Supplemental materials

Table e1: Patient characteristics – full list

	Men (n=11)	Women (n=11)	Total (n=22)
T stage (T1/T2/T3/T4)	1/7/3/0	1/6/2/2	2/13/5/2
N stage (N0/N1/N2/N3)	5/1/4/1	6/1/3/1	11/2/7/2
Age	63 ± 9.7 (48-75)	65 ± 9.8 (54-90)	64 ± 9.8 (48-90)
Chemo-radiotherapy*	CISPLATIN		

Tumour/organ *Volumes [cm^3]*

PTV-T	158 (137-231)	130 (116-244)	156
PTV-N	2030 (1900-2257)	1931 (1719-2112)	2014
Bowel	1135 (893-1328)	1557 (1483-1698)	1406
Bladder	168 (114-267)	76 (50-169)	139
Sacral bone†	291 (287-307)	293 (253-328)	291
Penile bulb	6.2 (4.4-7.4)	-	6.2
Testes	51 (46-68)	-	51
Vagina	-	24 (19-30)	24

PTV: Planning Target Volume, T: Tumor, N: elective volume. For all continuous measures, median values are reported with interquartile ranges in brackets. . * All patients received concurrent chemo-radiotherapy with CISPLATIN. † Data for 3 men and 4 women.

Appendix e2: Delineation and dose planning techniques.

Computed tomography (CT) simulation scans were acquired in the supine position on a carbon-fiber top with knee and feet supports, with CT slice thickness of 2.5 mm and standard intravenous contrast. A pelvic MRI was acquired directly after the CT, with similar setup. The delineation of clinical target volumes CTV-T, CTV-N, and bowel, bladder, femoral heads, and genitals was done by experienced radiation oncologists based on CT/MR fusion, in accordance with Danish Anal Cancer Group (DACG) guidelines, which were based on an atlas by Roels et al. See Table e3 and Figure e4 below for further details. Planned primary tumor planning volumes (PTV-T) ranged from 74 cm^3 to 568 cm^3 and elective nodal planning volumes (PTV-N) ranged from 1341 cm^3 to 2593 cm^3.

Step & Shoot IMRT delivery was employed. We used a library plan design, comprising eight co-planar 6 MV beams with gantry angles distributed evenly around the patient (i.e. 180, 140, 85, 30, 220, 275, 330, and 0 degrees) with the isocentre placed at the center of mass of the PTV-N. The collimator angle was manually selected to give the smallest open area in the Beam's Eye View (BEV) for each gantry angle. We did not allow for any segments with less than 4 MU and equivalent square aperture size smaller than 2 cm^2. This was in keeping with routine output and treatment planning commissioning limits. The average number of segments per beam was kept at 10 (maximum 80 segments in all), in order to avoid overly modulated plans. Treatment plans were normalized such that the average dose in the PTV-T volume was within 1% of the prescribed dose.

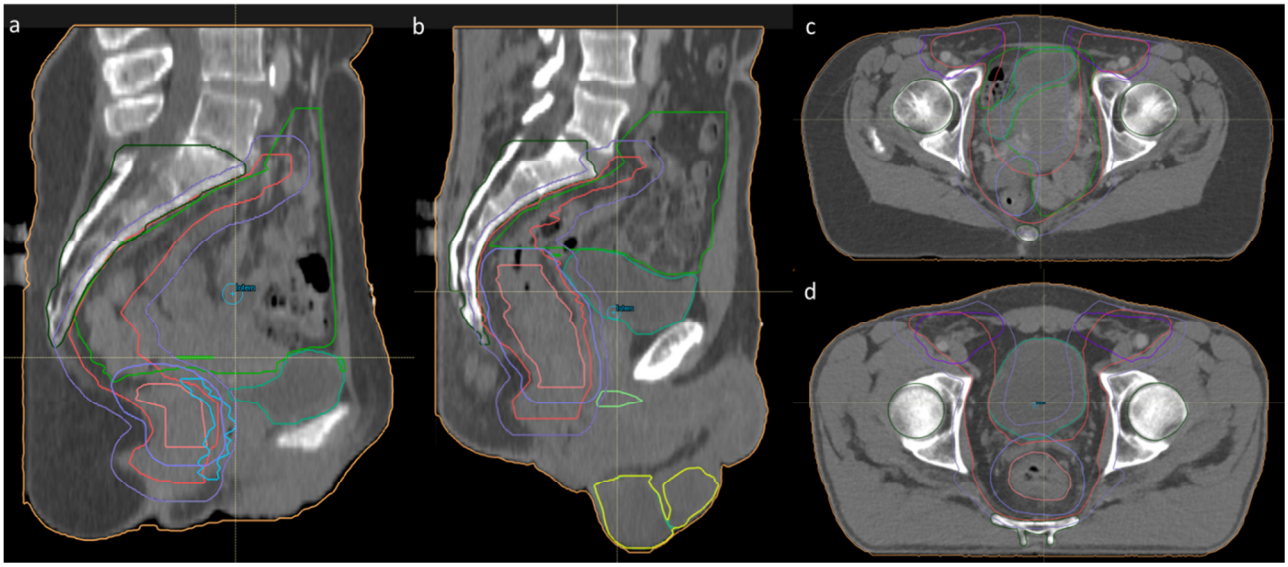
Table e3: Delineation guidelines.

OAR	**Delineation**
Bladder	The entire volume including the urine compartment
Bowel	The "peritoneal cavity" (representing the "potential" intestinal volume), excluding large vessels, bladder and musculature. The superior border was at the CT slice where L5 stops; at least 2 cm superior for CTV-N. The inferior border was at the most caudal CT slice containing intestine, excluding the rectum and mesorectum
Penile bulb, Testes, Vagina	Delineated with guidance from the MRI
Femoral heads	Consisted of the femoral heads, with the caudal border at the lower border of the femoral neck
Sacral bone	Includes the sacroiliac joints. The sagital reconstruction of the CT scan is used to check the delineation of the border between the sacral bone and the spine.

Target	**Delineation**
GTV	The primary tumor, delineated based on the MRI
CTV-T	The circumference of the anal canal at the plane of the GTV, including the GTV itself
PTV-T	10 mm margin isotropic to CTV-T
CTV-N	Contained the medial, right, and left superficial inguinal nodes, the external and internal iliac nodes, the pudendal nodes, and the obturator lymph nodes, and the perirectal adipose tissue containing the haemorrhoidale nodes and the presacral nodes, as well as the inferior, medial and superior rectal lymph nodes. The CTV-T was contained in the CTV-N
PTV-N	10 mm margin isotropic to CTV-N

GTV: Gross Tumor Volume, CTV: Clinical Target Volume, PTV: Planning Target Volume. T: Tumor, N: elective volume.

Figure e4: Example delineations



Sagittal view of delineation. a) female, b) male; and transversal view of delineation, c) male and d) female. Light pink: CTV-T, Dark pink: CTV-N, light purple: PTV-T, dark purple: PTV-N, green: bowel, blue-green: bladder, turquoise: vagina, light green: penile bulb, dark green: sacrum, yellow: testes.

Table e5: List of planning objectives

ROI	Objectives	Reference
PTV-T	$V_{95\%}$>98%	Department standard
	$V_{90\%}$>100%	
	$V_{107\%}$<1%	
PTV-N	$V_{95\%}$>98%	Department standard
	$V_{90\%}$>100%	
	$V_{107\%}$<3%†	
CTV-T and CTV-N	$V_{95\%}$=100%	Department standard
Bladder	V_{50Gy}<20%	Appelt et al. 2015
	V_{35Gy}<75%	Viswanathan et al. 2010
Bowel/intestine	V_{45Gy}<300cm^3	Devisetty et al. 2015
	V_{30Gy}<600cm^3	DeFoe et al. 2013
Body (hotspot)	V_{107}%<3cm^3	Department standard
Penile bulb	V_{50Gy}<50%*	Roach et al. 2010
Testes	D_{mean}<15Gy	Ogilvy-Stuart et al. 1993‡
Vagina	D_{max}<50Gy	Son et al. 2015‡

ROI: Region Of Interest, CTV: Clinical Target Volume, PTV: Planning Target Volume. T: Tumor, N: elective volume. † hotspots ($V_{107\ \%}$) in the elective volume (PTV-N) outside of PTV-T, * High dose volume as low as possible, ‡ The literature is sparse.

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Danish Anal Cancer Group (DACG) national treatment guidelines:
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Roach M, Nam J, et al. Radiation Dose-Volume Effects and the Penile Bulb. IJROBP 2010; 76 (3): 130-134. (QUANTEC).
Ogilvy-Stuart AL, et al. Effect of Radiation on the Human Reproductive System. Environmental Health Perspective Supplements. 1993; 101: 109-116.
Son CH, et al. Dosimetric Predictors of Radiation-Induced Vaginal Stenosis After Pelvic Radiation Therapy for Rectal and Anal Cancer. IJROBP 2015; 92(3): 548-554.

Table e6: Summary of results

Plan regimens	PTV-T [%]	PTV-Ns [%]	Bowel [ccm]		Bladder [%]		Hotspot [%]	PCI
	V95%	V95%	V30Gy	V45Gy	V35Gy	V50Gy	V107% PTV-Ns*	
High dose	98.9 (98.5-99.5)	98.2 (98.1-98.3)	892 (667-1076)	482 (409-633)	73.8 (68.5-78.8)	8.0 (3.5-13.5)	3.2 (2.6-3.7)	0.71 (0.69-0.74)
Low dose	99.0 (98.5-99.5)	98.9 (98.4-98.9)	861 (639-987)	248 (195-315)	63.6 (50.9-69.9)	0.0 (0.0-0.1)	5.0 (4.1-6.1)	0.71 (0.69-0.73)
Δ from high dose†	0.0	0.6	**-37**	**-234**	**-13.7**	-7.9	**2.0**	-0.01
Bladder sparing	99.3 (98.4-99.7)	98.6 (98.2-98.9)	880 (667-1063)	285 (220-351)	44.6 (32.5-56.3)	0.0 (0.0-0.4)	6.0 (5.5-6.7)	0.68 (0.66-0.70)
Δ from high dose†	0.1	0.4	**-20**	**-193**	**-30.3**	-7.9	**2.9**	**-0.03**
Δ from low dose†	0.0	-0.1	**12**	26	-13.6	0.0	**1.0**	**-0.03**
Bowel sparing	99.4 (99.0-99.9)	98.4 (98.2-98.5)	759 (552-886)	229 (159-269)	72.2 (63.4-81.9)	0.0 (0.0-1.1)	5.6 (4.9-6.6)	0.71 (0.69-0.74)
Δ from high dose†	0.2	0.1	**-128**	**-281**	-1.7	-7.8	**2.4**	0.0
Δ from low dose†	0.3	-0.3	**-77**	**-32.2**	**10.8**	0.0	0.6	0.0
Δ from bladder sparing†	0.2	-0.2	**-89**	**-71.4**	**28.3**	0.0	-0.4	**0.04**

Median values for all patients. PTV-N: Planning Target Volume – Elective nodes. Numbers in parenthesis are 1st and 3rd interquartile ranges. *$V_{107\%}$ of prescribed dose for the elective volume (PTV-N) outside of PTV-T. ‡ Plan Conformity Index (PCI) for PTV-N (defined as ($V_{95\%}$ PTV-Ns)/($V_{95\%}$ Body)). †A negative value means that the metric in question (in percentages or cubic centimetres) is lower compared to the regimen mentioned. Bold text: Significance at the 1% level (p<0.01) using paired rank tests. For femoral heads the median values for V50Gy for the high dose plans are 0.29 % and 0.14 % for dxt and sin, respectively. For the low dose plans including the bladder and bowel sparing plans the median values are 0.