mean dose was consistently below 28 Gy for the 3 strategies (B,C,D), with similar reduction entity[34]. In a previous study (also included in the present PhD project), we demonstrated, in anal cancer patients, that those having a LSBM-$V_{40} \geq 41$ % were more likely to develop $\geq$G3 HT[20]. Plan B,C and D were able to obtain LSBM-V_{40} values consistently below 30%, with no significant difference among the 3 planning strategies. Our data seem to show that, at least for a patient cohort of anal cancer patient as the one included in our planning comparison study, the optimization on BM as the whole osseous contour is able to spare BM similarly to that defined on ^{18}FDG-PET[34]. The paradigm in this setting, is that functional imaging (^{18}FDG-PET in this case) is able to correctly detect active BM within bony structures, identifying subvolumes smaller the those outlined by the whole bone contour and that may be optimized more easily without compromising target coverage and avoidance of other organs at risk[33,34] . The data outlined in the planning comparison study suggest that this assumption is not trivial and that optimization on whole bone contour may be as efficient. This may be due to the fact that ^{ACT}PBM dose reduction was driven in our study by ^{ACT}LSBM dose decrease. It has been shown that the relative proportion of active BM within LSBM is as high as 67% and hence in this case the outer contour of LSBM may be a valid surrogate of ^{ACT}LSBM[14,36]. Moreover LSBM and ^{ACT}LSBM are centrally located and usually in close proximity to primary tumor and macroscopic node treatment volumes and hence sparing one (mainly from high-dose) means sparing the other. Nevertheless, the other consideration is that BM distribution within the bones can be very different. Campbell et al investigated BM distribution according to ^{18}F-FLT-PET in a cohort of 51 lung cancer patients. Women had a higher proportion of functional BM in the pelvis, proximal femurs and skull, while men in the sternum and ribs, clavicles and scapulae. Elderly patients (> 75 years) had a higher relative proportion of active BM in the ribs, clavicles and scapulae[37]. Because of the slenderness of the sample size, we did not perform any subset analysis in the planning comparison study, but the relative proportion of active BM may be different among the 3 different subsites

(LSBM, IBM and LPBM) and within the same subsite, depending on patient's characteristics (sex and age for example) and intrinsic variability. The optimization of the whole bone contour is efficient but does not take into account individual variability, while the one based on functional imaging may be able to do it. Another point is that BM distribution within the pelvis may undergo substantial changes during the course of RT-CT, because of the clonal expansion of red marrow due to the trigger of antiblastic treatments. Functional imaging may be able to record and track this modifications[38]. However, the most appropriate quantitative imaging strategy to identify active BM has yet to be established. Several different methods have been investigated such as SPECT, ^{18}FDG-PET, ^{18}FLT-PET and quantitative MR. All the aforementioned tools have different characteristics with respect to sensitivity and specificity to detect active BM, magnitude and reliability of the quantitative information provided and availability among the radiation oncology facilities[39]. In this sense ^{18}FDG-PET is a reasonable choice in terms to cost-effectiveness. This is important because sparing pelvic BM as defined with ^{18}FDG-PET has clinical meaningfulness. This has been demonstrated in a prospective frame in the setting of cervical cancer, with the INTERTECC-2 trial, where patients treated with concurrent RT-CHT developed a lower rate of $\geq$ G3 neutropenia, if treated with a ^{18}FDG-PET-driven pelvic BM-sparing IMRT approach[40].

Conclusions

HT may be a consistent issue in anal cancer patients undergoing concurrent CT-RT, with potentially detrimental effects on clinical outcomes and patient's compliance to treatment. Radiotherapy is an important factor in determining HT and hence attention should be paid to BM during the treatment planning process. Nevertheless several aspects still need to be clarified. The most appropriate BM dose-volume parameters still need to be investigated. Some data stress the role of low doses to the whole pelvic osseous structures, some other medium to high doses. In general, Lyman-Kutcher-Burman (LKB) model confirm that BM act like a parallel organ and thus mean dose is a useful tool

to predict for the occurrence of acute HT[19]. The most important irradiated regions within the pelvis to enhance HT have yet to be determined. Those containing a large amount of active BM are for sure crucial, such as the sacrum and iliac subsites[32,33]. However, the dose to the whole PBM plays a role[18]. Probably both of them are important and an interaction between low doses to PBM and medium to high doses to specific subsites is a potential trigger for the development of HT[35]. Modern morphological and functional imaging modalities may enhance our ability to carefully define and delineate BM regions within treatment volume areas. Computed tomography-based delineation of the external aspect of bones prevents missing BM but may lead to extended normal tissue volumes to be spared, with challenging treatment plans in terms of both target coverage and organs at risk sparing. The incorporation of ^{18}FDG-PET in the diagnosis and staging of anal cancer is widespread and thus it is easy to implement its use for BM identification. Nevertheless its sensitivity and specificity in correctly identifying BM have been questioned[17]. In this sense, ^{18}F-FLT-PET may be a more adequate tool but its use in the clinical practice is still anecdotal. Adjunctively, the influence of CT on the relative distribution of active BM within osseous structures should also be taken into account, with potential differences compared to baseline status[38]. The most proper clinical endpoints to be used in this setting are still uncertain. Blood cell nadirs, acute HT as determined by a codified scoring scale or modification in the clinical management (CT dose reduction, treatment breaks, overall treatment time increase) have been used in the available studies, leading to different correlation with dosimetric parameters. Radiation oncologists have a crucial role in the prevention and management of HT in anal cancer patients. The systematic inclusion of BM volumes in the planning algorithm as avoidance structures should be strongly advised in patients undergoing RT for pelvic malignancies. However, the most appropriate imaging modalities for BM identification as the most proper dose-.volume parameters to be used and clinical endpoints to be addressed, still deserve investigation. Prospective clinical validation of BM-sparing treatment strategies is mandatory[40].

Five-year view

In the next 5 years, clinical research in the field of anal cancer should focus to find out the most reliable imaging modality to define and delineate BM within pelvic osseous structures to help its selective avoidance during RT treatments. Prospective trials addressing the issue of acute hematologic toxicity would be helpful to define robust endpoints with clinical meaningfulness and to better identify significant dosimetric parameters correlating with the toxicity profile to be incorporated within the treatment planning process to decrease this important side effect. Selection and definition of BM as an organ at risk should be advised on a routine basis to tailor sparing strategies and to increase the therapeutic index in this subset of patients.

Key issues

- Acute hematologic toxicity is an important side effects in anal cancer patients undergoing concurrent chemo-radiation
- Radiation is a consistent trigger for hematologic toxicity and pelvic bone marrow is a crucial organ at risk
- A dose-response relationship is evident but dose-volume parameters and robust clinical endpoints have yet to be determined
- The systematic inclusion of bone marrow in the planning algorithm as avoidance structures should be strongly advised, but prospective clinical validation is needed

Future perspectives

We are presently running a prospective phase II trial in order to reduce the acute HT profile in patients affected with squamous cell carcinoma of the anal canal undergoing concurrent CT-RT.

Study design

The study is based on the primary assumption, supported by data coming from the literature and from our previous studies, that ^{18}FDG-PET can be a valid tool to identify active bone marrow regions within pelvic bony structures and that the dose to these subsites is a strong predictor of acute HT in anal cancer patients undergoing definitive concurrent CT-RT. The hypotesis under investigation is that dose-painted IMRT, based on an optimization process addressed to active BM as defined through ^{18}FDG-PET, might be able to reduce the acute toxicity profile in anal cancer patients undergoing concurrent CT-RT, compared to our historical data.

Statistics

The comparative cohort will be based on our historical data of anal cancer patients treated with VMAT and concurrent 5-FU and Mitomycin C. In that series the rate of acute leukopenia $\geq$ Grade 2 was 66%[41]. We selected a one-armed optimal two-stage Simon's design to test the hypothesis that treatment modality under investigation (dose-painted IMRT with optimization addressed to BM as defined by ^{18}FDG-PET) would increase the rate of G0-G1 (vs G2,G3,G4) acute HT toxicity (> 34%) over the historical data obtained with the previous approach (VMAT-based IMRT with no optimization on BM) [null hypothesis (H_0): no difference in acute HT toxicity between treatment modalities][42].The present study is based on the following assumptions: 1- the historical data of success (p0) was represented by the 34% rate of G0-G1 acute HT toxicity (G2-G4:66%) detected in the previous study; 2- the threshold of successful trial (p1) with the treatment schedule under investigation was set to 54% of G0-G1 acute HT toxicity (G2-G4:46%); 3- the α error (one-sided type I error) was set at 5%; 4- the β error at 20% (type II error; power 80%). At the first stage, among 18 enrolled patients, at least 7 (39%) need to be scored as G0-G1 acute HT toxicity to further proceed with the trial. At the second stage, another 28 patients will be accrued for an overall sample size of 46 patients. A minimum of 20/46 (43.5%) with G0-G1 toxicity represent the threshold for the rejection of H_0 and the fulfilment of the criteria for the definition of a 'promising'

treatment for dose-painted IMRT with optimization addressed to BM as defined by ^{18}FDG-PET to decrease HT in anal cancer patients

Inclusion Criteria:

- Histologically confirmed stage I, II, IIIA, IIIB squamous cell carcinoma of the anus
- ^{18}FDG-PET performed during diagnostic and staging work-up before the beginning of combined CT-RT
- Chemotherapy with 5- fluorouracil (1000 mg/m2/day) given as continuous infusion along 96 hours (days 1-5 and 29-33) associated with mitomycin C (10 mg/m2) given as bolus (days 1 and 29)
- Blood cell count performed on a weekly basis.
- Intensity modulated radiotherapy delivered with volumetric approach and daily cone beam CT. Dose prescriptions for target volumes will be in accordance to Kachnich et al (cT2N0: 50.4 Gy/28 fractions /1.8 Gy daily to the gross tumor planning target volume and 42 Gy/28 fractions /1.5 Gy/daily to the elective nodal planning target volume. cT3-T4/N0-N3: 54 Gy/30 fractions /1.8-2 Gy daily) to the anal gross tumor planning target volume, 50.4 Gy/30 fr 1.68 Gy daily if sized <3cm or 54 Gy/30 fr /1.8 Gy daily if >3cm; elective nodal planning target volume: 45 Gy/30 fractions /1.5 Gy daily)[43].

Hematologic toxicity will be recorded according to CTCAE v4.02 acute radiation morbidity scoring criteria based on weekly complete blood count, considering $\geq$ G2 HT including leukopenia, neutropenia, anemia and thrombocytopenia. Other toxicities (gastrointestinal, genitourinary, skin and genitalia) will be also recorded.

Delineation of pelvic bone marrow osseous structures will be performed according to Mell et al[10].The external contour of pelvic bone marrow (PBM) on the planning CT employing bone

windows will be outlined. The PBM was delineated as a whole and then divided into 3 subsites: a) the iliac BM (IBM), extending from the iliac crests to the upper border of femoral head; b) lower pelvis BM (LPBM), accounting for bilateral pube, ischia, acetabula and proximal femura, from the upper limit of the femoral heads to the lower limit of the ischial tuberosities and c) lumbosacral BM (LSBM), extending from the superior border of L5 somatic body[10].

We will use ^{18}FDG-PET imaging to define areas of active bone marrow within the pelvis. Specifically ^{18}FDG-PET standardized uptake values (SUV) (corrected for body weight) will be evaluated and calculated with respect to the pelvic bones. The active bone marrow for each patient will be defined as the region within the total pelvic bones, with a SUV higher than the patient's individual mean SUV, while inactive bone marrow will be considered as the region within the total bone marrow with a SUV lower than this threshold, as defined by Rose et al[17].

Given the fact that squamous cell carcinoma on the anal canal is a rare cancer, we assume to complete patients enrollment in a 2-3 year timespan.

References

1. Franco P, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, et al. Intensity-modulated radiation therapy with simultaneous integrated boost combined with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series. Cancer Invest. 2015; 33: 259-66.
2. Nigro ND, Vaitkevicius VK, Considine B Jr. Combined therapy for cancer of the anal canal: a preliminary report. Dis Colon Rectum 1974; 17: 354-6.
3. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Early-stage node negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 1943-8.
4. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, et al. Locally advanced (T3-T4 or N+) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy. Anticancer Res 2016; 36: 2027-32.
5. Ajani JA, Winter KA, Gunderson LL, Pedersen J, Benson AB 3rd, Thomas CR Jr, et al. Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: a randomized controlled trial. JAMA. 2008; 299: 1914-21.
6. Julie DA, Oh JH, Apte AP, Deasy JO, Tom A, Wu AJ, Goodman KA. Predictors of acute toxicities during definitive chemoradiation using intensity-modulated radiotherapy for anal squamous cell carcinoma. Acta Oncol 2016; 55: 208-16.
7. Mauch P, Constine L, Greenberger J, Knospe W, Sullivan J, Liesveld JL, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys. 1995; 31: 1319-39.
8. Filippi AR, Franco P, Ricardi U. Is clinical radiosensitivity a complex genetically controlled event? Tumori 2006;92: 87-91.
9. Lujan AE, Mundt AJ, Yamada SD, Rotmensch J, Roeske JC. Intensity-modulated radiotherapy as a means of reducing dose to bone marrow in gynecologic patients receiving whole pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2003;57:516-21.
10. Mell LK, Kochanski JD, Roeske JC, Haslam JJ, Mehta N, Yamada SD, et al. Dosimetric predictors of acute hematologic toxicity in cervical cancer patients with concurrent cisplatin and intensity-modulated pelvic radiotherapy. Int J Radiat Oncol Biol Phys 2006; 66: 1356-65.
11. Ellis RE. The distribution of active bone marrow in the adult. Phys Med Biol 1961;5:255-8.
12. Jianyang W, Yuan T, Yuan T, Ning L, Hua R, Hui F, et al. A prospective phase II study of magnetic resonance imaging guided hematopoietical bone marrow-sparing intensity-modulated radiotherapy with concurrent chemotherapy for rectal cancer. Radiol Med 2016; 121: 308-314.
13. Mell LK, Tiryaki H, Ahn KH, Mundt AJ, Roeske JC, Aydogan B. Dosimetric comparison of bone marrow-sparing intensity modulated radiotherapy versus conventional techniques for treatment of cervical cancer. Int J Radiat Oncol Biol Phys 2008; 71: 1504-10.
14. Franco P, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, et al. Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemoradiation in anal cancer patients. Med Oncol 2016; 33: 72.
15. Robinson M, Sabbagh A, Muirhead R, Durrant L, Van den Heuvel F, Hawkins M. Modeling early haematologic adverse events in conformal and intensity-modulated pelvic radiotherapy in anal cancer. Radiother Oncol 2015; 117: 246-51.
16. Cheng JC, Bazan JG, Wu JK, Koong AC, Chang DT. Lumbosacral spine and marrow cavity modeling of acute hematologic toxicity in patients treated with intensity modulated radiation therapy for squamous cell carcinoma of the anal canal. Pract Radiat Oncol 2014; 4: 198-206.
17. Rose BS, Jee KW, Niemierko A, Murphy JE, Blaszkowsky LS, Allen JN, et al. Irradiation of FDG-PET-defined active bone marrow subregions and acute hematologic toxicity in anal cancer patients undergoing chemoradiation. Int J Radiat Oncol Biol Phys 2016; 94: 747-54.
18. Mell LK, Schomas DA, Salama JK, Devisetty K, Aydogan B, Miller RC, et al. Association between bone marrow dosimetric parameters and acute hematologic toxicity in anal cancer patients treated with concurrent chemotherapy and intensity-modulated radiotherapy. Int J Radiat Oncol Biol Phys 2008; 70: 1431-7.

19. Bazan JG, Luxton G, Mok EC, Koong AC, Chang DT. Normal tissue complication probability modeling of acute hematological toxicity in patients treated with intensity-modulated radiation therapy for squamous cell carcinoma of the anal canal. Int J Radiat Oncol Biol Phys 2012; 84: 700-6.
20. Franco P, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, et al. Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer. Clin Transl Oncol 2017;19:67-75.
21. Rose BS, Aydogan B, Liang Y, Yeginer M, Hassalle MD, Dandekar V, et al. Normal tissue complication probability modeling of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. Int J Radiat Oncol Biol Phys 2011; 79: 800-7.
22. Albuquerque K, Giangreco D, Morrison C, Siddiqui M, Sinacore J, Potkul R, et al. Radiation-related predictors of hematologic toxicity after concurrent chemoradiation for cervical cancer and implications for bone marrow-sparing pelvic IMRT. Int J Radiat Oncol Biol Phys. 2011;79:1043-47.
23. Yang TJ, Oh JH, Apte A, Son CH, Deasy JO, Goodman KA. Clinical and dosimetric predictors of acute hematologic toxicity in rectal cancer patients undergoing chemoradiotherapy. Radiother Oncol 2014; 113: 29-34.
24. Wan J, Liu K, Li K, Li G, Zhang Z. Can dosimetric parameters predict acute hematologic toxicity in rectal cancer patients treated with intensity-modulated pelvic radiotherapy? Radiat Oncol 2015; 10: 162.
25. Wang J, Tian Y, Tang Y, Wang X, Li N, Ren H, et al. A prospective phase II study og magnetic resonance imaging guided hematopoietical bone marrow-sparing intensity-modulated radiotherapy with concurrent chemotherapy for rectal cancer. Radiol Med 2016; 121: 308-14.
26. Wang J, Tian Y, Tang Y, Wang X, Li N, Ren H, et al. A Phase II prospective nonrandomized trial of magnetic resonance imaging-guided hematopoietic bone marrow-sparing radiotherapy for gastric cancer patients with concurrent chemotherapy. Onco Targets Ther 2016; 9: 2701-7.
27. Sini C, Fiorino C, Perna L, Noris Chiorda B, Deantoni CL, Bianchi M, et al. Dose-volume effects for pelvic bone marrow in predicting hematological toxicity in prostate cancer radiotherapy with pelvic node irradiation. Radiother Oncol 2016; 118: 79-84.
28. Deek MP, Benenati B, Kim S, Chen T, Ahmed I, Zou W, et al. Thoracic vertebral body irradiation contributes to acute hematologic toxicity during chemoradiation therapy for non-small cell lung cancer. Int J Radiat Oncol Biol Phys 2016; 94: 147-54.
29. Zhu H, Zakeri K, Vaida F, Carmona R, Dadachanji KK, Bair R, et al. Longitudinal study of acute hematologic toxicity in cervical cancer patients treated with chemoradiotherapy. J Med Imaging Radiat Oncol 2015; 59: 386-93.
30. Bazan JG, Luxton G, Kozak MM, Anderson EM, Hancock SL, Kapp DS, et al. Impact of chemotherapy on normal tissue complication probability models of acute hematologic toxicity in patients receiving pelvic intensity modulated radiation therapy. Int J Radiat Oncol Biol Phys 2013; 87: 983-91.
31. Mauch P, Constine L, Greenberger J, et al. Hematopoietic stem cell compartment: acute and late effects of radiation therapy and chemotherapy. Int J Radiat Oncol Biol Phys.1995;31: 319-39.
32. Hayman JA, Callahan JW, Herscjtal A, et al. Distribution of proliferating bone marrow adult cancer patients determined using FLT-PET imaging. Int J Radiat Oncol Biol Phys. 2011;79:847-52.
33. McGuire SM, Menda Y, Boles Ponto LL, et al. Spatial mapping of functional pelvic bone marrow using FLT PET. J Appl Clin Med Phys. 2014; 15: 4780.
34. Franco P, Fiandra C, Arcadipane F, et al . Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation. BMC Cancer 2017;17:110.
35. Franco P, Ragona R, Arcadipane F, et al. Lumbar-sacral bone marrow dose modeling for acute hematologic toxicity in anal cancer patients treated with concurrent chemo-radiation. Med Oncol 2016;33:137.
36. McGuire SM, Menda Y, Ponto LL, Gross B, Juweid M, Bayouth JE. A methodology for incorporating functional bone marrow sparing in IMRT planning for pelvic radiation therapy. Radiother Oncol. 2011;99:49-54.
37. Campbell BA, Callahan J, Bressel M, et al. Distribution atlas of proliferating bone marrow in non-small cell lung cancer patients measured by FLT-PET/CT imaging, with potential applicability in radiation therapy planning. Int J Radiat Oncol Biol Phys. 2015;92:1035-43.

38. Elicin O, Callaway S, Prior J, et al. [^{18}F]FDG-PET standard uptake value as a metabolic predictor of bone marrow response to radiation: impact on acute and late hematological toxicity in cervical cancer patients treated with chemoradiation therapy. Int J Radiat Oncol Biol Phys. 2014;90:1099-1107.
39. Wyss JC, Carmona R, Karunamuni RA, et al. [(18)F]Fluoro-2-deoxy-2-d-glucose versus 3'-deoxy-3'-[(18)F]fluorothymidine for defining hematopoietically active pelvic bone marrow in gynecologic patients. Radiother Oncol. 2016;118:72-78.
40. Mell LK, Sirak I, Wei L, et al. Bone marrow-sparing intensity modulated radiation therapy with concurrent cisplatin for stage IB-IVA cervical cancer: an international multicenter phase II clinical trial (INTERTECC-2). Int J Radiat Oncol Biol Phys. 2017;97:536-545.
41. Franco P, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, et al. Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients. Br J Radiol 2016;89(1060): 20150832.
42. Simon R (1989) Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989;10:1-10.
43. Kachnic LA, Winter K, Myerson RJ, et al. RTOG 0529: a phase 2 evaluation of dose-painted intensity modulated radiation therapy in combination with 5-fluorouracil and mytomycin C for the reduction of acute morbidity in carcinoma of the anal canal. Int J Radiat Oncol Biol Phys 2013; 86: 27-33.

List of results

Published manuscripts

1. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Hematologic toxicity in anal cancer patients during combined chemo-radiation: a radiation oncologist perspective.**

*Expert Rev Anticancer Ther 2017;17:335-345*1.

2. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M,Racca P, Ricardi U.
Early-stage node-negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy.
Anticancer Res 2016;36;1943-1948.

3. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M, Racca P, Ricardi U. **Locally Advanced (T3-T4 or N^+) Anal Cancer Treated with Simultaneous Integrated Boost Radiotherapy and Concurrent Chemotherapy.**

Anticancer Res 2016;36;2027-2032.

4. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, Rondi N, Morino M, Racca P, Ricardi U. **Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients.**

Br J Radiol 2016;89(1060):20150832.

5. Arcadipane F, **Franco P**, Ceccarelli M, Furfaro G, Rondi N, Trino E, Martini S, Iorio GC, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Image-guided IMRT with simultaneous integrated boost as per RTOG 0529 for the treatment of anal cancer.**

Asia Pac J Clin Oncol 2017 (in press).

6. **Franco P**, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, Di Muzio J, Morino M, Racca P, Ricardi U. **Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer.**

Clin Transl Oncol 2017;19:67-75.

7. **Franco P**, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, Cassoni P, Arena V, Bustreo S, Faletti R, Rondi N, Morino M, Ricardi U. **Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemo-radiation in anal cancer patients.**

Med Oncol 2016;33:72.

8. **Franco P**, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, Morino M, Racca P, Ricardi U. **Lumbar-sacral bone marrow dose modeling for acute hematologic toxicity in anal cancer patients treated with concurrent chemo-radiation.**

Med Oncol 2016;33:137.

9. **Franco P**, Fiandra C, Arcadipane F, Trino E, Giglioli FR, Ragona R, Ricardi U**. Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation**.

BMC Cancer 2017;17:110.

Selected abstracts

- **XXVII Italian Association of Radiation Oncology (AIRO) National Congress** – Rimini, Italy – November 11th-13th 2017- Oral communication - *Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation.*
- **European Society of Radiotherapy and Oncology (ESTRO) 36° Congress** – Vienna, Austria – May4th – May 9th 2017 – Poster viewing communication- *Dose to pelvic bone marrow defined with FDG-PET predicts for hematologic nadirs in anal cancer patients treated with concurrent chemo-radiation.*
- **European Society of Radiotherapy and Oncology (ESTRO) 35° Congress** – Turin, Italy – April 29th – May 3rd 2016 – Proffered papers – Oral communication- *Lumbar-sacral bone marrow modelling of acute hematologic toxicity in chemo-radiation for anal cancer.*

Awards

- **Early Career Investigator Award 2017** by the British Institute of Radiology for the paper: Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients, Br J Radiol 2016;89:20150832 (best paper 2016 within BJR).

Funding

- Research Grant funded by the University of Turin (ex 60%) 2017 - **A prospective phase II trial of bone-marrow sparing dose-painted IMRT to reduce hematologic toxicity in anal cancer patients undergoing concurrent chemo-radiation.**

Pierfrancesco Franco

Curriculum vitae

Personal Information

Name: Pierfrancesco Franco
Date of Birth: March 4th, 1977
Birthplace: Turin, Italy

Working address

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Education and Training

1996, admission at the Turin University School of Medicine
2000, January – start training in Medical Oncology at Valdese Evangelic Hospital – Turin
2002, January – start training in Radiation Oncology at Turin University
2003, March – MD degree
2003, April-September - Medical license training
2003, December – Medical license examination
2004, January – admission to the Radiation Oncology Residency Program of Turin University
2007, December - Radiation Oncology Specialization Degree
2014, January - admission to the PhD Program in Biomedical Sciences and Human Oncology – University of Turin

Working experiences

A 10-year experience as a full-staff consultant Radiation Oncologist in different Hospitals within the Italian National Healthcare System

- 2008 April-2009 April Fellowship – Radiation Oncology Department – University of Turin ('Dose escalation in lung cancer')

- 2009 May – 2009 December - Full Staff Position – Radiotherapy Department - Ospedale Sant' Andrea, Vercelli and Radiotherapy Department- University Hospital Maggiore della Carità, Novara, Italy

  Focused experience on prostate and breast cancer and translational research projects (single nucleotide polymorphism – SNP – for the prediction of late toxicity in breast cancer patients after radiotherapy)

- 2010 January –2014 April - Full Staff Position – Radiation Oncology Department – Tomotherapy Unit- Ospedale Regionale 'U. Parini'- AUSL Valle d'Aosta, Aosta, Italy

  Protracted experience in setting up a new facility equipped with a single-vault Tomotherapy platform. Developing new techniques to treat breast cancer patients with static angle tomotherapy (TomoDirect). Managing and organizational tasks also.

- 2014 April – November 2017 – Tenure-track Assistant Professor of Radiation Oncology – Department of Oncology – Radiation Oncology, University of Torino, Turin, Italy

  Academic position within the University Hospital with clinical, research and teaching duties. Implementation of head and neck, gastrointestinal and breast programs at the University of Turin.

- April 2017 – Italian National Scientific License for Associate Professorship (Scientific Field – MED 36 – Radiology, Radiation Oncology, Nuclear Medicine)

- **2017 December – present – Associate Professor of Radiation Oncology – Department of Oncology – Radiation Oncology, University of Torino, Turin, Italy**

Teaching experiences

- Teaching courses at the University of Torino School of Medicine for the Master Degree in Techniques of Radiology, Medical Imaging and Radiotherapy (since 2014)
  - Radiobiology (Turin branch)
  - Radiobiology (Cuneo branch)
  - Radiotherapy (Cuneo branch)
- Teaching courses at the University of Torino School of Medicine for the Residency Program in Radiation Oncology (since 2014)
  - Radiotherapy in gastrointestinal malignancies

  in Reconstructive and Plastic Surgery (since 2014)
  - Radiotherapy in breast cancer patients

- Teaching courses at the University of Torino School of Medicine for the Master in Deglutology (for speech language pathologist) (since 2014)
    - Dysphagia in radiotherapy for head and neck cancer
- Teaching courses at the University of Torino School of Medicine for the Master in Senology (for nurses) (since 2014)
    - Radiotherapy in breast cancer patients

Main interests

Head and Neck Oncology, Breast Cancer, Gastro-intestinal Cancer, Prostate Cancer, IMRT, IGRT, Functional Imaging.

Memberships

- member, European Society for Therapeutic Radiology and Oncology (ESTRO)
- member, European Organization for Research and Treatment of Cancer (EORTC)
- member, Associazione Italiana Radioterapia Oncologica (AIRO)

Professional Roles

- Since 2017 – Member of the Core Faculty and Teacher of the European School of Oncology (ESO)
- Since 2016 – Young Co-Chair of the Gastrointestinal Cancer Working Party for the Radiation Oncology Group (ROG) at the European Organization for Research and Treatment of Cancer (EORTC)
-Since 2016 – Member of the Young Committee of the European Society of Radiation Oncology (ESTRO)
-Since 2015- Co-Editor of the Young Corner within European Society of Radiation Oncology (ESTRO) Newsletter
-2012-2016: Italian Association of Radiation Oncology (AIRO) Giovani (AIRO-Young Members Working Group) - Member of Directive Council
-2013-2015: Italian Association of Radiation Oncology (AIRO) Head and Neck Working Group - Member of Directive Council
-2011-2013: Italian Association of Radiation Oncology (AIRO) Piemonte-Liguria-Valle d'Aosta- Member of Directive Council

Prizes and selections

- Early Career Investigator Award 2016 by the British Institute of Radiology for the paper: Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients, Br J Radiol 2016;89:20150832
- Selected for the ESTRO 2nd Agorá Meeting – 2016 - Mon San Benet, Spain
- Selected for an ESTRO Technology Transfer Grant (TTG) – 2014 - Optimization of breast radiation minimizing normal tissue dose - Department of Radiation Oncology – University of Pennsylvania School of Medicine- Abramson Cancer Center – Philadelphia – Pennsylvania – USA
- 'Vasario' prize for the best communication at XVI Regional Congress – Associazione Italiana Radioterapia Oncologica (AIRO) Piemonte Valle' d'Aosta - 2007

Grants and funding

- Research Grant funded by the University of Turin (ex 60%) 2017 - **A prospective phase II trial of bone-marrow sparing dose-painted IMRT to reduce hematologic toxicity in anal cancer patients undergoing concurrent chemo-radiation –** 25.000 euros

Invited National and International talks

- **European EUS Congress - 7° Congress** – EGEUS 2017- Turin, November 27-28th 2017 – **Satellite Symposium** – *EchoTip Ultra fiducial needle: mark the spot for radiation therapy.*

- **European EUS Congress - 7° Congress** – EGEUS 2017- Turin, November 27-28th 2017 – **Round Table on pancreatic cancer** – *Is there a role for fiducial markers during radiotherapy for pancreatic cancer?*

- **Piedmont Regional Oncological Network – Pre-clinical research and multidisciplinary approach in pancreatic cancer –** Turin, Italy – November 24th 2017 – *The role of radiation therapy in locally advanced pancreatic cancer.*

- **XXVII Italian Association of Radiation Oncology (AIRO) National Congress** – Rimini, Italy – November 11th-13th 2017- Oral communication - *Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation.*

- **Italian Society of Surgical Oncology (SICO)- 40° Congress** – Turin, June 30th 2017 – Oral communication - *Comparing simultaneous vs sequential boost strategies during concurrent chemo-radiation for anal cancer: results of a retrospective observational study.*

- **Piedmont Regional Oncological Network - News and controversies from ASCO 2017 –** Turin, Italy – June 23rd 2017 – *Head and neck.*

- **Breast Unit – AOU Citta' della Salute e della Scienza – Congress on 'Metastastic breast cancer: a multidisciplinary approach' –** Turin, Italy – May 25th 2017 – *Adjuvant radiotherapy after surgery.*

- **Plastic Surgery and Breast Unit, Turin – Continuos Medical Education** – Turin, May 17th 2017 - *Clinical data of radiotherapy in the treatment of breast cancer.*

- **European Society of Radiotherapy and Oncology (ESTRO) 36° Congress** – Vienna, Austria – May4th – May 9th 2017 – Poster viewing communication- *Dose to pelvic bone marrow defined with FDG-PET predicts for hematologic nadirs in anal cancer patients treated with concurrent chemo-radiation.*

- **What's new in gastric cancer? New horizons in the treatment of gastric and gastro-oesophageal cancer tumors** – Turin, Italy, January 25th 2017 – *New trends in radiation oncology.*

- **Piedmont Regional Oncological Network** – **Congress on the Role of nutrition in pancreatic tumors** – Turin, Italy – November 8th 2016- *Nutritional issues during radiotherapy for pancreatic cancer.*

- **Centre Hopitalier Universitaire Vadois (CHUV) – Journal Club –** Lausanne, Switzerland – October 25th 2016 – *Hematologic toxicity in anal cancer patients undergoing concurrent chemo-radiation: a radiation oncologist's perspective.*

- **European Association of Oral Medicine (EAOM) – 13th Biannual Congress** – Turin, Italy – September 15th-17th 2016- Workshop: Supportive Care in Oral Medicine – *Oral mucositis in head and neck cancer patients undergoing combined chemo-radiation.*

- **Piedmont Regional Oncological Network - News and controversies from ASCO 2016 –** Turin, Italy – June 17th 2016 – *Head and neck.*

- **Breast Unit – AOU Citta' della Salute e della Scienza – Congress on 'Breast Cancer Treatment: New challenges' –** Turin, Italy – May 26th 2016 – *The role of radiotherapy after neoadjuvant chemotherapy in breast cancer.*

- **European Society of Radiotherapy and Oncology (ESTRO) 35° Congress** – Turin, Italy – April 29th – May 3rd 2016 – Proffered papers – Oral communication- *Lumbar-sacral bone marrow modelling of acute hematologic toxicity in chemo-radiation for anal cancer.*

- **Think Tank Meeting on Research Challenges in Breast Cancer** – Assisi, Italy – February 5th-7th 2016 – *Radiation therapy of regional lymphnodes after primary systemic therapy, with or without ALND.*

- **Italian Society for Radiation Research (SIRR) –Workshop on Dose, Dose-rate and Biological Effects –** Rome, Italy – November 27th 2015 – *Radiobiology of hematologic toxicity in radiotherapy for pelvic malignancies.*

- **XXV Italian Association of Radiation Oncology (AIRO) National Congress** – Rimini, Italy – November 7th-10th 2015- *Late effects in oral cavity tumors after treatment.*

- **Academy of Medicine of Turin – Second Congress on Rare Tumors of the Gastrointestinal Tract –** Turin, Italy – October 17th 2015 – *Anal cancer tumors: an example of combination therapy.*

- **Teaching Course on Combined Modality Therapy in Locally Advanced Head and Neck Cancers –** Cuneo, Italy – October 12th-16th 2015- *Dose density and dose intensity in head and neck cancer chemo-radiation.*

- **VIII Italian Association of Radiation Oncology (AIRO) Young Members National Congress –** Montecatini Terme, Italy – June 13th 2015 – *Multimodality treatment as larynx-preservation strategy in larynx/hypopharynx cancers.*

- **Congress on Dysphagia and pain in head and neck cancer patients undergoing chemo-radiation** – Alessandria, Italy – March 23rd 2015 – *DARS- and XRS- sparing radiotherapy: advantages and pitfalls.*

- **9th International Congress on Diagnosis and Treatment of Breast Cancer** – Warsaw (Falenty), Poland – April 16th 2015 – *Breast Cancer Treatment with the Tomotherapy system.*

- **Piedmont Regional Oncological Network** – **Congress on the Role of nutrition in gastrointestinal tumors** – Turin, Italy – March 20th 2015- *Nutritional issues during radiotherapy for gastrointestinal malignancies.*

- **Accuray Taiwan Users Meeting** – Kaohsiung – Taiwan – November 30th 2014 - *Clinical experience of breast cancer treatments with the Tomotherapy System.*

- **Symposium on enhancing Tomotherapy** – Bangkok - Thailand – November 27th 2014 - *Clinical experience of breast cancer treatments with the Tomotherapy System.*

- **Italian Association of Medical Physics (AIFM) – Workshop on Breast Cancer Radiotherapy: physical and clinical perspective on diagnostic, dosimetry and radiotherapy –** Turin, Italy – June 13rd 2014 – *Clinical and technical issues in radiotherapy for breast cancer.*

- **Italian Association of Radiation Oncology (AIRO), Italian Association of Medical Oncology (AIOM), Italian Association of Surgical Oncology (SIURO) Workshop – Young Specialist Round-table** - Bologna, Italy - May 9th 2014 **–** *Metastatic prostate cancer: the role of radiotherapy in bone metastases.*

- **2014 Accuray-AERO Users' Meeting –** Minneapolis – Minnesota, USA **–** May 6th 2014 – *TomoDirect IMRT for hypofractionated whole breast irradiation with simultaneous integrated boost.*

- **18th Annual Swiss Society of Radiation Oncology (SASRO) Meeting** – Lugano – Switzerland – March 27th 2014 – *Can Tomotherapy treat all?*

- **Italian Association of Radiation Oncology (AIRO), Italian Association of Medical Oncology (AIOM), Italian Association of Surgical Oncology (SIURO) Workshop – Young Specialist Round-table** - Turin, Italy - November 12nd 2013 **–** *Low risk prostate cancer: the role of radiotherapy.*

- **Teaching Course on the use of Tomotherapy: general aspects on clinic and dosimetry –** Candiolo, Italy – January 12th 2013- *Tomotherapy and breast cancer.*

- **Advancing in Radiation Oncology: a Collaborative Forum** – Dallas – Texas, USA – November 28th-30th 2012 – *The use of static angle tomotherapy (TomoDirect) in the treatment of breast cancer.*

- **Italian Association of Radiobiology (AIRB) 28th Congress - Italian Association of Radiation Oncology (AIRO) Young Members IV National Congress** – Milan, Italy – June 15th-16th 2012- *Hypofractionation in lung cancer.*

- **Congress on Tomotherapy in Italy: comparative experiences –** Bard (AO), Italy – November 20th 2010 – *Experience with TomoDirect in Aosta.*

Publications

103. Iorio GC, **Franco P**, Gallio E, Martini S, Arcadipane F, Bartoncini S, Rondi N, Giglioli FR, Ala A, Airoldi M, Donadio M, De Sanctis C, Castellano I, Ricardi U. **Volumetric modulated arc therapy (VMAT) to deliver nodal irradiation in breast cancer patients.**
Med Oncol 2018 (in press).

102. **Franco P**, Fiandra C, Arcadipane F, Trino E, Giglioli FR, Ragona R, Ricardi U**. Incorporating ^{18}FDG-PET-defined pelvic active bone marrow in the automatic treatment planning process of anal cancer patients undergoing chemo-radiation**.
BMC Cancer 2017;17:110.

101. Cozzarini C, Rancati T, Palorini F, Avuzzi B, Garibaldi E, Balestrini D, Cante D, Munoz F, **Franco P**, Girelli G, Sini C, Vavassori V, Valdagni R, Fiorino C. **Patient-reported urinary incontinence after radiotherapy for prostate cancer: quantifying the dose-effect**.
Radiother Oncol 2017;125:101-106.

100. Arcadipane F, **Franco P**, Ceccarelli M, Furfaro G, Rondi N, Trino E, Martini S, Iorio GC, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Image-guided IMRT with simultaneous integrated boost as per RTOG 0529 for the treatment of anal cancer.**
Asia Pac J Clin Oncol 2017 (in press).

99. **Franco P**, Arcadipane F, Strignano P, Romagnoli R, Ricardi U. **Radiotherapy treatment strategies for squamous cell carcinoma of the cervical oesophagus: moving toward better outcomes.**
Annals Transl Med 2017;5:426.

98. Cante D, Petrucci E, Sciacero P, Piva C, Ferrario S, Bagnera S, Patania S, Mondini G, Pasquino M, Casanova Borca V, Vellani G, La Porta MR, **Franco P**. **Ten year results of accelerated hypofractionated adjuvant whole breast radiation with concomitant boost to the lumpectomy cavity after conserving surgery for early breast cancer.**
Med Oncol 2017;34:152.

97. Sciacero P, Cante D, Piva C, Casanova Borca V, Petrucci E, Gastaldi L, La Porta MR, **Franco P**. **The role of radiation therapy in vulvar cancer: review of the current literature**.
Tumori 2017 2017;103:422-429.

96. **Franco P**, Martini S, Di Muzio J, Cavallin C, Arcadipane F, Rampino M, Ostellino O, Pecorari g, Garzino Demo P, Fasolis M, Airoldi M, Ricardi U. **Prospective assessment of oral mucositis and its impact on quality of life and patient reported outcomes during radiotherapy for head and neck cancer.**
Med Oncol 2017;34:81.

95. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Racca P, Morino M, Ricardi U. **Hematologic toxicity in anal cancer patients during combined chemo-radiation: a radiation oncologist perspective.**
Expert Rev Anticancer Ther 2017;17:335-345.

94. Numico G, Fusco V, **Franco P**, Roila F**. Proton Pump Inhibitors in cancer patients: how useful they are? A review of the most common indications for their use.**
Crit Rev Oncol/Hematol 2017;111:144-151.

93. **Franco P**, Arcadipane F, Strignano P, Spadi R, Trino E, Martini S, Iorio GC, Satolli MA, Airoldi M, Romagnoli R, Camandona M, Ricardi U. **Preoperative treatments for adenocarcinoma of the lower oesophagus and gastro-esophageal junction: a review of the current evidence from randomized trials.** *Med Oncol 2017;34:40.*

92. Ghiggia A, Castelli L, Riva G, Tesio V, Provenzano E, Ravera M, Garzaro M, Pecorari G, **Franco P**, Potenza I, Rampino M, Torta R. **Psychological distress and coping in nasopharyngeal cancer: an exploratory study in Western Europe.**
Psychol Health Med 2017;22:449-461.

91. **Franco P**, Rampino M, Ostellino O, Schena M, Pecorari G, Garzino Demo P, Fasolis M, Arcadipane F, Martini S, Cavallin C, Airoldi M, Ricardi U. **Management of acute skin toxicity with hypericum perforatum and neem oil during platinum-based concurrent chemo-radiation in head and neck cancer patients**.
Med Oncol 2017;34:30.

90. **Franco P**, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, Di Muzio J, Morino M, Racca P, Ricardi U. **Dosimetric predictors of acute hematologic toxicity during concurrent intensity-modulated radiotherapy and chemotherapy for anal cancer.**
Clin Transl Oncol 2017;19:67-75.

89. **Franco P**, Freedman GM, Ricardi U, Poortmans P. **Simplicity is complexity resolved: the case of postoperative radiation after breast conservation.**
Transl Cancer Res 2016;5(Suppl 7):S1336-S1339.

88. Improta I, Palorini F, Rancati T, Cozzarini C, Avuzzi B, **Franco P**, Degli Esposti C, Del Mastro E, Girelli G, Iotti C, Vavassori V, Valdagni R, Fiorino C. **Bladder spatial-dose descriptors correlate with acute urinary toxicity after radiation therapy for prostate cancer**.
Phys Med 2016;32:1681-1689.

87. **Franco P**, Ragona R, Arcadipane F, Mistrangelo M, Cassoni P, Rondi N, Morino M, Racca P, Ricardi U. **Lumbar-sacral bone marrow dose modeling for acute hematologic toxicity in anal cancer patients treated with concurrent chemo-radiation.**
Med Oncol 2016;33:137.

86. **Franco P**, Fiorentino A, Dionisi F, Fiore M, Chiesa S, Vagge S, Cellini F, Caravatta L, Tombolini M, De Rose F, Meattini I, Mortellaro G, Apicella G, Marino L, Greto D on behalf of AIRO Giovani (Italian Association of Radiation Oncology -Young Members Working Group). **Combined modality therapy for thoracic and head and neck cancers: a review of updated literature based on a consensus meeting.** *Tumori 2016; 102:4 59-71.*

85. Guarneri A, **Franco P**, Romagnoli R, Trino E, Mirabella S, Molinaro L, Rizza G, Filippi AR, Carucci P, Salizzoni M, Ricardi U. **Stereotactic ablative radiation therapy prior to liver transplantation in hepatocellular carcinoma**.
Radiol Med 2016;121:873-881.

84. Cante D, Petrucci E, Piva C, Casanova Borca V, Sciacero P, Bertodatto M, Marta C, **Franco P**, Viale M, La Valle G, La Porta MR, Bertetto O; on behalf of Rete Oncologica Piemonte-Valle d'Aosta. **Delineation of the larynx as organ at risk in radiotherapy: a contouring course within 'Rete Oncologica Piemonte-Valle d'Aosta' network to reduce inter- and intraobserver variability**.
Radiol Med 2016;121: 867-872.

83. Garzino-Demo P, Zavattero E, **Franco P**, Fasolis M, Tanteri G, Mettus A, Tosco P, Chiusa L, Airoldi M, Ostellino O, Schena M, Rampino M, Ricardi U, Evangelista A, Merletti F, Berrone S, Ramieri G. **Parameters and outcomes in 525 patients operated on for oral squamous cell carcinoma.**
J Craniomaxillofac Surg 2016;44:1414-21.

82. Dionisi F, Guarneri A, Dell' Acqua V, Leonardi MC, Niespolo R, Macchia G, Comito T, Amichetti M, **Franco P**, Cilla S, Caravatta L, Alongi F, Mantello G. **Radiotherapy in the multidisciplinary treatment of liver cancer: a survey on behalf of the Italian Association of Radiation Oncology.**
Radiol Med 2016;121:735-743.

81. Guarneri A, **Franco P**, Trino E, Campion D, Faletti R, Mirabella S, Gaia S, Ragona R, Diotallevi M, Saracco G, Salizzoni M, Ricardi U, Carucci P. **Stereotactic ablative radiotherapy in the treatment of hepatocellular carcinoma > 3 cm**.
Med Oncol 2016;33:104.

80. Arcadipane F, **Franco P**, De Colle C, Rondi N, Di Muzio J, Pelle E, Martini S, Ala A, Airoldi M, Donadio M, De Sanctis C, Castellano I, Ragona R, Ricardi U. **Hypofractionation with no boost in early stage breast cancer patients**.
Med Oncol 2016;33:108.

79. Alterio D, **Franco P**, Numico G, Licitra L, Cossu Rocca M, Ferrari A, Pinto C, Russi EG, Ricardi U, Jereczek Fossa B . **Nonsurgical organ preservation strategies for locally advanced laryngeal tumors: what is the Italian attitude? Results of a national survey on behalf of AIRO and AIOM**.
Med Oncol 2016;33:76.

78. **Franco P**, Arcadipane F, Ragona R, Lesca A, Gallio E, Mistrangelo M, Cassoni P, Arena V, Bustreo S, Faletti R, Rondi N, Morino M, Ricardi U. **Dose to specific subregions of pelvic bone marrow defined with FDG-PET as a predictor of hematologic nadirs during concomitant chemo-radiation in anal cancer patients.**
Med Oncol 2016;33:72.

77. Cante D, **Franco P**, Sciacero P, Girelli G, Pasquino M, Casanova Borca V, Tofani S, La Porta MR, Ricardi U.
Hypofractionated whole breast radiotherapy and concomitant boost after breast conservation in elderly patients.
Tumori 2016;102:196-202.

76. De Bari B, **Franco P**, Niyazi M, Peruzzo Cornetto A, Qvortrup C, Navarro Martin A, Cacicedo J, Fernandez G, Louro LV, Lestrade L, Ciammella P, Greto D, Checkrine T, Youssef E, Filippi AR, Ostergaard Poulsen L, Alongi F.
The Pocketable Electronic Devices in radiation Oncology (PEDRO) Project: how the use of tools in medical decision making is changing?
Technol Cancer Res Treat 2016;15:365-376.

75. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M,Racca P, Ricardi U.
Early-stage node-negative (T1-T2N0) anal cancer treated with simultaneous integrated boost radiotherapy and concurrent chemotherapy.
Anticancer Res 2016;36;1943-1948.

74. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Rondi N, Morino M, Racca P, Ricardi U.
Locally Advanced (T3-T4 or N^+) Anal Cancer Treated with Simultaneous Integrated Boost Radiotherapy and Concurrent Chemotherapy.
Anticancer Res 2016;36;2027-2032.

73. **Franco P**, Arcadipane F, Ragona R, Mistrangelo M, Cassoni P, Munoz F, Rondi N, Morino M, Racca P, Ricardi U. **Volumetric modulated arc therapy (VMAT) in the combined modality treatment of anal cancer patients.**
Br J Radiol 2016;89(1060):20150832.

72. Palorini F, Rancati T, Cozzarini C, Improta I, Carillo V, Avuzzi B, Casanova Borca V, Botti A, Degli Esposti C, **Franco P**, Garibaldi E, Girelli G, Iotti C, Maggio A, Palombarini M, Pierelli A, Pignoli E, Vavassori V, Valdagni R, Fiorino C. **Multi-varable models of large International Prostate Symptom Score worsening at the end of therapy in prostate cancer radiotherapy**.
Radiother Oncol 2016;118:92-98.

71. Arcangeli S, Ramella S, De Bari B, **Franco P**, Alongi F, D'Angelillo R. **A cast of shadows on adjuvant radiotherapy for prostate cancer: a critical review based on a methodological perspective.**
Crit Rev Oncol/Hematol 2016;97:322-327.

70. Pietrantonio F, Aprile G, Rimassa L, **Franco P**, Lonardi S, Cremolini C, Biondani P, Sbicego EL, Pasqualetti F, Tomasello G, Niger M, Casagrande M, Ghidini M, Muni R, Montrone S, Bergamo F, Berenato R, Fontanella C, Bozzarelli S, Moretto R, Battaglin F, Di Bartolomeo M, de Braud F, Miceli R.
A new nomogram for estimating survival in patients with brain metastases secondary to colorectal cancer.
Radiother Oncol 2015;117:315-321.

69. Cozzarini C, Rancati T, Carillo V, Civardi F, Garibaldi E, **Franco P**, Avuzzi B, Esposti CD, Girelli G, Iotti C, Palorini F, Vavassori V, Valdagni R, Fiorino C.
Multiple-variable models predicting specific patient-reported acute urinary symptoms after radiotherapy for prostate cancer: results of a cohort study.
Radiother Oncol 2015;116:185-191.

68. **Franco P**, Potenza I, Schena M, Riva G, Pecorari G, Garzino Demo P, Fasolis M, Moretto F, Di Muzio J, Melano M, Airoldi M, Ragona R, Rampino M, Ricardi U.
Induction chemotherapy and sequential concomitant chemo-radiation in locally advanced head and neck cancers: how induction phase intensity and treatment breaks may impact on clinical outcomes.
Anticancer Res 2015;35:6247-6254.

67. Arcadipane F, Fiandra C, **Franco P**, Munoz F, Irgolini P, Trino E, Levis M, Guarneri A, Ricardi U.
Three-dimensional ultrasound-based target volume delineation and consequent dose calculation in prostate cancer patients with bilateral hip replacement: a report of 4 cases.
Tumori 2015;101:e133-e-137.

66. Potenza I, **Franco P**, Moretto F, Badellino S, Balcet V, Rossi G, Landolfo V, Riva G, Pecorari G, Ragona R, Rampino M, Ricardi U.
Exclusive radiotherapy for early-glottic cancer: a single-institution retrospective analysis with a focus on voice quality.
Anticancer Res 2015;35:4155-4160.

65. Girelli G, **Franco P**, Sciacero P, Cante D, Casanova Borca V, Pasquino M, Annoscia S, Tofani S, La Porta MR, Ricardi U.
Image-guided intensity-modulated radiotherapy for prostate cancer employing hypofractionation and simultaneous integrated boost: results of a consecutive case series with a focus on erectile function.
Anticancer Res 2015;35:4177-4182.
64. **Franco P**, Mistrangelo M, Arcadipane F, Munoz F, Sciacero P, Spadi R, Migliaccio F, Angelini V, Bombaci S, Rondi N, Numico G, Ragona R, Cassoni P, Morino M, Racca P, Ricardi U.
Intensity-modulated radiation therapy with simultaneous integrated boost with concurrent chemotherapy for the treatment of anal cancer patients: 4-year results of a consecutive case series.
Cancer Invest 2015;33:259-266.

63. Rovea P, Fozza A, **Franco P**, De Colle C, Cannizzaro A, Di Dio A, De Monte F, Rosmini C, Filippi AR, Ragona R. Ricardi U.
Once-weekly hypofractionated whole breast radiotherapy after breast conserving surgery in older patients: a potential alternative treatment schedule to daily 3-week hypofractionation.

Clin Breast Cancer 2015;15:270-276.

62. Numico G, Cristofano A, Mozzicafreddo A, Cursio OE, **Franco P**, Couthod G,Trogu A, Malossi A, Cucchi M, Sirotova' Z, Alvaro MR, Stella A, Grasso F, Spineazze' S, Silvestris N..
Hospital admission of cancer patients: avoidable practice or necessary care?
PLoS One2015;10:e0120827.

61.**Franco P**, Cante D, Sciacero P, Girelli G, La Porta MR, Ricardi U.
Tumor bed boost integration during whole breast radiotherapy: a review of the current evidence.
Breast Care 2015:10:44-49.

60. **Franco P**, Migliaccio F, Torielli P, Sciacero P, Girelli G, Cante D, Arrichiello C, Casanova Borca V, Peruzzo Cornetto A, Numico G, La Porta MR, Tofani S, Ricardi U.
Bilateral breast radiation delivered with static angle tomotherapy (TomoDirect).
Tumori 2015;101:e4-8.

59. Ricardi U, **Franco P**, Munoz F, Levis M, Fiandra C, Guarneri A, Moretto F, Bartoncini S, Arcadipane F, Badellino S, Piva C, Trino E, Ruggieri A, Filippi AR, Ragona R.
Three-dimensional ultrasound-based image-guided hypofractionated radiotherapy for intermediate-risk prostate cancer: results of a consecutive case series.
Cancer Invest 2015;33:23-28.

58. Filippi AR, Badellino S, Ceccarelli M, Guarneri A, **Franco P**, Monagheddu C, Racca P, Ricardi U.
Stereotactic Ablative Radiotherapy as first local therapy in patients with lung oligo-metastases from colorectal cancer.
Int J Radiat Oncol Biol Phys 2015;91:524-529.

57. D'Angelillo R, **Franco P**, De Bari B, Fiorentino A, Arcangeli S, Alongi F.
Combination of androgen deprivation therapy and radiotherapy for localized prostate cancer in the contemporary era.
Crit Rev Oncol/Hematol 2015;93:136-148.

56. Merlotti A, Alterio D, Vigna-Taglianti R, Muraglia A, Lastrucci L, Manzo R, Gambaro G, Caspiani O, Micciche' F, Deodato F, Pergolizzi S, **Franco P**, Corvo' R, Russi EG, Sanguineti G.
Technical guidelines for head and neck cancer IMRT on behalf of the Italian Association of Radiation Oncology – head and neck working group.
Radiat Oncol 2014;9:1165.

55. **Franco P**, Potenza I, Moretto F, Segantin M, Grosso M, Lombardo A, Taricco D, Vallario P, Filippi AR, Rampino M, Ricardi U.
Hypericum perforatum and neem oil for the management of acute skin toxicity in head and neck cancer patients undergoing radiation or chemo-radiation: a single-arm prospective observational study.
Radiat Oncol 2014;9:1164.

54. **Franco P**, De Bari B, Ciammella P, Fiorentino A, Chiesa S, Amelio D, Pinzi V, Bonomo P, Vagge S, Fiore M, Comito T, Cecconi A, Mortellaro G, Bruni A, trovo' M, Filippi AR, Greto D, Alongi F.
The role of stereotactic ablative radiotherapy in oncological and non-oncological clinical settings: highlights from the 7th Meeting of AIRO – Young Members Working Group (AIRO Giovani)
Tumori 2014;100:214e-229e.

53. De Bari B, **Franco P**, Ciammella P, Peruzzo Cornetto A, Greto D, Fundoni C, Filippi AR, Alongi F, on behalf of AIRO Giovani (Italian Association of Radiation Oncology-Young Members Working Group).

The PEDRO (Pocketable Electronic Devices in Radiation Oncology) project: how clinical practice is changing among young Radiation Oncologists?
Tumori 2014;100:236e-242e.

52. Alterio D, Ciardo D, Preda L, Argenone A, Caspiani O, Micera R, Ruo Redda MG, Russi EG, Bianchi E, Orlandi E, Bacigalupo E, Busetto M, Cante D, Deantonio L, De Sanctis V, **Franco P**, Lastrucci L, Marucci L, Merlotti A, Molteni M, Pajar F, Rampino M, Santoro L, Ferrari A, Bazzani F, Caputo M, Laudati A, Borzillo V, Favilene S, Simoni N, Vigo F, Iannacone E, Reali A, Bonanni A, Leone M, Gianello L, Vigna Taglianti R, Orecchia R.
Contouring of the pharyngeal superior constrictor muscle (PCSM). A cooperative study of the Italian Association of Radiation Oncology (AIRO) Head and Neck Group.
Radiother Oncol 2014;112:337-342.

51. **Franco P**, Migliaccio F, Angelini V, Cante D, Sciacero P, Peruzzo Cornetto A, Casanova Borca V, Zeverino M, Torielli P, Arrichiello C, Girelli G, La Porta MR, Tofani S, Numico G, Ricardi U.
Palliative radiotherapy for painful bone metastases from solid tumors delivered with static ports of tomotherapy (TomoDirect): feasibility and clinical results.
Cancer Invest 2014;32:458-463.

50. Numico G, Trogu A, Cristofano A, Mozicafreddo A, Courthod G, **Franco P**, Silvestris N.
Active treatment given in the last weeks of life: poor quality cancer care or justifiable behaviour?
Support Care Cancer 2014;22:2813-2819.

49. Carillo V, Cozzarini C, Rancati T, Avuzzi B, Botti A, Casanova Borca V, Cattari G, Civardi F, Degli Esposti C, **Franco P**, Girelli G, Maggio A, Muraglia A, Palombarini M, Pirelli A, Pignoli E, Vavassori V, Zeverino M, Valdagni R, Fiorino C.
Relationships between bladder dose-volume/surface histograms and acute urinary toxicity after radiotherapy for prostate cancer.
Radiother Oncol 2014;111:100-105.

48. Sciacero P, Girelli GF, Cante D, **Franco P**, Casanova Borca V, Grosso P, Marra A, Bombaci S, Tofani S, La Porta MR, Ricardi U.
Cerebellar glioblastoma multiforme in an adult woman.
Tumori 2014;100:e74-e78.

47. Ricardi U, Filippi AR, Piva C, **Franco P**.
The evolving role of radiotherapy in early stage Hodgkin's lymphoma.
Mediterr J Hematol Infect Dis 2014:6.

46. **Franco P**, Zeverino M, Migliaccio F, Torielli P, Angelini V, Sciacero P, Girelli G, Cante D, Arrichiello C, Casanova Borca V, Numico G, La Porta MR, Tofani S, Ricardi U.
Minimizing a tricky situation in breast irradiation with helical tomotherapy.
Tumori 2014;100:e35-e40.

45. Courthod G, **Franco P**, Palermo L, Pisconti S, Numico G.
The role of microRNA in head and neck cancer: current knowledge and perspectives.
Molecules 2014;19:5704-16.
44. De Bari B, Fiorentino A, Arcangeli S, **Franco P**, D'Angelillo RM, Alongi F.
From radiobiology to technology: what is changing in radiotherapy for prostate cancer.
Expert Rev Anticancer Ther 2014;14:553-564.

43. **Franco P**, Zeverino M, Migliaccio F, Cante D, Sciacero P, Casanova Borca V, Torielli P, Arrichiello C, Girelli G, La Porta MR, Tofani S, Numico G, Ricardi U.
Intensity-modulated and hypofractionated simultaneous integrated boost adjuvant breast radiation employing statics ports of tomotherapy (TomoDirect): a prospective phase II trial.
J Cancer Res Clin Oncol 2014;140:167-77.

42. Cante D, **Franco P**, Sciacero P, Girelli G, Marra AM, Pasquino M, Russo G, Casanova Borca V, Mondini G, Paino O, Numico G, Tofani S, La Porta MR, Ricardi U.
Hypofractionation and concomitant boost to deliver adjuvant whole-breast radiation in ductal carcinoma in situ (DCIS): a subgroup analysis of a prospective case series.
Med Oncol 2014;31:838.

41. De Bari B, Fiorentino A, Greto D, Ciammella P, Arcangeli S, Avuzzi B, D'Angelillo RM, Desideri I, Kirienko M, Marchiori D, Massari F, Fundoni C, **Franco P**, Filippi AR, Alongi F.
Prostate cancer as a paradigm of multidisciplinary approach? Highlights from the VI AIRO Giovani meeting.
Tumori 2013;99:637-49.

40. Cante D, La Porta MR, **Franco P**, Sciacero P, Girelli G, Marra A, Numico G, Denaro N, Russi EG, Ricardi U
The management of "in-field" skin toxicity in head and neck cancer patients treated with combined Cetuximab and radiotherapy.
Oncology 2013;85:257-61.

39. Ricardi U, Filippi AR, **Franco P**.
New concepts and insights into the role of radiation therapy in extra-cranial metastatic disease.
Expert Rev Anticancer Ther 2013;13:1145-55.

38. **Franco P**, Zeverino M, Migliaccio F, Sciacero P, Cante D, Casanova Borca V, Torielli P, Arrichiello C, Girelli G, Numico G, La Porta MR, Tofani S, Ricardi U.
Intensity-modulated adjuvant whole breast radiation delivered with static angle tomotherapy (TomoDirect): a prospective case series.
J Cancer Res Clin Oncol 2013;139:1927-36.

37. **Franco P**, Ciammella P, Peruzzo Cornetto A, De Bari B, Buglione M, Livi L, Alongi F, Filippi AR.
The STYRO 2011 Project: a Survey on perceived quality of training among young Italian radiation oncologists.
Med Oncol 2013;30:729.

36. Ciammella P, De Bari B, Fiorentino A, **Franco P**, Alongi F, Livi L, Filippi AR.
The 'BUONGIORNO project': Burnout Syndrome among young italian radiation oncologists.
Cancer Invest 2013;31:522-8.

35. Russi EG, Sanguineti G, Chiesa F, Merlotti A, Crosetti E, **Franco P**, Ansarin M, Vigna Taglianti R, Alterio D, Pergolizzi S, Reali A, Magrini SM, Ricardi U, Corvo' R.
Is there a role for postoperative radiotherapy after open-neck conservative laryngectomy with unfavourable prognostic factors at the pathological specimen? A Survey of the Head and Neck Study Group of the Italian Association of Radiation Oncology (AIRO).
Acta Otorhinolaryngol Ital 2013;33:311-319.

34. Filippi AR, **Franco P**, Ricardi U.
Surgery or stereotactic ablative radiotherapy in early-stage non-small cell lung cancer: time for a tailored approach?
Rep Pract Oncol Radiother 2013;19:275-279.

33. De Bari B, Alongi F, **Franco P**, Ciammella P, Chekrine T, Livi L, Jereczek-Fossa BA, Filippi AR on behalf of AIRO Young nad AIRO Prostate Working Group.
The "PROCAINA (PROstate CAncer INdication Attitudes) Project" (Part II) – A survey among Italian radiation oncologists on radical radiotherapy in prostate cancer.

Radiol Med 2013;118:1220-1239.

32. Alongi F, De Bari B, **Franco P**, Ciammella P, Chekrine T, Livi L, Jereczek-Fossa BA, Filippi AR on behalf of AIRO Young nad AIRO Prostate Working Group.
The "PROCAINA (PROstate CAncer INdication Attitudes) Project" (Part I) – A survey among Italian radiation oncologists on post-operative radiotherapy in prostate cancer.
Radiol Med 2013;118:660-678.

31. Cante D, **Franco P**, Sciacero P, Girelli G, Casanova Borca V, Pasquino M, Bombaci S, Migliaccio F, Marra A, Numico G, La Porta MR, Ricardi U
Combined chemo-radiation for head and neck region mixofibrosarcoma of the maxillary sinus.
Tumori 2013;99:e80-e83.

30. Ricardi U, Racca P, **Franco P**, Munoz F, Fanchini L, Rondi N, Dongiovanni V, Gabriele P, Cassoni P, Ciuffreda L, Morino M, Filippi AR, Aglietta M, Bertetto O.
Prospective phase II trial of neoadjuvant chemo-radiotherapy with oxaliplatin and capecitabine in locally advanced rectal cancer (XELOXART).
Med Oncol 2013;30:581.

29. Cante D, **Franco P**, Sciacero P, Girelli G, Marra AM, Pasquino M, Russo G, Borca VC, Mondini G, Paino O, Barmasse R, Tofani S, Numico G, La POrta MR, Ricardi U.
Five-years results of a prospective case series of accelerated hypofractionated whole breast radiation with concomitant boost to the surgical bed after conserving surgery for early breast cancer.
Med Oncol 2013;30:518.

28. Cante D, **Franco P**, Sciacero P, Girelli G, Casanova Borca V, Pasquino M, Migliaccio F, Tofani S, Grassi L, Marra A, Ozzello F, La Porta MR, Ricardi U.
Leptomeningeal metastasis from prostate cancer.
Tumori 2013;99:e6-e10.

27**.** Numico G**, Franco P**, Cristofano A, Migliaccio F, Spinazze' S, Cante D, Sciacero P, La Porta MR, Girelli G, Silvestris N, Ricardi U.
Is the combination of Cetuximab with chemo-radiotherapy regimes worthwhile in the treatment of locally advanced head and neck cancer? A review of current evidence.
Crit Rev Oncol/Hematol 2013;85:112-120.

26. **Franco P**, Ricardi U
TomoDirect to delivery static angle tomotherapy treatments.
J Nucl Med Radiother 2012;3:1000e107.

25. Filippi AR, **Franco P**, Ciammella P.
Role of modern radiation therapy in early stage Hodgkin's lymphoma: a young radiation oncologists' perspective.
Rep Pract Oncol Radiother 2012;17:259-261.

24. Filippi AR, Alongi F, Ciammella P, De Bari B, **Franco P**, Livi L.
The 'Young Group' of Italian Radiation Oncology Society (AIRO Giovani): past, present and future.
Rep Pract Oncol Radiother 2012;17:246-250.

23. Casanova Borca V, **Franco P,** Catuzzo P, Migliaccio F, Zenone F, Aimonetto S, Peruzzo A, Pasquino M, Russo G, La Porta MG, Cante D, Sciacero P, Girelli G, Ricardi U, Tofani S
Does TomoDirect 3DCRT represent a suitable option for post-operative whole breast irradiation? A hypothesis-generating pilot study
Radiat Oncol 2012;7:211.

22. Fiandra C, Filippi AR, Catuzzo P, Botticella A, Ciammella P, **Franco P**, Casanova Borca V, Ragona R, Tofani S, Ricardi U.
Different IMRT solutions vs 3D-conformal radiotherapy in early stage Hodgkin's lymphoma: dosimetric comparison and clinical considerations
Radiat Oncol 2012;7:186.

21. Russi EG, Corvo' R, Merlotti A, Alterio D, **Franco P**, Pergolizzi S, De Sanctis V, Ruo REdda MG, Ricardi U, Paiar F, Bonomo P, Merlano MC, Zurlo V, Chiesa F, Sanguineti G, Bernier J.
Swallowing disfunction in head and neck cancer patients treated by radiotherapy: Review and recommendations of the supportive task group of the Italian Association of Radiation Oncology.
Cancer Treat Rev 2012;38:1033-1049.

20. Catuzzo P, Zenone S, Aimonetto S, Peruzzo Cornetto A, Casanova Borca V, Pasquino M, **Franco P**, La Porta MR, Ricardi U, Tofani S.
Patient-specific quality assurance methods for TomoDirect™ whole breast treatment delivery.
Med Phys 2012;39: 4073-4078.

19. **Franco P**, Numico G, Migliaccio F, Catuzzo P, Cante D, Ceroni P, Sciacero P, Carassai P, Canzi P, La Porta MR, Girelli G, Casanova Borca V, Pasquino M, Tofani S, Ozzello F, Ricardi U.
Head and neck region consolidation radiotherapy and prophylactic cranial irradiation with hippocampal avoidance delivered with helical tomotherapy after induction chemotherapy for non-sinonasal neuroendocrine carcinoma of the upper airways.
Radiat Oncol 2012;7:21.

18. Terrazzino S, La Mattina P, Gambaro G, Masini L, **Franco P**, Canonico PL, Gennazzani AA, Krengli M.
Common variants of GSTP1, GSTA1 and TGFβ1 are associated with the risk of radiation-induced fibrosis in breast cancer patients.
Int J Radiat Oncol Biol Phys 2012;83:504-511.

17. Munoz F, Fiandra C, **Franco P**, Guarneri A, Ciammella P, De Stefanis P, Rondi , Moretto F, Badellini S, Iftode C, Ragona R, Ricardi U.
Tracking target position variability using intraprostatic fiducial markers and electronic portal imaging in prostate cancer radiotherapy.
Rad Med 2012;117:1057-1070.

16. Cante D, La Porta MR, Casanova-Borca V, Sciacero P, Girelli G, Pasquino M, **Franco P**, Ozzello F.
Accelerated hypofractionated adjuvant whole breast radiotherapy with concomitant photon boost after conserving surgery for early stage breast cancer: a prospective evaluation on 463 patients.
Breast J 2011;17:586-93.

15. **Franco P**, Catuzzo P, Cante D, La Porta MR, Sciacero P, Girelli G, Casanova Borca V, Pasquino M, Numico G, Tofani S, Meloni T, Ricardi U, Ozzello F.
TomoDirect: an efficient means to deliver radiation at static angles with Tomotherapy.
Tumori 2011; 97:488-92.

14. **Franco P**, Filippi AR, Ciammella P, Botticella A, De Crescenzo A, Tarella C, Namysl-Kaletka A, Ricardi U.
Primary duodenal follicular lymphoma: 6-years complete remission after combined radio-immunotherapy.
Acta Gastroenterol Belgica 2011; 74:337-42.

13. Rampino M, Ricardi U, Munoz FH, Reali A, Barone C, Musu AR, Balcet V, **Franco P**, Grillo R, Bustreo S, Pecorari G, Cavalot A, Garzino Demo P, Ciuffreda L, Ragona R, Schena M.
Concomitant adjuvant chemo-radiotherapy with weekly low dose cisplatin for high risk squamous cell carcinoma of the head and neck: a phase II prospective trial.

Clin Oncol 2011; 23:134-40.

12. **Franco P**, Filippi AR, Ciammella P, Botticella A, Namysl-Kaletka A, Ricardi U.
Polyostotic sclerosing histiocytosis (Erdheim-Chester disease) treated with combined vertebroplasty and radiation therapy.
Tumori 2010; 96:633-6.

11. **Franco P**, Beldi' D, Krengli M.
Dose per fraction and dose rate effect.
Tumori 2010;96:512-3.

10. Krengli M, **Franco P**, Terrone C, Volpe A, Marchioro G.
In Reply to Dr. Thoms et al.
Int J Radiat Oncol Biol Phys 2010;76:1277.

9. Ricardi U, Filippi AR, Guarneri A, Figlioli FR, Ciammella P, **Franco P**, Mantovani C, Borasio P, Scagliotti GV, Ragona R.
Stereotactic body radiation therapy for early-stage non-small cell lung cancer: Results of a prospective trial.
Lung Cancer 2010;68:72-7.

8. Ricardi U, Filippi AR; Biasin E, Ciammella P, Botticella A, **Franco P**, Corrias A, Vassallo E, Fagioli F.
Late toxicity in children undergoing hematopoietic stem cell transplantation with TBI-containing conditioning regimens fro hematological malignancies.
Strahlenther Onkol 2009;185 Suppl 2:17-20.

7. **Franco P**, Filippi AR, Ricca I, Rauchi C, Ricardi U.
Eyelid localisation in mantle-cell lymphoma: long-lasting complete remission after surface brachytherapy.
Tumori 2009;95:385-388

6. **Franco P**, Filippi AR, Ricardi U.
If sub-clinical turns into sub-optimal.
Tumori 2009;95:268-9.

5. Filippi AR, **Franco P**, Marinone C, Tarella C, Ricardi U.
Treatment options in skeletal localizations of hairy cell leukemia: a systematic review on the role of radiation therapy.
Am J Hematol 2007;82:1017-21.

4. Munoz F, **Franco P**, Ciammella P, Clerico M, Giudici M, Filippi AR, Ricardi U.
Squamous cell carcinoma of the prostate: long-term survival after combined chemo-radiation.
Radiat Oncol 2007;2:15.

3. Filippi AR, **Franco P**, Galliano M, Ricardi U.
Peripheral blood complete remission after splenic irradiation in mantle-cell lymphoma with 11q22-23 deletion and ATM inactivation.
Radiat Oncol 2006;1:35.

2. **Franco P,** Filippi AR, Fornari A, Marinone C, Ricardi U.
A case of bone involvement in hairy cell leukemia successfully treated with radiation therapy.
Tumori 2006;92:366-369.

1. Filippi AR, **Franco P**, Ricardi U.
Is clinical radiosensitivity a complex genetically controlled event?
Tumori 2006;92:87-91.

Reviewer for the following journals

-Lancet Oncology - Radiotherapy and Oncology, -Radiation Oncology, -BMC Cancer, -British Journal of Radiology, -Clinical Colorectal Cancer, -Future Oncology, -The Breast,- Breast Cancer Research and Treatment, -Breast Care, -Cancer Investigation, -Cancer Medicine,-Clinical and Translational Oncology, -Oncotarget- -Physica Medica, -Applied Radiation and Isotope Journal, -Oncotargets and Therapy, -Technology in Cancer Research and Treatment,-Journal of the Egyptian Cancer Institute, -Chemotherapy (Karger), - Clinical, Cosmetic and Investigational Dermatology, - ClinicoEconomics and Outcomes Research,- Asia-Pacific Journal of Clinical Oncology, -Tumori

Pierfrancesco Franco Turin, December 4th 2017