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Clinical Investigation

Parotid Gland Stem Cell Sparing Radiation Therapy for Patients With Head and Neck Cancer: A Double-Blind Randomized Controlled Trial

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Purpose: Radiation therapy for head and neck cancer frequently leads to salivary gland damage and subsequent xerostomia. The radiation response of the parotid glands of rats, mice, and patients critically depends on dose to parotid gland stem cells, mainly located in the gland's main ducts (stem cell rich [SCR] region). Therefore, this double-blind randomized controlled trial aimed to test the hypothesis that parotid gland stem cell sparing radiation therapy preserves parotid gland function better than currently used whole parotid gland sparing radiation therapy.

Methods and Materials: Patients with head and neck cancer (n = 102) treated with definitive radiation therapy were randomized between standard parotid-sparing and stem cell sparing (SCS) techniques. The primary endpoint was >75% reduction in

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Data sharing statement: Research data are stored in an institutional repository and will be shared upon request to the corresponding author.

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parotid gland saliva production compared with pretreatment production ($FLOW_{12M}$). Secondary endpoints were several aspects of xerostomia 12 months after treatment.

Results: Fifty-four patients were assigned to the standard arm and 48 to the SCS arm. Only dose to the SCR regions (contralateral 16 and 11 Gy [$P = .004$] and ipsilateral 26 and 16 Gy [$P = .001$] in the standard and SCS arm, respectively) and pretreatment patient-rated daytime xerostomia (35% and 13% [$P = .01$] in the standard and SCS arm, respectively) differed significantly between the arms. In the SCS arm, 1 patient (2.8%) experienced $FLOW_{12M}$ compared with 2 (4.9%) in the standard arm ($P = 1.00$). However, a trend toward better relative parotid gland salivary function in favor of SCS radiation therapy was shown. Moreover, multivariable analysis showed that mean contralateral SCR region dose was the strongest dosimetric predictor for moderate-to-severe patient-rated daytime xerostomia and grade ≥2 physician-rated xerostomia, the latter including reported alteration in diet.

Conclusions: No significantly better parotid function was observed in SCS radiation therapy. However, additional multivariable analysis showed that dose to the SCR region was more predictive of the development of parotid gland function−related xerostomia endpoints than dose to the entire parotid gland.

Introduction

Approximately 70% of all patients treated for head and neck cancer (HNC) receive radiation therapy. When administering radiation to cancerous tissue, adjacent normal tissues are inevitably coirradiated. This often leads to side effects. Exposure of the salivary glands to radiation frequently results in loss of gland function (hyposalivation) within the first weeks of radiation therapy.[1] Hyposalivation can cause xerostomia and other side effects, such as alterations in speech and taste and difficulties with mastication and deglutition.[2] Xerostomia is the most frequently reported side effect after HNC radiation therapy and has a major effect on quality of life of these patients.[2,3]

There is level I evidence that reducing parotid dose results in better posttreatment salivary production, less patient-rated xerostomia, and better general quality of life.[4-6] Unfortunately, adequate dose reduction to the parotid glands is not always possible.[7] Approximately 30% to 40% of all patients treated with intensity modulated radiation therapy (IMRT) develop life-long (permanent) xerostomia.[4,7,8] Multiple approaches are under investigation to lower the dose to the parotid glands and other organs at risk (OARs) compared with IMRT. Volumetric modulated arc therapy (VMAT),[9] adaptive radiation therapy,[10] intensity modulated proton therapy,[11,12] and spot-scanning proton arc[13] all result in lowering dose to the parotid glands and other OARs while meeting or even improving other treatment objectives. However, not all patients benefit from these approaches.

Clinical studies using IMRT have shown that radiation-induced xerostomia can partially recover over time,[3,8] indicating that in the first 2 years after radiation therapy the damaged parotid gland is capable of partly regaining its function.[14] This is consistent with preclinical and clinical observations that the salivary gland contains stem cells capable of regenerating damaged tissue.[15,16] Furthermore, dose to the parotid gland subvolume containing the major ducts was more damaging than dose to other parts of the gland.[17,18] Consistent with the finding that stem cells are critical to the radiation response of the glands, this region is rich in stem cells (stem cell rich [SCR] region) (Fig. 1a).[15] Indeed, in a clinical cohort, radiation dose delivered to this SCR region was the strongest predictor of salivary flow 1 year after treatment.[15] Interestingly, with recent high-precision radiation therapy technology, sparing such a subvolume of the parotid gland may be feasible in patients for whom sparing the whole gland is not feasible.

Therefore, the aim of this study was to test the hypothesis that an approach aimed at sparing the parotid gland SCR region preserves parotid gland function better than the currently used mean dose sparing of the parotid gland.

Methods and Materials

Study design

This investigator-initiated single-center double-blinded randomized controlled trial was conducted at the University Medical Center Groningen, the Netherlands. The institutional ethics review board approved the trial protocol. The trial was registered (ClinicalTrials.gov number NCT01955239) and performed according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.[19] Written informed consent was obtained from all patients before enrollment. Patients could withdraw consent at any time after enrollment. The trial was financially supported by the University Medical Center Groningen.

The sample size calculation was based on an expected reduction in risk of parotid gland dysfunction, defined as 75% or more compared with baseline parotid gland function. Demonstrating a reduction of this risk from 30% to 5% at a significance level of .05 and a power of 80% required 76 evaluable patients (38 per arm). This estimated clinical gain was based on the results of our retrospective analysis. Because the 1-year survival of these patients is approximately 75%, randomization of 102 patients was needed.

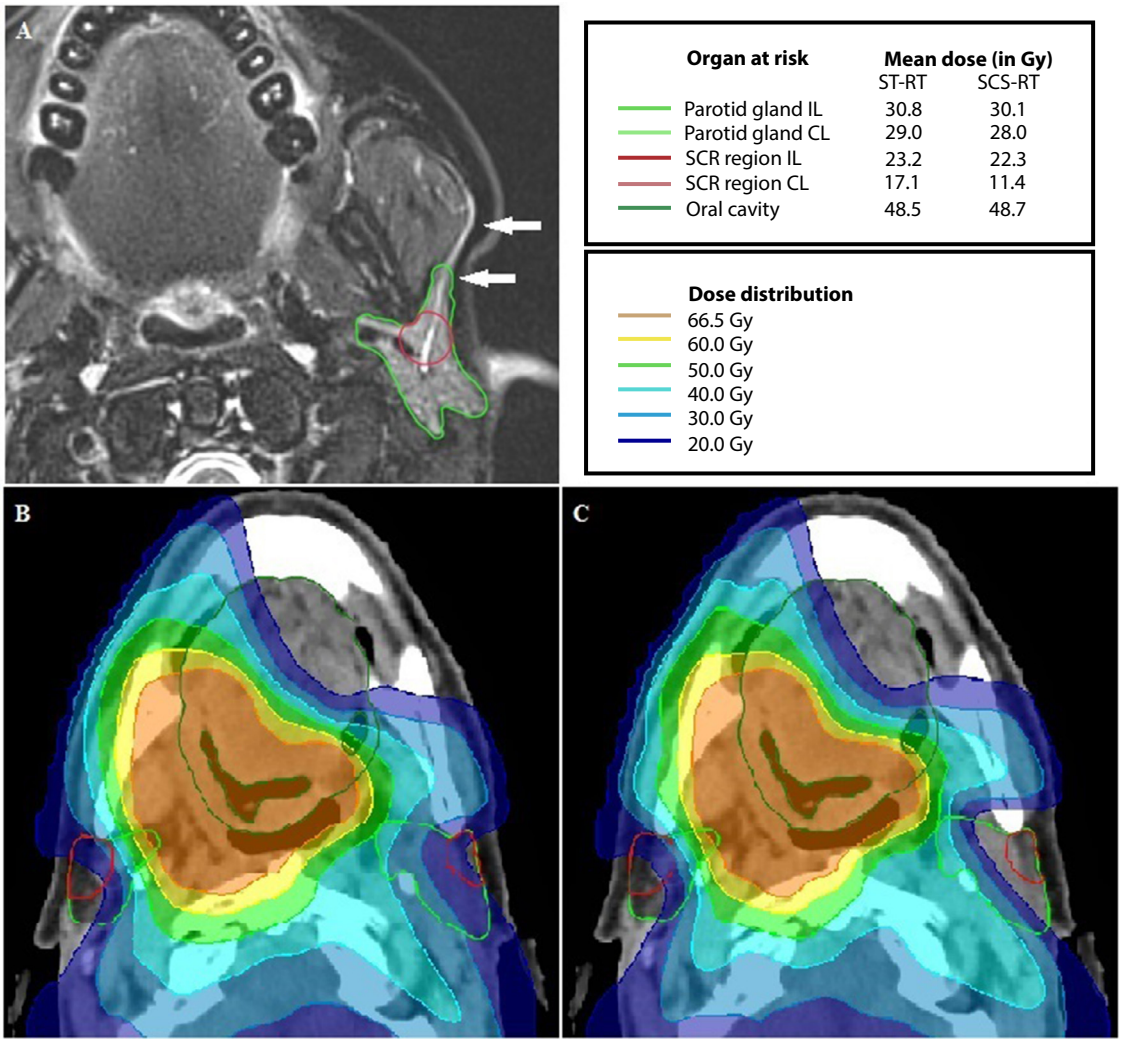

Fig. 1. Sparing the stem cell rich region during radiation therapy. (a) Axial magnetic resonance image (T2-blade with fat suppression) with left parotid gland, automatically generated SCR region and main salivary duct (white arrows). Axial computed tomography image with dose distribution based on (b) standard parotid gland sparing radiation therapy and (c) stem cell sparing radiation therapy. In this example patient, the contralateral SCR region dose was lowered substantially, and the ipsilateral SCR region dose could be reduced to a limited extent. *Abbreviations:* IL = ipsilateral; CL = contralateral; SCR region = stem cell rich region.

Patients with HNC of at least 18 years old and scheduled for curative definitive radiation therapy (70 Gy in 35 fractions) with or without systemic treatment were eligible. Other inclusion criteria were squamous cell carcinoma originating from the mucosa of the head and neck area or nasopharyngeal carcinoma, World Health Organisation performance score between 0 and 2, prophylactic or therapeutic irradiation of both sides of the neck (at least level II to IV), and pretreatment parotid gland saliva production stimulated with 5% citric acid ≥0.2 mL/min.[20] This threshold was adjusted to ≥0.1 mL/min because the pretreatment flow was lower than expected based on historical results in our center. Exclusion criteria were postoperative radiation therapy, previous radiation therapy of the head and neck region, unilateral radiation therapy, and salivary gland tumors.

Patients were enrolled by their treating physician. Next, for every patient a standard whole parotid gland sparing standard radiation therapy (ST-RT) and a parotid gland stem cell sparing radiation therapy (SCS-RT) plan were generated and evaluated by the treating physician. After approval of both treatment plans, patients underwent randomization in a 1:1 ratio by data management using an in-house developed minimization algorithm. The stratification factors were sex, age, tumor location, tumor stage, nodal stage, systemic treatment, and baseline xerostomia score. Only the radiation technologist who prepared the treatment plan for clinical administration was informed by the data manager about the assigned study arm per patient. Thus, physician and medical personnel who had contact with the patient and research personnel involved in the analysis

were blinded for study arm allocation, constituting a double-blind design.

The primary endpoint was defined as more than 75% reduction in parotid gland saliva production compared with pretreatment production at 12 months after radiation therapy. Secondary endpoints were change in relative stimulated parotid gland salivary flow corrected for baseline and calculated as a percentage at 6 and 12 months after treatment. Secondary endpoints also included moderate-to-severe patient-rated xerostomia defined by question 41 of the European Organization for Research and Treatment for Cancer Quality of Life Questionnaire Head and Neck (QLQ-H&N35) ("Do you have a dry mouth?" dichotomized by a 4-point Likert scale: not at all/a bit vs quite a bit/a lot),[21] moderate-to-severe patient-rated daytime and nighttime xerostomia defined by questions 1 and 7 of the Groningen Radiation Induced Xerostomia questionnaire ("Do you experience a dry mouth during daytime?" and "Do you experience a dry mouth during nighttime?" respectively, dichotomized likewise),[22] and grade ≥2 physician-rated xerostomia defined by the Common Terminology Criteria for Adverse Events version 4.0[23] at 6, 12, and 24 months after treatment.

Trial procedures

Before start of treatment, all patients underwent computed tomography (CT), magnetic resonance imaging, and 18F-fluorodeoxyglucose positron-emission tomography with an immobilization mask. Target volumes and OARs were delineated according to international guidelines[24,25] using coregistered CT, magnetic resonance imaging, and 18F-fluorodeoxyglucose positron-emission tomography.

For each parotid gland, the center of the SCR region was identified using a script written in MATLAB R2013a, based on our previous publication.[15] Based on knowledge of the localization of the main duct of the parotid gland, this center was manually corrected in the x-y plane. To this end, it was shifted to a position 0.5 cm in a caudal-lateral direction from the junction of the mandible, the masseter muscle, and parotid gland (Fig. E1A). A volume around this center was generated with a margin of 1 cm in the anteroposterior and lateral-medial directions and 2 cm in the craniocaudal direction (Fig. E1B). The SCR region is defined as the overlap of this oval-shaped volume and the parotid gland volume (Fig. E1C,D).

For every patient, an ST-RT plan and an SCS-RT plan were generated (Fig. 1b,c). The dose to the SCR regions in the SCS-RT plan was reduced as much as possible while keeping the whole mean parotid gland dose the same. No compromises were made on the required dose to the planning target volumes. Details about the radiation therapy regimens used are given in previous studies.[26,27] In summary, radiation therapy consisted of IMRT or VMAT using a simultaneous integrated boost technique to a total prescribed dose of 70 Gy with fractions of 2 Gy in 6 to 7 weeks. All patients received bilateral neck radiation with a prophylactic dose of 54.25 Gy in fractions of 1.55 Gy. Patients with locally advanced disease and <70 years of age who were deemed fit enough received concurrent chemotherapy, or cetuximab if chemotherapy was contraindicated. If given, chemotherapy was administered concurrently with carboplatin on the first day of radiation therapy (300-350 mg/m^2 in 30 minutes) and 5-fluorouracil on day 1 to 4 with continuous infusion (600 mg/m^2/24 hours) or with cisplatin (100 mg/m^2 in 30 minutes). In total, 3 courses of chemotherapy were given with an interval of 3 weeks. Treatment with cetuximab consisted of a loading dose of 400 mg/m^2 1 week before radiation therapy and a weekly dose of 250 mg/m^2 during radiation therapy.

Parotid gland salivary flow measurements were performed with the use of Lashley cups to specifically evaluate parotid gland function. These measurements were performed by well-trained personnel. Before placement of the Lashley cups, the buccal mucosa was dried with gauze. The cups were placed on the exit of Stenson's duct. The lengths of the tubes connecting the cups with the suction pump and the vial were standardized to the preweighed sampling container. Salivary flow was stimulated with 5% (w/v) citric acid, applied with a micropipette (50 μL) to both lateral borders of the tongue at 30-second intervals. Parotid saliva was collected for 10 minutes. The volume of the collected saliva was determined by weight, assuming a specific gravity of 1.0 g/mL.[20,28] Parotid gland salivary flow measurements were performed twice before treatment and averaged and once at 6 and 12 months after treatment. To prevent a potential influence of intravenous fluids on measured salivary production, baseline measurements were performed before the execution of contrast-enhanced CT and the administration of cetuximab loading dose. In addition, follow-up measurements were performed at least 6 months after completion of treatment.

Statistical analysis

All clinical parameters and dose parameters were complete. Clinical parameters were derived from the electronic patient files and dosimetric parameters from the treatment planning system. However, salivary flow measurements and xerostomia endpoints were incomplete due to, for example, tumor recurrence, death, or noncompliance.

Continuous variables were presented as mean ± standard deviation or median (interquartile range), and discrete variables were presented as frequency count (percentage). All analyses were performed 2-sided with a significance level $\alpha = 0.05$. One-way analysis of variance, the Kruskal-Wallis test, and the Fisher's exact test were used to calculate differences in patient and treatment characteristics between treatment arms. Changes in salivary flow were analyzed with one-way analysis of variance and the Fisher's exact test. Correlations between salivary flow and dose were analyzed with Spearman's rank correlation coefficients.

Comparison of prevalence of xerostomia endpoints between study arms was performed using the Fisher's exact tests. Univariable and multivariable analyses of the dichotomized xerostomia endpoints were performed using logistic regression with forward selection, based on the likelihood-ratio test. The following known confounders were considered in multivariable analysis: mean dose to the SCR regions, non-SCR parotid glands (parotid gland minus SCR region), submandibular glands, oral cavity, buccal mucosa, and baseline xerostomia (dichotomized as any vs none). If applicable, OARs were considered contralateral, ipsilateral, and combined. The analyses were performed in RStudio Version 1.1.463 and IBM SPSS Statistics Version 23.

Results

Between September 2013 and May 2017, 249 patients were assessed for eligibility. After exclusion of 143 patients, mostly due to insufficient pretreatment parotid gland salivary flow production, 106 patients were randomized between the 2 study arms (Fig. E2). Four patients were excluded before completion of treatment because of changing treatment to palliative radiation therapy (n = 1) or primary surgery (n = 1), meeting an exclusion criterion due to metastases at restaging (n = 1), or withdrawal of informed consent (n = 1). Fifty-four patients received ST-RT and 48 received SCS-RT. Study patients were followed up to 24 months after treatment. Twelve months after radiation therapy, 6 patients had died and another 3 patients were lost to follow-up because of disease progression in the ST-RT arm. In the SCS-RT arm, 6 patients died. Around 21% of the endpoints were not available at 12 months after treatment (Table E1). This was due to 15% being lost to follow-up and 6% due to noncompliance. Therefore, a total of 88 patients were analyzed (Fig. E2). No harm or unintended effects were observed.

Patients treated with ST-RT experienced more pretreatment patient-rated daytime xerostomia than patients treated with SCS-RT ($P = .01$) (Table 1). In the SCS-RT arm, dose to the SCR region was significantly reduced, as intended, compared with the ST-RT arm (Table 1). The mean dose in the whole parotid gland and other relevant OARs was comparable between the treatment arms (Table E2). As such, because SCR region dose was the only treatment-related risk factor that differed significantly between the arms, the 2 study arms were well balanced, allowing the hypothesized benefit of specifically reducing dose to the SCR region to be tested.

To test the benefit of SCS-RT on parotid gland function, stimulated saliva production was compared. At 12 months after radiation therapy, no difference was observed in the prevalence of reduction in parotid gland saliva production to 75% or more compared with baseline (Table 2). As such, the trial was negative for its primary endpoint. Although the relative parotid gland flow was consistently better in the SCS-RT arm, this difference did not reach significance (Table 2). Finally, apart from moderate-to-severe patient-rated nighttime xerostomia 6 months after treatment ($P = .03$), no significant differences were observed in the prevalence of the other secondary xerostomia endpoints (Figs. E3-6).

The present study showed that ipsilateral parotid gland function was correlated with dose to both SCR regions (Table 3). Contralateral relative parotid gland flow was only significantly correlated with mean dose to the contralateral SCR region at 6 months after radiation therapy (Table 3). However, neither dose to the parotid glands nor dose to their SCR regions was associated with moderate-to-severe patient-rated xerostomia (Tables 4 and E3).

Given the role of the parotid glands during stimulation,[29] daytime xerostomia seems to be a more appropriate endpoint for assessing the impact of parotid gland preservation on patient symptoms. Indeed, univariate analysis showed that dose to the contralateral SCR region was associated with moderate-to-severe patient-rated daytime xerostomia 12 and 24 months after treatment (Table E4). After testing for possible confounders in multivariable analysis, contralateral SCR region dose remained a significant, independent predictor for daytime xerostomia at 12 and 24 months; in the latter, dose to the ipsilateral SCR region remained significant as well (Table 4). After correction for pretreatment daytime xerostomia, dose to the combined SCR regions was the only dosimetric parameter remaining significantly associated with daytime xerostomia 6 months after treatment (Table 4). No significant associations were found between dose to the SCR regions and moderate-to-severe patient-rated nighttime xerostomia (Tables E5 and 4). Finally, in the univariate analysis, dose to the combined SCR regions was associated with grade ≥2 physician-rated xerostomia 6 and 12 months after radiation therapy (Table E6). For treatment plan optimization purposes, more detailed information on the role of the individual SCR regions is needed to determine which SCR region should receive the highest priority during radiation therapy planning. It was observed that dose to the contralateral SCR region was univariately associated with grade ≥2 physician-rated xerostomia at 6, 12, and 24 months, and dose to the ipsilateral SCR region was univariately associated with grade ≥2 physician-rated xerostomia at 12 months after treatment (Table E6). After correction for possible confounders, the contralateral SCR region dose remained an independent predictor for grade ≥2 xerostomia 12 and 24 months after radiation therapy (Table 4). In addition, dose to the submandibular glands and contralateral buccal mucosa were also independent predictors for grade ≥2 xerostomia at 24 months (Table 4).

Discussion

This double-blind randomized controlled trial hypothesized that SCS-RT would preserve parotid gland function better than ST-RT. Although a trend toward better parotid gland

Table 1 Baseline characteristics of patients

	ST-RT n = 54	SCS-RT n = 48	*P* value
Clinical parameters			
Sex (n [%])			.39*
Female	18 (33)	12 (25)	
Male	36 (67)	36 (7)	
Age at diagnosis (mean ± SD), y	61 ± 10	63 ± 11	.36†
WHO PS (n [%])			.35*
WHO PS 0	39 (72)	39 (81)	
WHO PS 1-2	15 (28)	9 (19)	
Tumor location (n [%])			.21*
Oropharynx	34 (63)	36 (75)	
Other	20 (37)	12 (25)	
Tumor stage (n [%])			.69*
T0-T2	26 (48)	21 (44)	
T3-T4	28 (52)	27 (56)	
Nodal stage (n [%])			.57*
N0	6 (11)	8 (17)	
N1-N3	48 (89)	40 (83)	
Clinical tumor stage (n [%])			.28*
Stage I-II	6 (11)	2 (4)	
Stage III-IV	48 (89)	46 (96)	
Treatment modality (n [%])			.54*
Radiation therapy alone	17 (31)	18 (38)	
Radiation therapy with systemic treatment	37 (69)	30 (62)	
Xerostomia before radiation therapy			
Patient-rated xerostomia (n [%])			.07*
None	28 (52)	33 (69)	
Some	26 (48)	14 (29)	
Unknown	0 (0)	1 (2)	
Patient-rated daytime xerostomia (n [%])			.01*,§
None	31 (58)	37 (77)	
Some	19 (35)	6 (13)	
Unknown	4 (7)	5 (10)	
Patient-rated nighttime xerostomia (n [%])			.10*
None	22 (41)	27 (56)	
Some	28 (52)	17 (36)	
Unknown	4 (7)	4 (8)	
Physician-rated xerostomia (n [%])			1.00*
Grade 0	44 (81)	40 (83)	
Grade ≥1	10 (19))	8 (17)	
Mean dose (in Gy) to parotid gland structures			
Parotid gland contralateral (mean ± SD)	23 ± 8.3	23 ± 7.0	.70†
Parotid gland ipsilateral (mean ± SD)	32 ± 11	31 ± 10	.68†
Parotid glands (mean ± SD)	28 ± 8.6	27 ± 7.5	.63†
SCR region contralateral (median [IQR])	16 (13)	11 (5.4)	.004‡,§
SCR region ipsilateral (median [IQR])	26 (17)	16 (10)	.001‡,§
SCR regions (median [IQR])	22 (13)	14 (7.7)	.001‡,§

Abbreviations: ANOVA = analysis of variance; CTCAE = Common Terminology Criteria for Adverse Events; EORTC = European Organization for Research and Treatment for Cancer; GRIX = Groningen Radiation Induced Xerostomia; IQR = interquartile range; SCR = stem cell rich; SCS-RT = stem cell sparing radiation therapy; SD = standard deviation; ST-RT = standard parotid gland sparing radiation therapy; QLQ-H&N35 = Quality of Life Questionnaire Head and Neck; WHO PS = World Health Organization performance score.

Patient-rated xerostomia according to EORTC QLQ-H&N35 question 41[21]; patient-rated daytime and nighttime xerostomia according to GRIX questions 1 and 7[22]; physician-rated xerostomia according to the CTCAE v4.0.[23]

* Fisher's exact test

† One-way ANOVA

‡ Kruskal-Wallis test

§ Significant, $\alpha \leq 0.05$.

Table 2 Changes in stimulated parotid gland salivary flow

	ST-RT n		SCS-RT n		*P* value
At 6 mo after RT, contralateral parotid gland					
Difference in relative flow rate (mean [SEM]) (%)	48	−12 (10)	38	19 (15)	.08*
Reduction to <25% of baseline function (n [%])	48	5 (10)	38	3 (7.7)	.73†
At 6 mo after RT, ipsilateral parotid gland					
Difference in relative flow rate (mean [SEM]) (%)	48	−35 (8.4)	39	−17 (13)	.23*
Reduction to <25% of baseline function (n [%])	48	12 (25)	39	9 (24)	1.00†
At 6 mo after RT, parotid glands together					
Difference in relative flow rate (mean [SEM]) (%)	48	−26 (7.7)	38	−1.9 (12)	.08*
Reduction to <25% of baseline function (n [%])	48	7 (15)	38	3 (7.9)	.50†
At 12 mo after RT, contralateral parotid gland					
Difference in relative flow rate (mean [SEM]) (%)	43	8.2 (13)	38	16 (14)	.70*
Reduction to <25% of baseline function (n [%])	43	1 (2.3)	38	1 (2.6)	1.00†
At 12 mo after RT, ipsilateral parotid gland					
Difference in relative flow rate (mean [SEM]) (%)	42	−21 (10)	36	−16 (8.5)	.74*
Reduction to <25% of baseline function (n [%])	42	8 (19)	36	3 (8.3)	.21†
At 12 mo after RT, parotid glands together					
Difference in relative flow rate (mean [SEM]) (%)	41	−13 (10)	36	−2.9 (10)	.50*
Reduction to <25% of baseline function (n [%])	41	2 (4.9)	36	1 (2.8)	1.00†

Abbreviations: ANOVA = analysis of variance; RT = radiation therapy; SCS-RT = stem cell sparing radiation therapy; SEM = standard error of the mean; ST-RT = standard parotid gland sparing radiation therapy.

Relative parotid gland salivary flow (mL/min) was corrected for baseline and calculated as a percentage.

* One-way ANOVA test

† Fisher's exact test

salivary flow in patients treated with SCS-RT was shown, no difference in prevalence of ≥75% reduction in stimulated parotid gland saliva production between SCS-RT and ST-RT was shown. As such, the study was negative for the primary endpoint.

Several factors may have contributed to the lack of significant differences in salivary flow between the study arms. First, pretreatment parotid gland salivary flow was lower than observed in our historical cohort used for the initial power analysis. Second, the mean dose to the parotid glands and consequently the SCR regions was relatively low. In both the study used for the sample size calculation[30] and the study used for the definition of the primary endpoint,[1] the parotid glands received around 40 Gy, whereas in the current study the mean dose in the parotid glands was 27 Gy. The rapid changes in radiation techniques (eg, the transition to different techniques, such as IMRT/VMAT[8,9] or intensity modulated proton therapy[11,12]) or the possibilities of adaptive radiation therapy[10] and dose escalation[31] resulting in lower doses to OARs are possible explanations and known issues in the field of radiation therapy research and probably affecting the results.[32] This might have

Table 3 Decreased relative parotid gland salivary flow is correlated with increased mean dose to the parotid glands and SCR regions

	Mean dose to the parotid glands			Mean dose to the SCR regions		
	CL	IL	Combined	CL	IL	Combined
Contralateral parotid gland						
Difference (%) relative flow, M06	−0.30 (*P* = .005*)	−0.09 (*P* = .41)	−0.17 (*P* = .12)	−0.27 (*P* = .02*)	−0.11 (*P* = .33)	−0.17 (*P* = .12)
Difference (%) relative flow, M12	−0.14 (*P* = .20)	0.00 (*P* = .98)	−0.03 (*P* = .80)	−0.08 (*P* = .50)	−0.04 (*P* = .74)	−0.07 (*P* = .55)
Ipsilateral parotid gland						
Difference (%) relative flow, M06	−0.37 (*P* < .001*)	−0.47 (*P* < .001*)	−0.49 (*P* < .001*)	−0.40 (*P* < .001*)	−0.44 (*P* < .001*)	−0.49 (*P* < .001*)
Difference (%) relative flow, M12	−0.20 (*P* = .09)	−0.40 (*P* < .001*)	−0.36 (*P* = .001*)	−0.23 (*P* = .05*)	−0.39 (*P* < .001*)	−0.41 (*P* < .001*)

Abbreviations: CL = contralateral; IL = ipsilateral; M06 = 6 months after treatment; M12 = 12 months after treatment; SCR = stem cell rich.

* Significant according to Spearman's rank correlation rho, $\alpha \leq 0.05$.

Table 4 Multivariable analysis of the xerostomia endpoints

	M06	M12	M24
Moderate-to-severe patient-rated xerostomia	n = 84, 50 events	n = 81, 39 events	n = 71, 30 events
Pretreatment patient-rated xerostomia (any)	3.00 (1.14-7.89)*	4.28 (1.62-11.3)†	-
Mean dose to submandibular gland IL	-	-	0.94 (0.88-1.01)
Mean dose to buccal mucosa CL	-	-	1.09 (1.02-1.15)†
Moderate-to-severe patient-rated daytime xerostomia	n = 78, 36 events	n = 75, 25 events	n = 67, 18 events
Pretreatment daytime xerostomia (any)	5.13 (1.66-15.9)†	-	-
Mean dose to SCR regions	1.06 (1.01-1.12)*	-	-
Mean dose to SCR region CL	-	1.11 (1.02-1.21)*	1.34 (1.12-1.59)‡
Mean dose to SCR region IL	-	-	0.93 (0.86-1.00)*
Moderate-to-severe patient-rated nighttime xerostomia	n = 78, 45 events	n = 76, 33 events	n = 67, 27 events
Pretreatment nighttime xerostomia (any)	3.95 (1.53-10.2)†	3.38 (1.30-8.74)*	5.87 (1.88-18.3)†
Mean dose to submandibular glands	-	-	1.13 (1.00-1.29)
Grade ≥2 physician-rated xerostomia	n = 88, 28 events	n = 84, 17 events	n = 66, 11 events
Pretreatment physician-rated xerostomia (any)	5.32 (1.56-18.2)†	-	-
Mean dose to non-SCR parotid gland CL	1.09 (1.03-1.16)†	-	-
Mean dose to SCR region CL	-	1.11 (1.02-1.20)*	1.20 (1.04-1.39)*
Mean dose to submandibular glands	-	-	0.84 (0.72-1.00)*
Mean dose to buccal mucosa CL	-	-	1.15 (1.04-1.28)†

Abbreviations: CL = contralateral; CTCAE = Common Terminology Criteria for Adverse Events; EORTC = European Organization for Research and Treatment for Cancer; GRIX = Groningen Radiation Induced Xerostomia; IL = ipsilateral; M06, M12, and M24 = 6, 12, and 24 months after treatment; QLQ-H&N35 = Quality of Life Questionnaire Head and Neck; SCR = stem cell rich.

Odds ratios with 95% confidence interval. Patient-rated xerostomia according to EORTC QLQ-H&N35 question 41[21]; patient-rated daytime and nighttime xerostomia according to GRIX questions 1 and 7[22]; physician-rated xerostomia according to the CTCAE v4.0.[23]

Significant after logistic regression (forward selection, likelihood-ratio test).

* $P \leq .05$

† $P \leq .01$

‡ $P \leq .001$

reduced the loss of salivary flow rate in the current study (Table 2) compared with the 80% reduction as described by Burlage et al.[1] Third, the challenges of a randomized controlled trial for testing approaches to reduce radiation-induced side effects[32] might partially explain the absence of significant differences between the 2 study arms. Intra-arm variability is known to reduce the power for detecting differences between study arms of randomized controlled trials.[33] In addition to variation in response to the intervention, studies aiming to spare normal tissues in radiation therapy suffer from an inherent variation in the dose to OARs. In the present study, this led to strongly overlapping distributions of dose in the SCR regions between the 2 study arms (eg, dose to the contralateral SCR region ranged from 5.9-31 Gy and 4.5-39.4 Gy, respectively, in the ST-RT and the SCS-RT arms). This is different from, for example, drug-testing studies in which patients either do or do not receive the investigational product or dose-escalation studies in which patients receive either the current treatment dose or the escalated treatment dose. The inherent intra-arm variation in radiation therapy studies aiming at proving the efficacy of strategies of sparing normal tissues reduces the power of these studies. Therefore, multivariable analysis is a useful alternative to investigate the impact of dose to an OAR on outcome.

In preclinical models, the radiation response of parotid glands critically depended on dose to the stem cells, primarily located in the main salivary gland ducts.[15] The present study showed that dose to this SCR region can be significantly lowered while keeping the dose to the whole parotid gland the same.

Moderate-to-severe patient-rated xerostomia (assessed by the question "Do you have a dry mouth?"[21]) was not associated with dose to the SCR regions. However, the sensation of oral dryness is caused by a lack or change in salivary mucins, part of the mucinous saliva produced by salivary glands other than the parotid glands.[34] The parotid glands produce serous saliva and are mainly responsible for stimulated salivary flow.[29] Therefore, this question might be too general to allow evaluation of the impact of better preservation of the parotid gland by reducing dose to the SCR region. The role of the parotid glands is primarily during eating and drinking.[29] Because grade ≥2 physician-rated xerostomia included the impact of xerostomia on alterations in the oral intake,[23] this endpoint might be more parotid gland function specific than general patient-rated xerostomia.

In addition, the need to consider the different aspects of xerostomia separately was shown in relation to salivary flow. Dijkema et al[35] demonstrated that parotid saliva production was associated with daytime xerostomia 1 year after radiation therapy, whereas submandibular saliva production was associated with nighttime xerostomia. Therefore, we investigated moderate-to-severe patient-rated

daytime xerostomia according to the Groningen Radiation Induced Xerostomia questionnaire.[22] Indeed, in line with previous work,[15] multivariable analysis showed dose to the contralateral SCR region to be a stronger dosimetric predictor than dose to the entire parotid gland for both of these endpoints at 12 and 24 months after treatment (Table 4). Furthermore, the impact of the contralateral SCR region on development of xerostomia became larger over time (Table 4). This is in line with the observation in mice and rats that regeneration of acinar cells by progenitor cells and stem cells was only visible after longer latency times.[15,36]

In addition, for daytime xerostomia an association was found for dose to the combined SCR regions at 6 months after treatment (Table 4). At 24 months, an odds ratio smaller than 1 was found for the ipsilateral SCR region (Table 4). This may have resulted from the decision to power the current study for 12 months after treatment. Therefore, fewer patients and events were available at 24 months after treatment (Tables E3-6 and 4), resulting in common statistical issues for multivariable analysis (eg, model instability). As such, in general, the results from the analysis of data obtained at 24 months after treatment should be interpreted with caution. The same statistical issues applied to patient-rated xerostomia and grade $\geq$2 physician-rated xerostomia at 24 months, resulting in odds ratios smaller than 1 for, respectively, the predictors dose to the ipsilateral submandibular glands and dose to the combined submandibular glands (Table 4).

Finally, we found a dose-effect relation between dose to the SCR regions and ipsilateral parotid function loss at 6 and 12 months after radiation therapy. In addition, such a relationship was found for contralateral relative parotid gland flow and dose to the contralateral SCR region at 6 months after radiation therapy (Table 3). Moreover, dose to the contralateral SCR region was most predictive for experiencing daytime xerostomia (Table 4). These results may suggest that xerostomia is only experienced if damage to the contralateral gland, by dose to the contralateral SCR region, prevents it from compensating for loss of function of the ipsilateral parotid gland. In addition, in the entire study population a significant correlation between reduced relative ipsilateral parotid gland flow and increased relative contralateral parotid gland flow was present at 12 months after treatment (R^2 value 0.48) (Fig. E7). A potential role for such compensation by a contralateral salivary gland was suggested earlier for the submandibular glands in rats after surgery[37] and for the parotid and submandibular glands in patients after radiation therapy.[38]

Elucidating the role of specific structures in the development of side effects

Multiple OARs were found to be relevant for the development of xerostomia. The parotid glands, the submandibular glands, the oral cavity, and specific regions of the parotid glands were investigated.[8,15,38-46] The next step is prioritization of relevant OARs for treatment optimization, currently often determined using a prospective or retrospective cohort study. However, a major issue in elucidating the role of a potential structure is collinearity between doses to different OARs.[47] Therefore, it is often difficult, and sometimes even impossible, to elucidate the role of a specific structure in the development of side effects.

The current randomized clinical study solved this problem by reducing collinearity between the SCR region and the entire parotid gland in the administered treatment plan. As a result, the correlation coefficients in our study cohort were 0.63 and 0.75, respectively, for the contralateral and ipsilateral SCR region and parotid gland, compared with R^2 values of 0.80 and 0.88, and 0.81 and 0.86 in, respectively, 97 patients 1 year before and 96 patients 1 year after the current study treated with ST-RT in our center (Fig. E8). This made it possible to use multivariable analysis to elucidate the contributions of the doses to the SCR regions and the entire parotid glands to the development of xerostomia.

Sparing the contralateral stem cell dense region to prevent xerostomia

The selection of the contralateral SCR region as most predictive for xerostomia (Table 4) implies the importance of further lowering dose to the SCR region already receiving a low dose (Table 1). This was supported by laboratory and modeling research.[18,42,44,48,49] Van Luijk et al[18] demonstrated a bath and shower effect in the parotid glands: Administration of a low-dose bath to the cranial part containing the SCR region of the glands and a high-dose shower to the caudal part of the glands resulted in significantly more function loss than irradiating the caudal part alone. Nagle et al[48] confirmed this effect by showing that human stem cells were disproportionally affected by low doses. In addition, Han et al[42] suggested that injury (ie, the development of side effects) is induced by parotid subvolumes receiving higher dose, whereas the ability to recover can be sustained by further reducing dose to parotid subvolumes receiving lower dose. Jiang et al[44] and Robertson et al[49] confirmed the important role of low dose to the parotid glands for development of xerostomia. This decrease of the low-dose bath is particularly possible for the contralateral SCR region, because dose is already low and, though sometimes limited, can be lowered more easily considering the distance to the target volumes.

However, aside from dose to the contralateral SCR region, dose to other OARs, pretreatment xerostomia, and additional clinical risk factors (eg, age, tumor location and classification, and administration of systemic therapy) are also relevant for the development of xerostomia.[8,15,38-46] Therefore, the development of normal tissue complication probability models for the different xerostomia endpoints, considering the role of the SCR region in addition to other risk factors, will contribute to prioritizing the sparing of

different OARs and might be an aid for the selection of treatment techniques, such as adaptive radiation therapy or proton therapy.[10,11]

Sparing subvolumes of the parotid glands

Finally, in other studies, associations between dose to parotid gland subvolumes and parotid function were also investigated (ie, salivary flow and/or xerostomia).[42-44,50] Using an unbiased approach, studies found several subvolumes related to optimal preservation of parotid function, such as the lateral and cranial portion,[43] superior[42,44] and middle-anterior[42] portion of the contralateral parotid gland, superoposterior portion of the ipsilateral parotid gland,[42]and caudal-anterior portion.[50] In addition, Miah et al[45] showed that bilateral superficial lobe parotid-sparing IMRT reduced the risk of developing xerostomia compared with contralateral parotid-sparing IMRT. All of these subvolumes contained the SCR region as defined in the present study based on an explanatory mechanism from the radiobiology studies.[15,17] Moreover, Beuttner et al[43] were able to confirm the bath-and-shower effect of parotid glands in patients, as earlier shown by van Luijk et al[18] in rats. They were able to show that a relative high dose to the medial-caudal part of the parotid gland is preferred over a homogenous low-dose bath in the entire parotid gland,[43] confirming the importance of sparing the cranial part containing the major ducts, as shown in our current and previous work.[15]

Conclusions

To our knowledge, no other study has evaluated the sparing of a subvolume of the parotid glands by performing a double-blind randomized controlled trial. The primary study outcome was negative. In addition, no significant differences were observed between the study arms for the secondary endpoints. However, we were able to confirm in multivariable analysis that mean dose to this SCR region was the strongest dosimetric predictor for side effect endpoints specific for parotid function—such as, patient-rated daytime xerostomia and physician-rated grade ≥2 xerostomia, the latter including alterations in oral intake.

References

1. Burlage FR, Coppes RP, Meertens H, Stokman MA, Vissink A. Parotid and submandibular/sublingual salivary flow during high dose radiotherapy. *Radiother Oncol* 2001;61:271–274.
2. Sciubba JJ, Goldenberg D. Oral complications of radiotherapy. *Lancet Oncol* 2006;7:175–183.
3. Langendijk JA, Doornaert PAH, Leeuw IM de, Leemans CR, Aaronson NK, Slotman BJ. Impact of late treatment-related toxicity on quality of life among patients with head and neck cancer treated with radiotherapy. *J Clin Oncol* 2008;26:3770–3776.
4. Beetz I, Schilstra C, Schaaf A van der, et al. NTCP models for patient-rated xerostomia and sticky saliva after treatment with intensity modulated radiotherapy for head and neck cancer: The role of dosimetric and clinical factors. *Radiother Oncol* 2012;105:101–106.
5. Dijkema T, Raaijmakers CPJ, Ten Haken RK, et al. Parotid gland function after radiotherapy: The combined Michigan and Utrecht experience. *Int J Radiat Oncol Biol Phys* 2010;78:449–453.
6. Eisbruch A. IMRT reduces xerostomia and potentially improves QoL. *Nat Rev Clin Oncol* 2009;6:567–568.
7. Beetz I, Steenbakkers RJHM, Chouvalova O, et al. The QUANTEC criteria for parotid gland dose and their efficacy to prevent moderate to severe patient-rated xerostomia. *Acta Oncologica* 2014;53:597–604.
8. Nutting CM, Morden JP, Harrington KJ, et al. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): A phase 3 multicentre randomised controlled trial. *Lancet Oncol* 2011;12:127–136.
9. Osborn J. Is VMAT beneficial for patients undergoing radiotherapy to the head and neck? *Radiography* 2017;23:73–76.
10. Brouwer CL, Steenbakkers RJHM, Langendijk JA, Sijtsema NM. Identifying patients who may benefit from adaptive radiotherapy: Does the literature on anatomic and dosimetric changes in head and neck organs at risk during radiotherapy provide information to help? *Radiother Oncol* 2015;115:285–294.
11. Water TA van de, Lomax AJ, Bijl HP, et al. Potential benefits of scanned intensity-modulated proton therapy versus advanced photon therapy with regard to sparing of the salivary glands in oropharyngeal cancer. *Int J Radiat Oncol Biol Phys* 2011;79:1216–1224.
12. Dijk LV van, Steenbakkers RJHM, ten Haken B, et al. Robust intensity modulated proton therapy (IMPT) increases estimated clinical benefit in head and neck cancer patients. *PLOS ONE* 2016;11 e0152477.
13. Liu G, Li X, Qin A, et al. Improve the dosimetric outcome in bilateral head and neck cancer (HNC) treatment using spot-scanning proton arc (SPArc) therapy: A feasibility study. *Radiat Oncol* 2020;15:21.
14. Li Y, Taylor JMG, Ten Haken RK, Eisbruch A. The impact of dose on parotid salivary recovery in head and neck cancer patients treated with radiation therapy. *Int J Radiat Oncol Biol Phys* 2007;67:660–669.
15. Luijk P van, Pringle S, Deasy JO, et al. Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer. *Science Translational Medicine* 2015;7:305ra147.
16. Martinez PS, Cinat D, Luijk P van, et al. Mouse parotid salivary gland organoids for the in vitro study of stem cell radiation response. *Oral Dis* 2021;27:52–63.
17. Konings AWT, Cotteleer F, Faber H, Luijk P van, Meertens H, Coppes RP. Volume effects and region-dependent radiosensitivity of the parotid gland. *Int J Radiat Oncol Biol Phys* 2005;62:1090–1095.
18. Luijk P van, Faber H, Schippers JM, et al. Bath and shower effects in the rat parotid gland explain increased relative risk of parotid gland dysfunction after intensity-modulated radiotherapy. *Int J Radiat Oncol Biol Phys* 2009;74:1002–1005.
19. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA* 2013;310:2191–2194.
20. Burlage FR, Pijpe J, Coppes RP, et al. Variability of flow rate when collecting stimulated human parotid saliva. *Eur J Oral Sci* 2005;113:386–390.
21. Bjordal K, Graeff A de, Fayers PM, et al. A 12 country field study of the EORTC QLQ-C30 (version 3.0) and the head and neck cancer specific module (EORTC QLQ-H and N35) in head and neck patients. *Eur J Cancer* 2000;36:1796–1807.
22. Beetz I, Burlage FR, Bijl HP, et al. The Groningen Radiotherapy-Induced Xerostomia questionnaire: Development and validation of a new questionnaire. *Radiother Oncol* 2010;97:127–131.
23. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. 2009. Available at: https://www.eortc.be/services/doc/ctc/ctcae_4.03_2010-06-14_quickreference_5x7.pdf. Accessed March 19, 2021.

24. Grégoire V, Ang K, Budach W, et al. Delineation of the neck node levels for head and neck tumors: A 2013 update. DAHANCA, EORTC, HKNPCSG, NCIC CTG, NCRI, RTOG, TROG consensus guidelines. *Radiother Oncol* 2014;110:172–181.
25. Brouwer CL, Steenbakkers RJHM, Bourhis J, et al. CT-based delineation of organs at risk in the head and neck region: DAHANCA, EORTC, GORTEC, HKNPCSG, NCIC CTG, NCRI, NRG Oncology and TROG consensus guidelines. *Radiother Oncol* 2015;117:83–90.
26. van der Laan HP, Christianen MEMC, Bijl HP, Schilstra C, Langendijk JA. The potential benefit of swallowing sparing intensity modulated radiotherapy to reduce swallowing dysfunction: An in silico planning comparative study. *Radiother Oncol* 2012;103:76–81.
27. Christianen MEMC, Schilstra C, Beetz I, et al. Predictive modelling for swallowing dysfunction after primary (chemo)radiation: Results of a prospective observational study. *Radiother Oncol* 2012;105:107–114.
28. Vissink A, Wolff A, Veerman E. Saliva collectors. In: Wong DT, ed. *Saliva Diagnostics*. Hoboken: Wiley-Blackwell; 2008:37–59.
29. Aps JKM, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. *For Sci Int* 2005;150:119–131.
30. Burlage FR, Roesink JM, Kampinga HH, et al. Protection of salivary function by concomitant pilocarpine during radiotherapy: A double-blind, randomized, placebo-controlled study. *Int J Radiat Oncol Biol Phys* 2008;70:14–22.
31. Atwell D, Elks J, Cahill K, et al. A review of modern radiation therapy dose escalation in locally advanced head and neck cancer. *Clin Oncol* 2020;32:330–341.
32. Pignol J, Janus C. The evaluation of innovation in radiation oncology – what can we do and what should we do? *Acta Oncol* 2015;54:1251–1253.
33. Vamvakas EC. Rationale, objectives, and interpretation of randomized controlled trials. *J Clin Aph* 1997;12:130–139.
34. Tabak LA. Structure and function of human salivary mucins. *Crit Rev Oral Biol Med* 1990;1:229–234.
35. Dijkema T, Raaijmakers CPJ, Braam PM, Roesink JM, Monninkhof EM, Terhaard CHJ. Xerostomia: A day and night difference. *Radiother Oncol* 2012;104:219–223.
36. Lombaert IMA, Brunsting JF, Wierenga PK, et al. Rescue of salivary gland function after stem cell transplantation in irradiated glands. *PLOS ONE* 2008;3:e2063.
37. Schwartz-Arad D, Michaelp Y, Zajicek G. Compensatory hyperplasia of the rat submandibular gland following unilateral extirpation. *J Dental Res* 1991;70:1328–1331.
38. Eisbruch A, Kim HM, Terrell JE, Marsh LH, Dawson LA, Ship JA. Xerostomia and its predictors following parotid-sparing irradiation of head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 2001;50:695–704.
39. Van den Bosch L, Schaaf A van der, Laan HP van der, et al. Comprehensive toxicity risk profiling in radiation therapy for head and neck cancer: A new concept for individually optimised treatment. *Radiother Oncol* 2021;157:161–162.
40. Little M, Schipper M, Feng FY, et al. Reducing xerostomia after chemo-IMRT for head-and-neck cancer: Beyond sparing the parotid glands. *Int J Radiat Oncol Biol Phys* 2012;83:1007–1014.
41. Hawkins PG, Lee JY, Mao Y, et al. Sparing all salivary glands with IMRT for head and neck cancer: Longitudinal study of patient-reported xerostomia and head-and-neck quality of life. *Radiother Oncol* 2018;126:68–74.
42. Han P, Lakshminarayanan P, Jiang W, et al. Dose/volume histogram patterns in salivary gland subvolumes influence xerostomia injury and recovery. *Sci Rep* 2019;9:3616.
43. Buettner F, Miah AB, Gulliford SL, et al. Novel approaches to improve the therapeutic index of head and neck radiotherapy: An analysis of data from the PARSPORT randomised phase III trial. *Radiother Oncol* 2012;103:82–87.
44. Jiang W, Lakshminarayanan P, Hui X, et al. Machine learning methods uncover radiomorphologic dose patterns in salivary glands that predict xerostomia in patients with head and neck cancer. *Adv Radiat Oncol* 2018;4:401–412.
45. Miah AB, Gulliford SL, Morden J, et al. Recovery of salivary function: Contralateral parotid-sparing intensity-modulated radiotherapy versus bilateral superficial lobe parotid-sparing intensity-modulated radiotherapy. *Clin Oncol* 2016;28:69.
46. Sari SY, Yilmaz MT, Elmali A, et al. Parotid gland stem cells: Mini yet mighty. *Head & Neck* 2020;43:1122–1127.
47. Luijk P van, Langendijk JA, Coppes RP. Understanding mechanisms yields novel approaches to reduce radiotherapy-related xerostomia. *Ann Transl Med* 2017;5:27.
48. Nagle PW, Hosper NA, Barazzuol L, et al. Lack of DNA damage response at low radiation doses in adult stem cells contributes to organ dysfunction. *Clin Cancer Res* 2018;24:6583–6593.
49. Robertson SP, Quon H, Kiess AP, et al. A data-mining framework for large scale analysis of dose-outcome relationships in a database of irradiated head and neck cancer patients. *Med Phys* 2015;42:4329–4337.
50. Clark HD, Thomas SD, Reinsberg SA, Moiseenko VV, Hovan AJ, Wu JS. Heterogeneous radiotherapy dose-outcomes response in parotid glands. *Con Sci Phys Oncol* 2018;4 035001.