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Original Article

Proton and photon radiotherapy in stage III NSCLC: Effects on hematological toxicity and adjuvant immune therapy

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ABSTRACT

Background and purpose: Concurrent chemo-radiotherapy (CCRT) followed by adjuvant durvalumab is standard-of-care for fit patients with unresectable stage III NSCLC. Intensity modulated proton therapy (IMPT) results in different doses to organs than intensity modulated photon therapy (IMRT). We investigated whether IMPT compared to IMRT reduce hematological toxicity and whether it affects durvalumab treatment.
Materials and methods: Prospectively collected series of consecutive patients with stage III NSCLC receiving CCRT between 06.16 and 12.22 (staged with FDG-PET-CT and brain imaging) were retrospectively analyzed. The primary endpoint was the incidence of lymphopenia grade ≥ 3 in IMPT vs IMRT treated patients.
Results: 271 patients were enrolled (IMPT: n = 71, IMRT: n = 200) in four centers. All patients received platinum-based chemotherapy. Median age: 66 years, 58 % were male, 36 % had squamous NSCLC. The incidence of lymphopenia grade ≥ 3 during CCRT was 67 % and 47 % in the IMRT and IMPT group, respectively (OR 2.2, 95 % CI: 1.0–4.9, $P = 0.03$). The incidence of anemia grade ≥ 3 during CCRT was 26 % and 9 % in the IMRT and IMPT group respectively (OR = 4.9, 95 % CI: 1.9–12.6, $P = 0.001$). IMPT was associated with a lower rate of Performance Status (PS) ≥ 2 at day 21 and 42 after CCRT (13 % vs. 26 %, $P = 0.04$, and 24 % vs. 39 %, $P = 0.02$). Patients treated with IMPT had a higher probability of receiving adjuvant durvalumab (74 % vs. 52 %, OR 0.35, 95 % CI: 0.16–0.79, $P = 0.01$).
Conclusion: IMPT was associated with a lower incidence of severe lymphopenia and anemia, better PS after CCRT and a higher probability of receiving adjuvant durvalumab.

Introduction

The standard-of-care for fit patients with unresectable stage III NSCLC is concurrent chemo-radiation (CCRT) with a radiation dose of 60 Gray (Gy) in 2 Gy daily fractions followed by adjuvant durvalumab for 12 months. In the PACIFIC study, adjuvant durvalumab led to 5-year overall survival (OS) and progression free survival (PFS) rates of 43 % and 33 %, respectively[1]. CCRT is a toxic treatment with acute and late side effects that may also compromise treatment efficacy and survival [2]. The radiation of primary (bone marrow) and secondary lymphoid organs such as the spleen and lymph nodes may also compromise the immune response[3]. Radiotherapy (RT) directly kills circulating

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immune cells[4]. Circulating "naive" lymphocytes are highly sensitive to radiation: in vitro, a dose of about 3 Gy has been shown to kill 90 % of the irradiated naïve T-cells (CD4 + and CD8 +)[5]. Consistently, Bradley et al. ($N = 554$) reported acute lymphopenia in 27 % (21 % grade ≥ 3) of patients undergoing CCRT for stage III NSCLC[2]. The radiation effects on lymphocytes and bone marrow cells are lasting up to 10 years[6,7]. The function of T lymphocytes might also be impaired by RT[8,9]. In the pre-durvalumab era several studies showed that severe lymphopenia is correlated with worse OS in patients receiving CCRT for NSCLC[4,10–12]. Preserving the immune system during CCRT is key to optimize durvalumab efficacy. In two recent retrospective multicenter studies ($N = 151$ and $N = 309$), the baseline lymphocyte count, not developing lymphopenia during CCRT, and lymphocyte count recovery at the start of adjuvant durvalumab, were all associated with better OS and PFS[13,14]. Intensity Modulated Proton Therapy (IMPT) represents a RT delivery technique which, compared to Intensity Modulated Photon Therapy (IMRT), could limit the exposure of healthy tissues, due to a more selective energy deposition in depth[15,16]. In patients receiving CCRT for esophageal cancer ($N = 144$, retrospective study), IMPT reduced the incidence of grade 4 lymphopenia compared to IMRT (56 % vs 22 %)[17]. Currently, there is no solid data about the effect of IMPT in stage III NSCLC in terms of haematological toxicity and immunotherapy efficacy[18,19,20]. To fill this knowledge gap, we herein present data about our cohort of patients with stage III NSCLC treated with CCRT, with either IMRT or IMPT.

Material and methods

Patients and study design

This is a retrospective data completion and analysis of prospectively collected series of consecutive patients with stage III NSCLC receiving CCRT in four centers (Netherlands and Italy). The study has been approved by the ethics committee of the Maastricht University Medical Center+ (MUMC +), MAASTRO radiotherapy clinic, Groningen University Medical Center+ (UMCG +) and University Hospital of Udine. Details about ethical approvals are reported in **Supplementary material S1**. Eligible patients had histologically or cytological diagnosed stage III, unresectable, NSCLC according to the Staging Manual in Thoracic Oncology, version 8, of the International Association for the Study of Lung Cancer[21]. These patients were treated with CCRT, with or without adjuvant durvalumab, between June 2016 and December 2022. Patients were eligible for the study if the treatment plan consisted of two or more cycles of platinum-doublet chemotherapy, according to clinical practice of each center, concurrent with radiotherapy. Radiotherapy could be delivered either with protons or photons, and the radiotherapy plan should consist of 60–66 Gy (60 Gy relative biological equivalent for IMPT) delivered in 25–30 fractions. All patients included in the present study and treated with IMRT received arc IMRT (VMAT). Patients were staged with fluorodeoxyglucose-positron emission tomography (FDG-PET) and brain imaging, either magnetic resonance imaging (MRI) or computed tomography (CT) before the start of CCRT. Key exclusion criteria were previous lung radiotherapy, previous exposure to chemotherapy or to anti-programmed death ligand (PDL)-1 antibodies, and diagnosis of another invasive cancer within the previous 2 years. Patients who received a mixed treatment (a certain number of fractions with photons and a certain number with protons, done for technical reasons) were allocated to the IMPT group if they received at least 30 % of the radiotherapy treatment with IMPT, since we assumed that this amount of IMPT could still translate in a clinically significant difference in hematological toxicity. The *total bone marrow* was defined as the sum of the following bone structures: sternum, scapulae, clavicles, thoracic vertebrae – from T1 to T12 - and ribs - delineated until the level of vertebrae T12-L1 disc[22]. We defined *other bones* the bone marrow included in all the above mentioned structures but the vertebrae (Fig. 1). In all centers, patients were selected for receiving IMPT through Normal Tissue Complication Probability (NTCP) models. These models predict the expected benefit of IMPT compared to IMRT[23,24]. Details about proton therapy facilities and NTCP based selection are reported in **supplementary material S3**. The primary endpoint of this study was the incidence of lymphopenia grade ≥ 3 in proton treated vs photon treated patients. We assumed a reduction of grade ≥ 3 lymphopenia from 25 % in the IMRT arm to 7 % in the IMPT arm, thus an alpha error of 0.05 and a power of 94 % (type II error of 0.06) was expected. Considering the retrospective nature of the study, a propensity score stratification analysis was performed for incidence of hematological toxicity and PS after CCRT[25]. The propensity score model was created with the variables age, sex, GTV, performance status at baseline, tumor stage and type of chemotherapy. Details about the propensity stratification model are reported in **supplementary material S8**. Continuous variables were reported as median and interquartile range (IQR) and differences were tested using T-tests or Mann-Whitney test as appropriate. Categorical variables were described by counts and frequency distribution with 95 % confidence intervals and differences were analysed using Chi-square analyses. The prognostic impact of clinical variables and hematological toxicity on survival were investigated through uni- and multi-variate Cox proportional hazards regression models. Further details about statistical considerations are reported in **supplementary material S5**.

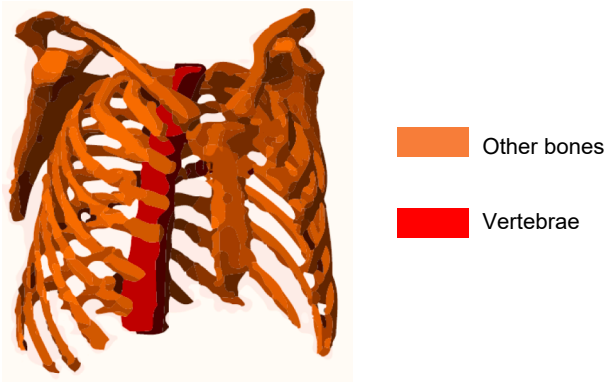

Fig. 1. Bone marrow delineation.

Results

A total of 271 patients were enrolled in the study (71 patients received IMPT and 200 received IMRT). A total of 159 patients were excluded (n = 156 not meeting inclusion criteria). Baseline characteristics were well balanced between the two groups and are reported in Table 1. PDL-1 expression level was unknown in 33 % of the patients. 96 % of patients completed the planned RT. No statistically significant differences were noted in the baseline white blood count and hemoglobin levels.

The incidence of lymphopenia grade ≥ 3 during CCRT in the IMRT arm was 67 % and 47 % in the IMPT arm (OR = 2.2, 95 % CI: 1.0–4.9, $P = 0.032$; Fig. 2**A**). In the multi-variable logistic model, IMRT vs IMPT remained significantly associated with higher risk of developing lymphopenia grade ≥ 3 (adjusted OR = 2.6, 95 % CI: 1.1–6.2, $P = 0.029$). The Hosmer-Lemeshow test indicated that the model was a good fit ($P = 0.4$). In addition, the GTV volume was significantly associated with the risk of developing lymphopenia (0.4 % higher risk of lymphopenia for every cm^3 increase in the GTV, $P = 0.02$). Weekly chemotherapy versus a Q21 schedule was not associated with a lower incidence of lymphopenia grade ≥ 3 ($P = 0.29$). The causal OR for lymphopenia grade ≥ 3 calculated using the propensity score stratification model was 1.9 (95 % CI: 0.8–4.4, $P = 0.14$). The incidence of anemia grade ≥ 3 during CCRT in the IMRT and IMPT arm was 26 % and 9 %, respectively (Fig. 2**A**) (OR 3.8, 95 % CI: 1.6–9.3, $P = 0.003$). In the multi-variable logistic model,

Table 1
Baseline characteristics of enrolled patients.

Characteristics	IMPT (n = 71)	IMRT (n = 200)	Total (*N* = 271)	*P* value
Age (years)				
Median	67	66	66	0.74
Range	35–81	37–80	35–81	
Gender (%)				
Male	63	55	58	0.27
Female	37	45	42	
Disease stage (%)				
IIIA	35	43	41	0.37
IIIB	58	48	51	
IIIC	7	9	8	
WHO performance status (%)				
0–1	87	90	89	0.33
2–3	13	10	11	
Tumor histology (%)				
Squamous	42	35	36	0.15
Non squamous	58	65	64	
PDL-1 (%)				
< 1	31	37	35	0.30
1–49	38	40	40	
> 50	31	23	25	0.19
Chemotherapy (%)				
Carboplatin	68	45	51	**0.001**
Cisplatin	32	55	49	
3 cycles Q21 (1 induction, 2 CCRT)	84	74	76	**0.034**
1 induction Q21 → weekly CCRT	11	9	10	
Weekly CCRT	4	17	14	
Radiotherapy dose (Gy)				
Median	60	60	60	0.09
Range	31–61	8–66	8–66	
Radiotherapy fractions (n)	30	30	30	0.28
Median	13–32	4–33	4–33	
Range				
Baseline WBC (10^9/L)				
Median	9.2	8.9	8.9	0.84
Range	2.4–19.7	3.6–28	2.4–28	
Baseline Lymphocytes (10^9/L)	2.0	1.7	1.77	0.32
Median	0.7–7.5	0.21–10.3	0.21–10.3	
Range				
Baseline Neutrophils (10^9/L)	6.3	5.9	6.0	0.85
Median	2.7–15.2	1.23–25	1.23–25	
Range				
Baseline Hb (mmol/L)				
Median	8.6	8,4	8.4	0.29
Range	5.7–10.3	5,2–10.6	5.2–10.6	

Abbreviations: CCRT = concurrent chemo-radiotherapy; IMRT = intensity modulated photon therapy; IMPT = intensity modulated proton therapy; Q21 = chemotherapy every 21 days; PDL-1 = Programmed death ligand-1.

IMRT vs IMPT remained significantly associated with a higher risk of developing anemia grade ≥ 3 (adjusted OR = 4.9, 95 % CI: 1.9–12.6, *P* = 0.001). Q21 chemotherapy was also associated with a higher risk of anemia grade ≥ 3 (adjusted OR = 29.4, 95 % CI: 3.9–220, *P* = 0.001). The causal OR for anemia grade ≥ 3 was 3.1 (95 % CI: 1.2–8.0, *P* = 0.02). Subgroup analysis are shown in Fig. 3**.** Anemia grade ≥ 3 was significantly associated with worse overall survival (OS): median OS was NR, 95 CI% NR-NR, vs 27 months, 95 % CI: 12–41, *P* = 0.002), while lymphopenia grade ≥ 3 was not associated with worse survival (median OS NR, 95 % CI: NR-NR, vs 32 months, 95 % CI: 21–42, *P* = 0.11). No differences were showed in the incidence of either febrile neutropenia, neutropenia grade ≥ 3 or thrombocytopenia grade ≥ 3 between IMPT and IMRT.

IMPT vs IMRT was associated with a lower rate of PS ≥ 2 at day 21 (d21) after CCRT (13 % vs. 26 %, OR = 0.44, 95 % CI: 0.2–0.96, *P* = 0.04) and at day 42 (d42) after CCRT (24 % vs. 39 %, OR = 0.49, 95 % CI: 0.26–0.91, *P* = 0.024) as shown in Fig. 2**B**. PS ≥ 2 at d21 and at d42 were also associated with the development of anemia (*P* = 0.003 and *P* < 0.001, respectively). These findings remained significant also in the multi-variable model (adjusted OR = 0.4, 95 % CI: 0.16–0.98, *P* = 0.045 and adjusted OR = 0.4, 95 % CI: 0.2–0.78, *P* = 0.008, respectively). WHO PS at baseline (>1 vs ≤ 1) was associated with higher WHO PS at d21 (adjusted OR = 15.1, 95 % CI: 5.4–41*P* < 0.001) and at d42 (adjusted OR = 3.5, 95 % CI: 1.4–8.4, *P* = 0.004). Causal OR were also calculated for WHO PS at d21 and at d42. They were 2.2 (95 % CI: 0.9–5.0, *P* = 0.07) and 2.1 (95 % CI: 1.1–4.2, *P* = 0.03) respectively. WHO PS < 2 at d21 and d42 was associated with better survival (mOS 54.4, 95 CI% 37–71, vs 28 months, 95 CI% 14–42, OR 0.5, 95 % CI 0.32–0.78*P* = 0.003; and mOS 54.4, 95 CI% NR-NR, vs 29.7 months, 95 % CI: 12.4–46.9, OR = 0.49, 95 % CI: 0.33–0.72, *P* < 0.001, respectively).

The median time from end of CCRT and start of adjuvant durvalumab was 41 days (range: 10–156) in the IMRT group and 31 days (range: 6–97) in the IMPT group (p = 0.013). IMPT was also associated with a higher probability of receiving adjuvant durvalumab within 42 days (72 % vs 51 %, respectively, OR = 2.57, 95 % CI: 1.19–5.5, *P* = 0.015). In the multi-variable logistic model (including age and PS at d42) IMPT was the only predictive factor for receiving durvalumab within 42 days (adjusted OR = 0.4, 95 % CI:0.16–0.95, *P* = 0.04, Hosmer-Lemeshow test, *P* = 0.32). Among patients enrolled after June 2018, when durvalumab was available in clinical practice in all Institutions involved, baseline characteristics were well balanced and are reported in **Supplementary Table 1.** Also in this subgroup, IMPT was associated with lower incidence of anemia grade ≥ 3 (8.5 % vs 19.4 %, OR = 0.38, 95 % CI: 0.15–0.90, *P* = 0.044), lower incidence of lymphopenia grade ≥ 3 (47 % vs 67 %, OR = 0.44, 95 % CI: 0.19–0.90, *P* = 0.044), higher probability to have a PS < 2 at d21 after CCRT (86 % vs 73 %, OR = 0.41, 95 % CI: 0.18–0.91, *P* = 0.029 and at d42 after CCRT (76 % vs 64 %, OR = 0.56, 95 % CI: 0.29–1.06, *P* = 0.079). In this subgroup, patients treated with IMPT vs IMRT also had a higher probability of receiving adjuvant durvalumab (74 % vs 52 %, *P* = 0.002). These findings remained significant in the multi-variable model – including tumor stage, WHO PS, type of chemotherapy (carboplatin vs cisplatin and weekly vs Q21 schedule), age, PDL-1, and GTV- (adjusted OR = 0.35, 95 % CI: 0.16–0.79, *P* = 0.012). Baseline characteristics among patients treated with durvalumab after IMRT vs. IMPT were well balanced (**Supplementary Table 2**). Median FU was 30.4 months (95 % CI: 28–32) in the IMRT arm and 15.5 months (95 % CI: 14.3–16.6) in the IMPT arm (P < 0.001). No differences were noted between IMPT and IMRT in terms of immune-related adverse events (irAEs) (Table 2). Immune related pneumonitis occurred in 7.8 % and 9.8 % in the IMRT and IMPT arm respectively (*P* = 0.48). All immune related pneumonitis were grade 2 or grade 3. All-cause pneumonitis grade ≥ 2 during durvalumab was 22.1 % and 23.5 % in the IMRT and IMPT arm respectively, *P* = 0.506.

The mean body dose showed a trend of association with lymphopenia grade ≥ 3 (*P* = 0.08) and it was significantly associated with and anemia grade ≥ 3 (*P* = 0.023). A trend towards association between the V20 Gy – the volume that receive at least 20 Gy - for the total bone marrow and lymphopenia grade ≥ 3 was observed (*P* = 0.088). Total bone marrow mean dose (*P* = 0.015), V1 (*P* = 0.001), V2 (*P* = 0.002), V3 (*P* = 0.002), V4 (*P* = 0.003), V5 (*P* = 0.006), and vertebrae V1 (*P* = 0.01) were associated with the risk of developing anemia grade ≥ 3 as well as heart V1 (*P* = 0.005), V2 (*P* = 0.003), V3 (*P* = 0.003), V4 (*P* = 0.003), V5 (*P* = 0.048), the whole body V1 (*P* = 0.023), V2 (*P* = 0.021). All the associations between radiation volumes and hematological toxicities are reported in **Supplementary Table S3.**

IMPT compared to IMRT significantly improved the mean dose delivered at the target lymph nodes (GTVn) (56 vs 55 Gy, *P* = 0.01) and at the target primary (GTVp) (58.3 vs 56.9 Gy, *P* < 0.001). At the same time, IMPT significantly reduced the radiation dose to OARs correlated

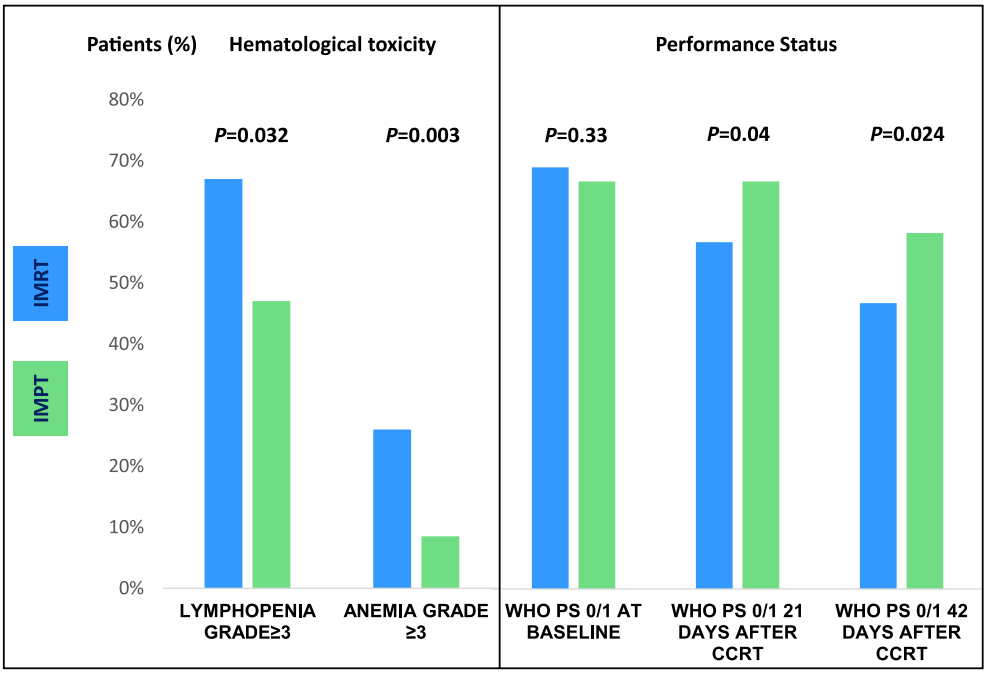

Fig. 2. A) Incidence of anemia and lymphopenia grade ≥ 3 during CCRT in patients treated with IMPT and IMRT. B) WHO PS at baseline, at 21 days, and at 42 days after CCRT, according to IMPT vs IMRT. Abbreviations: CCRT = concurrent chemo-radiotherapy; IMRT = intensity modulated photon therapy; IMPT = intensity modulated proton therapy; WHO PS =.World Health Organization performance status.

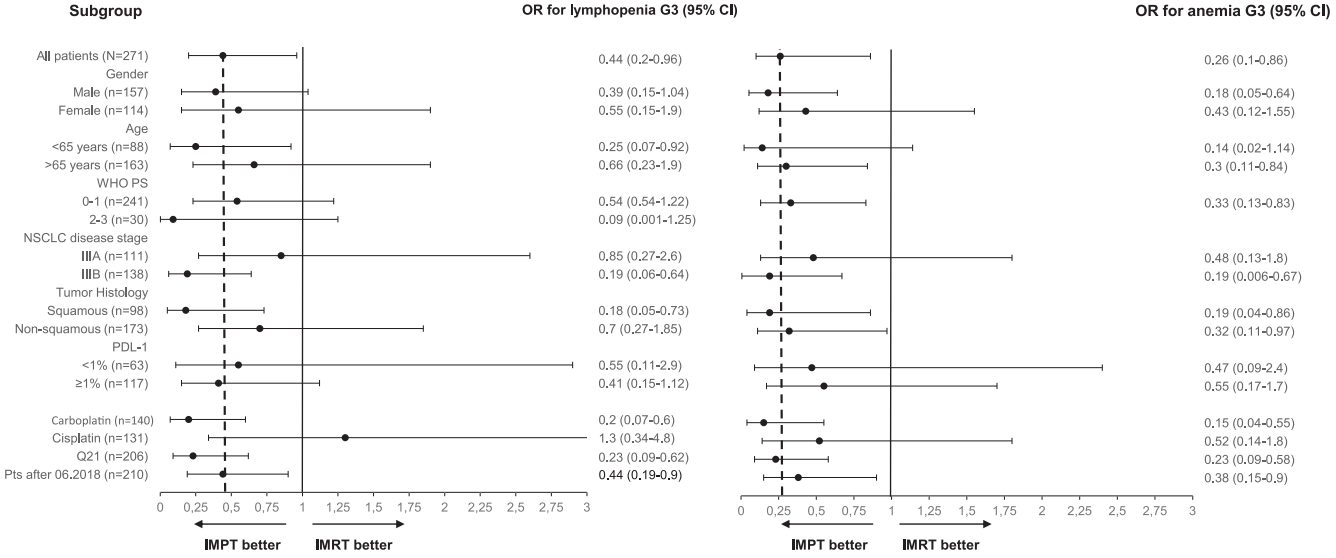

Fig. 3. Subgroup analysis of prognostic factors for developing severe anemia and severe lymphopenia. Abbreviations: OR = odds ratio; G3 = grade 3 according to CTCAE version 5.0; WHO PS = World Health Organization performance status; NSCLC = Non-small cell lung cancer; Q21 = chemotherapy every 21 days; PDL-1 = Programmed death ligand-1.

with toxicities. Of note, IMPT significantly reduced the mean body dose compared to IMRT ($P < 0.001$), (Fig. 4). All the volume comparison between IMPT and IMRT plans are reported in Supplementary Table S4.

Logistic regression confirmed that mean body dose was significantly associated with the development of anemia G3 ($P = 0.004$) and possibly associated with the development of lymphopenia grade 3 ($P = 0.08$) Supplementary Figure S1.

Discussion

Our study revealed that IMPT, compared to IMRT, reduced the incidence of lymphopenia grade ≥ 3 (47 % vs 67 %) and anemia grade ≥ 3 (9 % vs 26 %) in patients with stage III NSCLC receiving CCRT. IMPT was also associated with a lower rate of PS ≥ 2 after CCRT (13 % vs. 26 % after 21 days, and 24 % vs. 39 % after 42 days). Furthermore, IMPT was associated with higher probability of patients receiving adjuvant durvalumab (74 % vs 52 %) and of receiving durvalumab within 42 days

Table 2
Durvalumab eligibility and safety after IMPT or IMRT.

Characteristics	IMPT (n = 51)	IMRT (n = 77)	Total (*N* = 128)	*P* value
Median time from end CCRT start Durvalumab – days (range)	31 6–97	41 10–156	37 6–156	**0.013**
Durvalumab within 14 days – %	12	4	7	0.09
Durvalumab within 28 days – %	40	25	30	0.057
Durvalumab within 42 days – %	72	51	57	**0.015 OR 2.57 (95 % CI: 1.19–5.5)**
All grade irAEs	29.4	36.6	33.6	0.87
Grade ≥ 2 irAEs	21	26	24.2	0.36
Grade ≥ 3 irAEs	5.9	7.8	7	0.48
Grade ≥ 4 irAEs	0	0	0	–
Immune related pneumonitis	9.8	7.8	8.6	0.48
All cause pneumonitis during Durvalumab (Grade ≥ 2)	23.5	22.1	22.7	0.51
Hypo/hypertiroidism (any grade)	15.7	18.3	17.2	0.64
Colitis (any grade)	2	1.3	1.6	–
Arthritis/myositis	2	9.1	5.5	–
Dermatitis	3.9	3.9	3.9	–
Median duration of Durvalumab – months (range)	5.6 (1–14.9)	10.5 (1–19.2)	8.4 (1–19.2)	**0.01**
Median FU - months (95 % CI)	15.5 (14.3–16.6)	30.4 (28–32)	22.6 (19.6–25.5)	**< 0.001**

Abbreviations: CCRT = concurrent chemo-radiotherapy; IMRT = intensity modulated photon therapy; IMPT = intensity modulated proton therapy; irAEs = immune related adverse events; FU = follow-up; CI = confidence interval.

(72 % vs 51 %). A higher mean body dose was significantly associated with anemia grade ≥ 3 ($P = 0.023$) and showed a trend of association with lymphopenia grade ≥ 3 ($P = 0.08$). A trend towards associations between lymphopenia was shown also for higher V20 total bone marrow, V4 other bones and V5 other bones. Bone marrow volumes and heart volumes were significantly associated with the development of anemia grade ≥ 3. We showed that IMPT increased the mean dose delivered to the tumor, while reducing the radiation dose to organs at risk. The lower dose delivered with protons to heart, bone marrow and whole body explains the lower incidence of severe anemia and lymphopenia in patients treated with IMPT.

A previous retrospective study ($N = 901$). showed that the mean dose to lungs and heart and vertebrae V20 were the strongest predictors for developing severe lymphopenia during CCRT[4]. The association was incrementally lower for higher volumes, which is in line with our findings and consistent with radio-sensitivity of both circulating lymphocytes and stem cells[26]. After 1 Gy of radiation, the T and B-lymphocytes apoptosis rate is 50 %, explaining why the lower volumes, which are also the ones most reduced by IMPT compared to IMRT, were associated with lymphopenia in our study [27]. Neutrophils are more radio-resistant, explaining why we did not note any differences in neutropenia incidence between IMPT and IMRT[28]. Investigating the effects of radiation on lymphocytes in vivo is challenging since they circulate continuously between peripheral blood and tissue (it is estimated that about 5 % of total lymphocytes are in the blood flow, the rest being in the peripheral tissue and lymphoid organs), they continuously mix within the blood flow, and the dose to precursor stem-cells may lower the production of lymphocytes[29,30]. For all these factors, we have to rely on proxy parameters. Heart and lung doses may be representative of the radiation delivered to blood volume while dose to bone marrow might be more related to the effect of radiation on stem cells [26]. The whole body dose might represent even a more reliable proxy parameter to predict the effects of radiation in terms of hematological toxicity, since all blood filled organ as well as bone marrow harbor and/or produce lymphocytes[31]. Notably this is an easy radiation parameter to implement in clinical practice. Total body radiation is used as conditioning regimen prior to hematopoietic cell transplantation, supporting the correlation between total body radiation dose and myelosuppression[32]. Lymphopenia has shown to be a negative prognostic factor also in patients who experienced major trauma and who were otherwise healthy[33]. In light of this, the healthy tissues inflammation generated by RT might contribute in impairing the immune system after CCRT. Thus, proton therapy, reducing healthy tissue exposure, could positively affect survival by mitigating immune suppression. In future studies lymphocytes sub-population must be analyzed prospectively in order to reach a deeper understanding of the radiotherapy effects on the immune system[34]. The protective effect of IMPT on lymphocytes and the immune system may translate into increased durvalumab eligibility and improved durvalumab efficacy[35].

The selection of patients trough NCTP models, introduces a selection bias. However, the selection bias in our population worked against the IMPT arm since the patients who received IMPT were the ones expected to have greater toxicity. The present study is the first study investigating the safety of durvalumab after IMPT, showing no significant increased toxicity. The finding that the pneumonitis incidence was the same in IMPT and IMRT is positive, considering the greater lung toxicities expected in the IMPT arm. However, the different median follow-up in the two treatment groups (IMRT vs IMPT) could have affected this analysis and longer follow-up in the IMPT group should be awaited before drawing firm conclusions. On the other side the different median FU did not impact acute toxicity since the time of detection was the same (the duration of CCRT treatment). The shorter FU in the IMPT arm also explains the shorter median durvalumab time in the IMPT arm.

The main limit of the PROMETHEuS study is its retrospective nature. This shortcoming might be mitigated because we included prospectively collected consecutive patients and the investigators were blind to treatment received (IMPT vs IMRT) while computing the data and delineating the bone marrow. Moreover, our findings are supported by a mechanistic rationale and are coherent throughout the whole analysis (**Supplementary Table 4**).

Reaching scientific evidence of the benefit of proton therapy using randomized clinical trials is hampered by the current limited capacity of proton therapy centers and the fact that late radiation induced toxicities would take years to develop while the radiotherapy technology is rapidly evolving[36]. On top of that, randomizing patients to a clearly unfavorable arm in term of toxicity (IMRT) represents an ethical issue [37]. Thus, observational studies and *in silico* models represent the only realistic ways to implement IMPT. Another important limitation is that the lymphocytic count was not assessed routinely for all the patients and lymphopenia information was only available for 55 % (N = 148) of patients. However, the proportionality of proton vs photon treated patients was preserved among patients with a lymphocytic count assessment available ($P = 0.18$). This was not an issue for anemia, performance status and durvalumab administration. The fact that 70 % patients enrolled in the IMPT arm did receive also some radiation fractions with photons reflects a real word scenario, showing that IMPT is beneficial even when imbricated with photon therapy.

Conclusions

The present study showed that IMPT can reduce severe lymphopenia and anemia in patients with unresectable locally advanced NSCLC. Patients treated with IMPT had a better performance status after CCRT and they were more likely to receive adjuvant durvalumab.

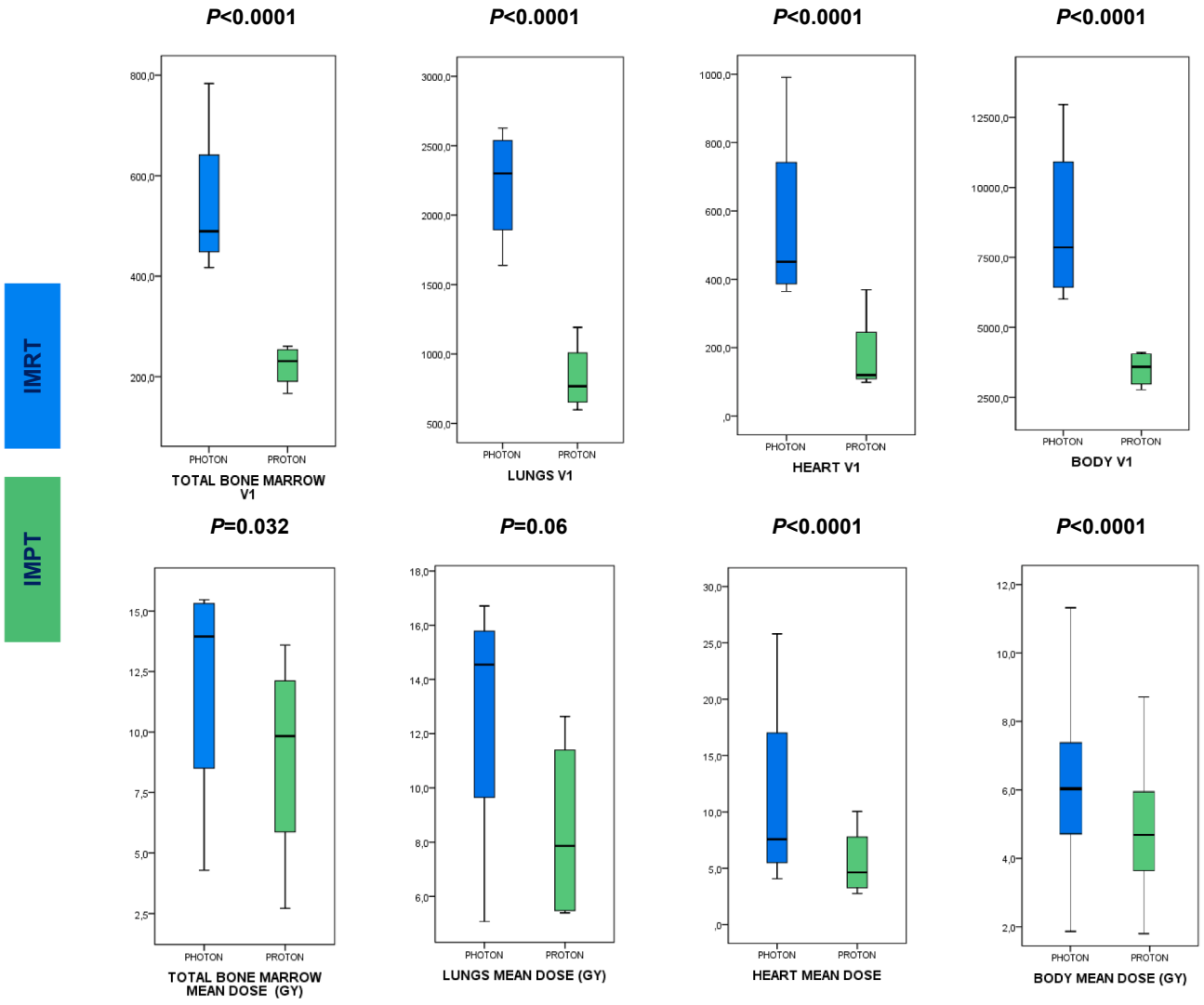

Fig. 4. Comparison of irradiated volumes with IMPT vs IMRT. Abbreviations: IMRT = intensity modulated photon therapy; IMPT = intensity modulated proton therapy; Gy = Grey; V1 = volume that received at least 1 Gy.

Disclosures

During the preparation of this work the authors did NOT use any Generative AI and/or AI-assisted technologies in the writing process.

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CRediT authorship contribution statement

Francesco Cortiula: Investigation, Formal analysis, Data curation, Writing – original draft. **Lizza E.L. Hendriks:** Investigation, Methodology, Writing – review & editing, Supervision. **Robin Wijsman:** Investigation. **Ruud Houben:** Formal analysis. **Michelle Steens:** Investigation. **Sarah Debakker:** Investigation. **Richard Canters:** Investigation, Supervision, Data curation. **Marco Trovò:** Investigation. **Nanna M. Sijtsema:** Methodology. **Anne G.H. Niezink:** Investigation. **Mirko Unipan:** Data curation, Methodology. **Susanna Urban:** Investigation. **Anna Michelotti:** Investigation. **Safiye Dursun:** Investigation. **Gerben Bootsma:** Investigation. **Djoya Hattu:** Data curation. **Joost J. Nuyttens:** Writing – review & editing. **Eugenia Moretti:** Investigation. **Vicki T. Taasti:** Data curation, Methodology. **Dirk De Ruysscher:** Conceptualization, Writing – review & editing, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2023.110019.

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