

Radiotherapy induced xerostomia, pre-clinical promise of LMS-611

Author(s): Paterson C ^[1], Caldwell B ^[1], Porteous S ^[2], McLean A ^[2], Messow CM ^[3],

Thomson M ^[1]

[1]

Beatson West of Scotland Cancer Centre
1053 Great Western Road
Glasgow
G12 0YN

[2]

Lamellar Biomedical Limited
Caledonian House
Phoenix Crescent
Bellshill
ML4 3NJ

[3]

Robertson Centre for Biostatistics
University of Glasgow
Boyd Orr Building
University Avenue
Glasgow
G12 8QQ

Corresponding author:

Claire Paterson
Beatson West of Scotland Cancer Centre
1053 Great Western Road
Glasgow
G12 0YN
UK

Claire.paterson2@ggc.scot.nhs.uk

Telephone 00 44 141 301 7068

ABSTRACT

PURPOSE

Radiotherapy induced xerostomia (RIX) is the most common permanent side effect of radiotherapy (RT) to the head and neck (H&N).

There is no effective topical treatment.

LMS-611 is a mimetic of a natural lamellar body which prevents thick secretions like saliva from congesting organs.

Primary objective - assess saliva properties before and during RT to the H&N.

Secondary objectives - re-assess saliva properties with the addition of LMS-611, measure inter-patient variability, correlate patient reported symptoms with laboratory measurements and design subsequent first-in-human clinical trial of LMS-611.

METHODS

Patients with H&N cancer receiving RT as primary treatment were recruited.

Patients completed the Groningen RIX (GRIX) questionnaire and provided saliva samples at baseline, weeks 2, 4 and 6 of RT. Saliva adhesiveness and viscosity was tested by measuring time taken to travel 5cm down an inclined plane.

RESULTS

30 patients were enrolled.

The inclined plane test (IPT) results (seconds) were as follows: baseline 31.3, week-2: 49.7, week-4: 51.1, week-6: 55.7. Wide inter-patient variability was seen at baseline. GRIX scores increased as RT progressed. Spearman rank correlation coefficient of inclined plane tests with GRIX scores was -0.06 at baseline, week-2 0.25, week-4 0.12 and week-6 0.08.

LMS-611 concentrations of 10mg/ml and 20mg/ml significantly reduced IPT times on saliva samples.

CONCLUSIONS

Saliva becomes more visco-adhesive and RIX worsens as RT progresses. There is little correlation between objective and subjective measures of RIX. The addition of LMS-611 to thick, sticky saliva restores its fluidity ex-vivo. This warrants in-vivo analysis of the effect of LMS-611 upon RIX.

KEYWORDS

Radiation induced xerostomia; LMS-611; Visco-ease; GRIX; RIX;

INTRODUCTION

Radiotherapy (RT) or chemo radiotherapy (CRT) is well established as an alternative to surgery in squamous cell carcinoma (SCC) of the head & neck (H&N), with the dual aims of tumour cure and organ preservation [1]. Unfortunately, high doses of radiation are needed for tumour control; so long term sequelae of radiotherapy are frequently observed and impact significantly upon patients' quality of life [2-4].

Radiotherapy induced xerostomia (RIX) is the most commonly reported late and permanent side effect of RT to the H&N [5]. RT preferentially damages the fluid secreting serous cells, rather than the mucin secreting cells, of the salivary glands, so patients experience a build-up of thick, sticky mucus and a dry mouth [6]. This can cause discomfort, taste alteration, speech and swallowing difficulties and accelerates dental caries [7].

There is currently no effective topical treatment for RIX and a Cochrane review (2011) concluded that '*Well designed, adequately powered randomized controlled trials of topical interventions for dry mouth are required to provide evidence to guide clinical care*' [8].

The changing epidemiology of H&N cancer, mainly due to a rise in oropharyngeal cancer caused by human papilloma virus means that patients are often younger with little co-morbidity [9].

This group have a significantly improved response to treatment and overall survival [10-12] and will therefore live much longer with the consequences of treatment. [13, 14] With no effective topical agent, there remains an unmet clinical need for this group who will experience RIX to some degree over a long period of time.

Reducing xerostomia with parotid sparing intensity modulated radiotherapy (IMRT) has resulted in modest improvements in observer-rated and patient reported xerostomia. Despite this, grade 2 (Radiation Therapy Oncology Group scale) or worse, xerostomia rates of 40% are typical at 12 months post IMRT. [15, 16] Clinically significant RIX remains a problem therefore for many patients.

Lamellar bodies have surface active properties and are an essential lubricant of the body's tissues, preventing mucosal surfaces from sticking to each other and sticky secretions, like mucous and thick saliva, from congesting the hollow organs. LMS-611 is a multi-lipid mimetic of a naturally occurring lamellar body with an identical 3D microstructure and biophysical properties to the natural substance. A small, pilot, ex-vivo study, has previously shown that LMS-611 has the potential to reduce the 'stickiness' of oral cavity secretions from patients following radiation for H&N cancer [unpublished data] with its mode of action being biophysical rather than pharmacological.

This pre-clinical study of LMS-611 was designed as an ex-vivo, proof of concept study and as a preparatory step towards a clinical study of LMS-611 in H&N cancer patients with RIX.

MATERIALS & METHODS

Participants

Patients with H&N cancer, who were scheduled to commence radical RT or CRT as primary treatment, were recruited to this single centre study.

Eligible patients were 18 years or older and were judged to be at high risk of radiation induced xerostomia. Exclusion criteria included known pre-existing xerostomia, use of any other investigational drug or product within 30 days and primary surgery (other than neck dissection alone) for SCC H&N.

The protocol was approved by the national South West Wales Research Ethics Committee (MREC 13/WA/0153). Written informed consent was obtained from all participants. The study was sponsored by NHS Greater Glasgow and Clyde and funded by Lamellar Biomedical Limited (LBL). The study was conducted according to the principles of Good Clinical Practice and the 1964 Declaration of Helsinki.

Procedures

All patients received radical RT or CRT delivered with volumetric modulated arc therapy (VMAT). Gross tumour and the entirety of involved nodal levels received 65Gy/30# over 6 weeks. Prophylactic dose to areas considered at high risk of occult disease was 54Gy/30# over 6 weeks. Selection and delineation of target volumes was carried out according to international guidelines [17]. Cisplatin was delivered at 100mg/m² on day 1 and 22 of treatment for those receiving concurrent chemotherapy.

Whole, unstimulated saliva samples and xerostomia questionnaires were collected from patients prior to radiotherapy (baseline) then 2 weeks, 4 weeks and 6 weeks into radiotherapy.

Saliva adhesiveness and viscosity was tested by LBL using the inclined plane test (IPT) and by measuring surface tension (pendant drop) and contact angle (sessile drop) by goniometry.

The IPT measures the time taken for saliva to travel 5 cm down an inclined plane (IP), held at 90 degrees to the horizontal. This is used as a marker of saliva viscosity/adhesiveness where short transit times indicate less visco-adhesive saliva and longer times the converse. Saliva samples were stored between 2-8°C before being removed from refrigerated storage and allowed to reach ambient room temperature prior to carrying out the IPT. All samples were tested within 5 days of production by the patients. Some samples were so visco-adhesive that even after several minutes there was no movement down the slope. In these cases the IP times were truncated at 60 seconds.

Surface tension and contact angle measurements were taken using a KSV Theta CAM101 goniometer operating with OneAttension software.

Patient reported xerostomia scores were collected using the Groningen Radiotherapy-Induced Xerostomia Questionnaire (GRIX) [18]. This is a validated 14 item questionnaire which asks about dry mouth and sticky saliva during the day and night. All scores were converted linearly to a 0-100 scale where higher scores represent more xerostomia.

The primary objective was to measure the adhesive and viscoelastic properties of saliva samples pre and post RT to the H&N area. Secondary objectives were to

validate the findings of the pilot study with further ex-vivo efficacy data on differing concentrations of LMS-611, to measure the inter-patient differences in saliva properties, to correlate patient reported symptoms with laboratory measurements, and to inform the design of the subsequent clinical study.

Statistical Analysis

Continuous variables are summarised as mean, standard deviation, median, interquartile range and range, or a subset of these. Categorical variables are summarised as number and percentage per category. Violin plots are used to present the results at each time point.

Values at follow up have been compared to baseline values and values at the previous visit using paired Wilcoxon tests.

The relation between GRIX scores and other results is described using Spearman correlation coefficients with bootstrap 95% confidence intervals calculated from 10000 bootstrap samples.

In the IPT, there are many truncated times where the sample did not travel the full distance within the observed time. Therefore the results of the IPT have been additionally analysed as survival data, considering travelling the full distance as the event of interest, and any recorded time of 60 seconds as censored observation. The relation of other variables to the IPT results has been analysed using proportional hazards models accounting for repeated measurements within a patient.

P-values have not been adjusted for multiple testing.

All analyses have been carried out in R version 3.0.1[19].

Role of the Funding Source

The funding source (Lamellar Biomedical Ltd) carried out the laboratory tests on the saliva samples obtained.

All laboratory work was performed at Lamellar Biomedical in compliance with the QMS system in accordance with ISO 9001:2008, ISO 13485:2003 and 21 CFR Part 820.

The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

RESULTS

30 patients were recruited to the study between September 2013 and April 2014. 29 patients completed the GRIX questionnaires and provided saliva samples at baseline and weeks 2, 4 and 6 of RT. One patient died from pneumonia during week 3 of RT treatment and therefore did not complete the study beyond week 2.

Demographics

Patient demographics are summarised in table 1. All patients had a pathologically confirmed diagnosis of SCC of the oropharynx with staging carried out as per local protocol with examination under anaesthetic, CT and MRI as indicated.

Saliva Adhesiveness and Viscosity Tests

INCLINED PLANE TEST:

The IPT results are summarised in table 2 and figure 1. This demonstrates increasing time taken for the IPT, and therefore increasing saliva adhesiveness and viscosity, when RT is commenced. The increase was significant from baseline to week-2, $p=0.001$. Values increase only moderately from week-2 to week-4 and from week-4 to week-6 with $p=0.250$ and $p=0.297$ respectively.

Wide inter-patient variability with a large range of values at baseline was observed. This variability appeared to decrease as treatment continued. This is at least partly due to the values being truncated at 60 seconds.

The number of values included at each time point is less than the original sample size as not all saliva samples were suitable for testing. Some samples were so viscous that it was not possible for them to be handled in the laboratory and hence were excluded from the inclined plane test.

SURFACE TENSION AND CONTACT ANGLE:

As the volume of each sample directly impacted the level of testing performed a test priority was established: IP measurements were prioritised then surface tension and contact angle measurements would be assessed if possible.

Where samples did not allow analysis, it was recorded.

Analysis of the surface tension and contact angle of patient saliva proved to be particularly challenging due to the nature of the saliva samples received. Several samples presented both quantitative and qualitative limitations which restricted the analysis of both surface tension and contact angle measurements.

As a result of this the number of samples that underwent goniometry assessment to assess surface tension and contact angle was limited.

The results of contact angle and surface tension measurements taken on untreated saliva samples are not included here as meaningful interpretation is not possible due to the limitations described above.

Fig. 1 Violin plots of the time taken to descend the IP against treatment duration. P-values refer to the comparison with previous visit by wilcoxon test

Inclined Plane Tests with Addition of LMS-611

Table 3 summarises, for each time point of assessment, the time taken for saliva to descend the IP where saline or LMS-611 has been added.

As previously described, the time taken for untreated saliva to descend the IP increased from baseline to week-6, indicating increasing saliva adhesiveness and viscosity; this acted as the control.

The addition of saline or LMS-611 at concentrations of 2.5mg/ml and 5mg/ml to the saliva samples did not reduce IPT times. However, when LMS-611 at concentrations of 10mg/ml and 20mg/ml were added significant reductions were seen in the IPT at each time point, as seen in the video of the IPT [Online Resource 1, Addition of LMS-611 to RIX Saliva]

Analysing the time to descend the IP as survival data separately for each time point (not shown) and overall adjusting for week of radiotherapy (table 4) demonstrates these statistically significant differences. The hazard ratio refers to the likelihood of

saliva travelling the 5cm; therefore a small hazard ratio indicates stickier saliva. Interestingly the addition of saline or LMS-611 2.5mg/ml to saliva seems to produce significantly stickier saliva than no treatment. It is difficult to account for this effect.

Patient Reported Xerostomia

Patient reported xerostomia scores collected using the GRIX questionnaire are summarised in figure 2. GRIX scores increased from one time point to the next as RT progressed. There is a statistically significant increase ($p < 0.001$) from baseline to week-2 then week-2 to week-4 of RT with only a small further increase from week-4 to week-6. RIX scores demonstrated modest inter-patient variability at baseline with a wide range of scores observed. This variability remained constant throughout treatment.

Fig. 2 Violin plots of GRIX Scores by treatment duration. P-values refer to the comparison with the previous visit by wilcoxon test.

Correlation of Saliva Adhesiveness/Viscosity with Xerostomia Scores

The IPT results were correlated with patient reported GRIX scores obtained for all time points. No relevant correlation was seen between the 2 measurements, with Spearman Correlation Co-efficient of -0.06 (-0.43 – 0.33, 95% CI) at baseline; 0.25 (-0.18 – 0.60, 95% CI) at week 2; 0.12 (-0.33 – 0.54, 95%CI) at week 4 and 0.08 (-0.39 – 0.52, 95% CI) at week 6.

Treating the IPT results as survival data, a model predicting the IPT results from the GRIX scores adjusting for time did not show a significant relationship (hazard ratio of 0.990, 95% confidence interval 0.975 – 1.004, $p = 0.170$).

DISCUSSION

RIX is the most frequently reported late toxicity following RT to the H&N area. It remains a clinically significant problem for many patients despite advances in radiation technology [15, 16] and there is currently no effective topical treatment [8]. The aim of this study was to assess viscosity and adhesiveness of saliva before and during RT to the H&N area and evaluate whether addition of LMS 611 changed these properties. Inter-patient variability in saliva properties was also examined and the objective and subjective measurements of RIX correlated.

Patient characteristics are as expected for locally advanced SCC of the H&N, with more males than females and most patients receiving combined chemoradiotherapy suggesting that the results are applicable to this group of patients generally.

As expected and previously reported, saliva adhesiveness and viscosity increased as RT treatment progressed [5, 20]. The largest difference in saliva properties was observed between baseline and 2 weeks into RT, suggesting that the serous cells of the salivary glands are affected by the relatively low doses of radiation received in the first two weeks of RT. This is in keeping with previous work demonstrating a sharp reduction in salivary flow rates during the first week of RT delivered with conventional fractionation [5, 6, 20-25]. The mechanism behind this is thought to be

due to early damage to the plasma membrane in acinar cells rather than cell death which occurs later in the course of RT damage [26].

Wide inter-patient variability in saliva properties was observed pre-treatment; this may be due to age, medication [27-29] or smoking. [30, 31] These possible confounding factors were not explored further. This variability lessened with time as the entire sampled population developed RIX. This was at least partly due to a ceiling effect, since there were an increasing number of samples that did not travel the full distance within 60 seconds as RT progressed.

The GRIX questionnaire was chosen for this study as it specifically includes questions about sticky saliva, which is the component of RIX that LMS-611 is most likely to influence. It has been previously validated for use in RIX and is currently being used in a study in the USA to assess the impact of '*Acetylcysteine Rinse in Reducing Saliva Thickness and Mucositis in Patients with Head and Neck Cancer Undergoing Radiation Therapy*' [32], i.e. in the same setting as this study. GRIX scores indicate that, subjectively, xerostomia worsened as patients went through RT. Significant differences were seen between pre-treatment scores and each subsequent time point. The largest differences reported in RIX occurred between baseline and week-2 then week-2 and week-4. There was little further worsening of patient reported xerostomia between week-4 and 6 of RT. Again, this may reflect high sensitivity of salivary glands to relatively low doses of radiation delivered during the initial weeks of treatment.

Most of the literature reports on established RIX post RT and there appears to be only one previous report describing worsening quality of life due to RIX during RT [33]. However, that study used a non-validated, physician reported assessment tool whereas a patient reported score such as the GRIX questionnaire is generally accepted as the preferred measure [34]. Most studies assessing interventions for RIX are carried out in the late phase of xerostomia. As demonstrated in this study however, xerostomia does occur in the acute phase and therefore it is also valid to evaluate a novel intervention for RIX during RT as done here.

Some inter-patient variability in GRIX scores is noted at each time point. This variability remains constant over the course of RT and is likely to reflect differences in patients' perception of the symptom. Significant variation in reporting of xerostomia has been previously documented in this setting [21, 35] and also in the palliative care setting where dry mouth is also a common symptom [36].

No relevant correlation was observed between the objectively assessed saliva properties and patient reported xerostomia questionnaires. This is the first study examining saliva visco-adhesive properties and correlating with patient reported measures. Weak or no correlation between patients' assessment of xerostomia and salivary flow rate has previously been reported by several authors [5, 21 & 37]. The reasons for this and for the current results are unclear. A possible explanation may be that subjective xerostomia assessments in this study and others encompass all components contributing to the patients' feeling of xerostomia whereas the objective measures of salivary flow rate or visco-adhesive properties isolate only that

particular aspect. To find a relevant correlation one may have to assess all objective components that contribute to the symptom of xerostomia. This is beyond the scope of this study but this finding reinforces the importance of including patient reported measures in xerostomia studies.

This study has demonstrated that saliva became more adhesive and viscous as RT progressed. However, the addition of LMS-611 at concentrations of 10mg/ml and 20mg/ml reversed this change in visco-adhesive properties and restored its fluidity. The addition of saline to saliva samples did not, therefore ruling out the possibility that the addition of fluid alone, rather than an active mucokinetic preparation, may cause this change. Indeed the data suggests that the addition of saline to saliva samples makes the saliva more visco-adhesive than with no additive at all. Furthermore LMS-611 at concentrations of 2.5mg/ml and 5mg/ml had little or no impact on the saliva properties. The 10mg/ml and 20mg/ml preparations demonstrated significant efficacy. As a result the 2.5mg/ml and 5mg/ml concentrations have been removed from the forthcoming clinical study. The effects of LMS-611 in concentrations of 10mg/ml and 20mg/ml on xerostomia will be assessed in-vivo.

Previous pre-clinical work with LMS-611 [unpublished data] has shown that it acts on the biophysical properties of saliva by changing its external bonds and therefore its visco-adhesive properties. The effect is almost instantaneous and can be seen in the video [Online Resource 2, Addition of LMS-611 to Mucin].

Although parotid sparing IMRT is now commonplace in H&N cancer, leading to improvements in late toxicities and quality of life, RIX remains a significant clinical problem for many patients. Rates of clinically significant late xerostomia up to 40% are seen, despite constraining the dose delivered to the contralateral parotid gland. [15, 16, 37-39] For some patients with bilateral cervical nodal metastases or bulky primary disease crossing midline, it is not possible to deliver parotid sparing RT for fear of compromising dose to tumour and subsequent disease control. Most of these patients will develop RIX as a late, permanent and significant toxicity. Furthermore, many centres are not yet able to offer IMRT to all patients who might benefit from it. In April 2013 it was reported that only 22.3% of all patients receiving radical RT in England were treated with IMRT [40]. Globally it is estimated that less than 10% of the population have access to this technology [41].

Currently available interventions for RIX remain unsatisfactory with no evidence that any topical therapy is effective in relieving the symptom of dry mouth [8]. Salivary stimulants are more effective in treating RT induced hypo-salivation than salivary substitutes, hyperbaric oxygen, or acupuncture but may cause significant side effects. Other novel interventions which aim to regenerate salivary gland tissue post radiotherapy e.g. stem cell transplant and gene therapy remain at a preliminary investigational stage and are likely to take many years to be widely available in clinical practice. [16] Salivary gland transfer is a further option but is also experimental, requires a surgical procedure and may not be suitable for all patients. [42] There remains a need, therefore, for further studies examining topical

interventions for RIX and in particular to assess patient reported symptom scores and quality of life measures when assessing efficacy [35].

LMS-611 oral spray is an attractive option for the treatment of RIX. Its mode of action is biophysical rather than pharmacological and therefore has an excellent safety and side effect profile [unpublished data]. Compared to other novel approaches, the timeline for its development from bench to bedside is significantly shorter; it is non-invasive and can be made widely available. This warrants in-vivo analysis of the effects of LMS-611 upon RIX.

CONCLUSIONS

Saliva becomes more adhesive and viscous as RT progresses. There is wide inter-patient variability in these saliva properties pre-treatment.

Patient reported xerostomia worsens as RT progresses with the largest change within the first two weeks of radiotherapy. Inter-patient variability in reported xerostomia remains constant throughout treatment.

No relevant correlation between patient reported xerostomia and laboratory measurements of saliva properties was demonstrated.

This data suggests that concentrations of 10mg/ml and 20mg/ml merit in-vivo testing in a forthcoming clinical study.

Current topical measures for the management of RIX in H&N cancer are unsatisfactory and new interventions for RIX remain relevant in the parotid-sparing IMRT era.

TABLES

Table 1: Patient Demographics

Total Number of Patients		30
Mean Age (years)		54.8
Age range (years)		42-67
Gender	Male	24 (80%)
	Female	6 (20%)
Stage	II	1 (3.3%)
	III	4 (13.3%)
	IV	25 (83.3%)
Radiotherapy alone		3 (10%)
Chemoradiotherapy		27 (90%)

Table 2: Inclined Plane Test Results

		Time point during radiotherapy			
		Baseline	Week 2	Week 4	Week 6
Inclined Plane Test (seconds)	Mean (SD)	31.3 (22.5)	49.7 (14.0)	51.1 (14.9)	55.7 (9.0)
	Median (IQR)	31.0 (6.8, 56.0)	57.5 (44.8, 60.0)	60.0 (42.2, 60.0)	60.0 (57.2, 60.0)
	Range	2.0 – 60.0	16.0 – 60.0	5.0 – 60.0	32.0 – 60.0
	Mean difference from baseline		18.3	19.1	25.3
	Comparison to baseline using wilcoxon test		p=0.001	p=0.003	p<0.001
	Comparison to baseline using proportional hazards regression		p=0.005	p=0.004	p<0.001
	Mean difference from previous visit		18.3	3.3	5.5
	Comparison to previous visit using wilcoxon test		p=0.001	p=0.250	p=0.297
	Number samples assessable	28	26	22	22

Table 3: Inclined Plane Test Results with addition saline or LMS-611

			Time point during radiotherapy		
			Week 2	Week 4	Week 6
Inclined Plane Test (seconds)	Untreated	N _{OBS} Mean (SD) Median (IQR)	26 49.7 (14.0) 57.5 (44.8, 60.0)	22 51.1 (14.9) 60.0 (42.2, 60.0)	22 55.7 (9.0) 60.0 (57.2, 60.0)
	Saline	N _{OBS} Mean (SD) Median (IQR)	23 59.0 (4.4) 60.0 (60.0, 60.0)	18 59.3 (2.4) 60.0 (60.0, 60.0)	22 60.0 (0.0) 60.0 (60.0, 60.0)
	LMS-611 2.5 mg/ml	N _{OBS} Mean (SD) Median (IQR)	23 57.6 (5.3) 60.0 (59.0, 60.0)	17 58.6 (4.9) 60.0 (60.0, 60.0)	18 58.9 (4.7) 60.0 (60.0, 60.0)
	LMS-611 5 mg/ml	N _{OBS} Mean (SD) Median (IQR)	23 47.2 (18.4) 60.0 (36.0, 60.0)	16 54.4 (10.3) 60.0 (53.2, 60.0)	19 58.3 (7.3) 60.0 (60.0, 60.0)
	LMS-611 10 mg/ml	N _{OBS} Mean (SD) Median (IQR)	23 24.2 (20.2) 16.0 (10.5, 37.0)	18 17.3 (14.6) 14.5 (10.0, 17.8)	22 32.5 (16.7) 29.0 (18.8, 44.5)
	LMS-611 20 mg/ml	N _{OBS} Mean (SD) Median (IQR)	23 4.3 (2.9) 3.0 (2.0, 6.0)	18 7.1 (8.0) 4.0 (2.0, 9.0)	22 11.0 (9.6) 8.5 (5.2, 12.8)

N_{OBS} = Number of Observations Assessable

Table 4: All inclined plane test results with addition of LMS-611 or saline, adjusted for week of radiotherapy. Cox proportional hazards model.

	Hazard Ratio	95% Confidence Interval	p-value
Saline vs. untreated	0.115	(0.045, 0.294)	p<0.001
2.5 mg/ml vs. untreated	0.283	(0.125, 0.639)	p=0.002
5 mg/ml vs. untreated	0.593	(0.337, 1.042)	p=0.069
10 mg/ml vs. untreated	4.957	(3.132, 7.846)	p<0.001
20 mg/ml vs. untreated	30.687	(17.852, 52.750)	p<0.001

ACKNOWLEDGEMENTS

The authors acknowledge Graham Park, Head of Technical Development, Lamellar Biomedical Limited who contributed to study design and saliva sample analysis but sadly passed away prior to study completion.

Other contributors include:

Study design: R Jones, J Paul, C Bray (all Cancer Research UK Clinical Trials Unit, Beatson WoSCC). N Brittain & J Lang (NHS Greater Glasgow & Clyde Research & Development Department)

Statistical Analysis: A McConnachie (Robertson Centre for Biostatistics, University of Glasgow)

Staff Funding: Scottish Cancer Research Network and NHS Research Scotland Career Researcher Fellowship, both from Chief Scientists Office, Scottish Government Health Directorate

Assistance with preparation of manuscript: A James (Beatson WoSCC) and A J Chalmers (Institute of Cancer Sciences, University of Glasgow)

CONFLICT OF INTEREST

Funding for this study was provided by Lamellar Biomedical Limited. Dr Claire Paterson has no conflicts of interests to declare and had full access to all of the primary data. Review of the data by the journal is welcome.

REFERENCES

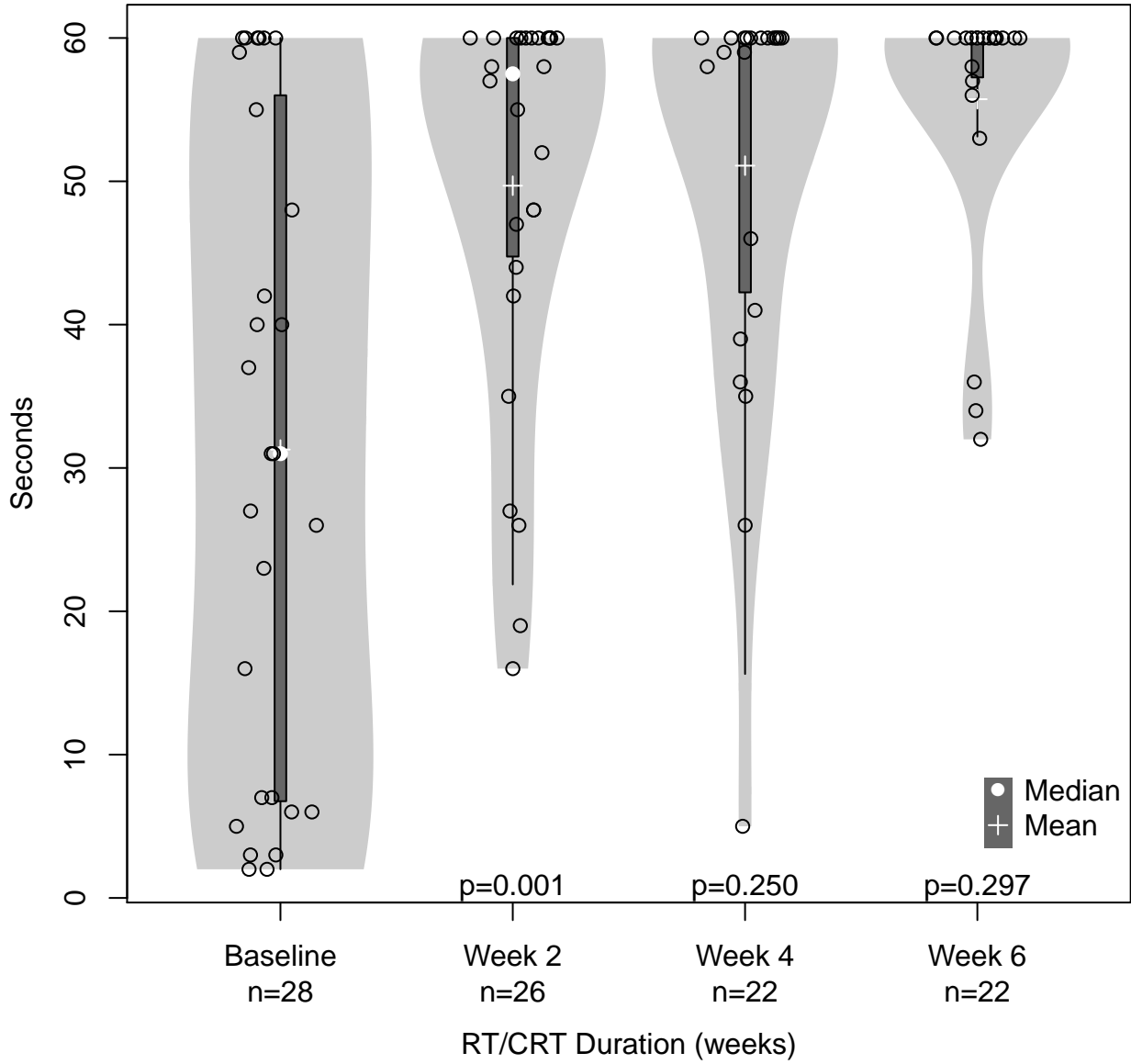
1. Bhide SA, Nutting CM.
(2010) Advances in radiotherapy for head and neck cancer
Oral Oncol Jun; **46 (6)**: 439–41
2. Jensen AB, Hansen O, Jorgensen K, Bastholt L.
(1994) Influence of late side-effects upon daily life after radiotherapy for laryngeal and pharyngeal cancer
Acta Oncol; **33 (5)**: 487–91.
3. Bjordal K, Kaasa S.
(1995) Psychological distress in head and neck cancer patients 7–11 years after curative treatment
Br J Cancer; **71 (3)**: 592–97
4. Dirix P, Nuyts S, Vander Poorten V, Delaere P, Van Den Bogaert W
(2008) The influence of xerostomia after radiotherapy on quality of life Results of a questionnaire in head and neck cancer
Support Care Cancer 16 (2):171–179
5. Dirix P, Nuyts S, Van den Bogaert W
(2006) Radiation-induced xerostomia in patients with head and neck cancer: a literature review
Cancer **107 (11)**:2525-2534
6. Makkonen TA, Tenovuo J, Vilja P, Heimdahl A
(1986) Changes in the protein composition of whole saliva during radiotherapy in patients with oral or pharyngeal cancer
Oral Surg Oral Med Oral Pathol **62 (3)**:270-275
7. Wijers OB, Levendag PC, Braaksmma MM, Boonzaaijer M, Visch LL, Schmitz PI
(2002) Patients with head and neck cancer cured by radiation therapy: a survey of the dry mouth syndrome in long-term survivors
Head Neck; **24 (8)**: 737–47.
8. Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R
(2011) Interventions for the management of dry mouth: topical therapies
Cochrane Database Syst Rev. Dec 7;(12)
9. Mehanna H, Jones TM, Gregoire V, Ang KK
(2010) Oropharyngeal carcinoma related to human papilloma virus
BMJ 340:c1439
10. Gillison ML, Harris J, Westra W et al
(2009) Survival outcomes by tumour human papilloma virus (HPV) status in stage III-IV oropharyngeal cancer (OPC) in RTOG 0129
J Clin Oncol ASCO Meeting Abstracts 27(15S): 6003
11. Fakhry C, Westra WH, Li S, Cmelak A et al
(2008). Improved survival of patients with human papilloma virus-positive head and neck squamous cell carcinoma in a prospective clinical trial
J Natl Cancer Inst 100(4): 261-9.
12. Ang KK, Harris J, Wheeler R et al
(2010) Human papilloma virus and survival of patients with oropharyngeal cancer
N Engl J Med Jul 1;363(1):24-35.

13. Goldstein DP, Hynds Karnell L, Christensen AJ and Funk GF
(2007) Health-related quality of life profiles based on survivorship status for head and neck cancer patients
Head Neck 29(3): 221-9
14. Semple C
(2001) The role of the CNS in head and neck oncology
Nurs Stand 15(23): 39-42
15. Little M, Schipper M, Feng FY et al
(2012) Reducing Xerostomia after Chemo-IMRT for Head-and-Neck Cancer: Beyond Sparing the Parotid Glands
Int J Radiation Oncol Biol Phys, Vol. 83, No. 3, 1007-1014
16. Vissink A, Mitchell JB, Baum BJ et al,
(2010) Clinical management of salivary gland hypofunction and xerostomia in head-and neck cancer patients: Successes and barriers
Int. J. Radiation Oncology Biol. Phys., Vol. 78, No. 4, 983–991
17. Gregoire V, Coche E, Cosnard G, Hamoir M, Reyckler H.
(2000) Selection and delineation of lymph node target volumes in head and neck conformal radiotherapy. Proposal for standardizing terminology and procedure based on the surgical experience.
Radiother Oncol; **56 (2)**: 135–50
18. Beetz I, Burlage FR, Henk P et al
(2010) The Groningen Radiotherapy-Induced Xerostomia Questionnaire: Development and Validation of a new Questionnaire
Radiotherapy & Oncology 97; 127-131
19. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/> Accessed September 2014
20. DA Freitas, AD Caballero, MM Pereira et al
(2011) Oral sequelae of head and neck radiotherapy
CEFAC, vol 13 (6); 1103-1108
21. L Franzen, U Funeg rd, T Ericson, R Henriksson
(1992) Parotid Gland Function During and Following Radiotherapy of Malignancies in the Head and Neck: A consecutive study of salivary flow and patient discomfort
Eur J Cancer, 28 (2-3); 457-462,
22. Dreizen S, Daly TE, Drane JB, Brown LR
(1977) Oral complications of cancer radiotherapy
Postgrad Med, 61 (2), 85-92
23. Wescott WB, Mira JG, Starcke EN, Shannon IL, Thornby JI
(1978) Alterations in whole saliva flow rate induced by fractionated radiotherapy
Am J Roentgenol 130 (1), 145-149.
24. Shannon IL, Trodahl JN, Starcke EN
(1978) Radio sensitivity of the human parotid gland
Proc Soc Exp Biol Med, 157 (1), 50-53

25. JG Mira, WB Wescott, EN Starcke, IL Shannon
(1981) Some factors influencing salivary function when treating with radiotherapy
Int J Radiat Oncol Biol Phys, 7 (4), 535-541
26. AWT Konings, RP Coppes, A Vissink
(2005) On the mechanism of salivary gland radio sensitivity
Int. J. Radiation Oncology Biol. Phys., Vol. 62, No. 4, 1187–1194,
27. Porter S, Scully C, Hegarty AM
(2004) An update of the etiology and management of xerostomia
Oral medicine, oral surgery, oral pathology Vol. 97 No. 1: 28-46
28. Villa A, Abati S*
(2011) Risk factors and symptoms associated with xerostomia: a cross-sectional study
Australian Dental Journal; 56: 290–295
29. Thomson WM
(2015) Dry mouth and older people
Australian Dental Journal; 60:(1 Suppl): 54–63
30. Zuabi O, Machtei EE, Ben Aryeh H et al
(1999) The effect of smoking and periodontal treatment on salivary composition in patients with established periodontitis.
J Periodontol.70:1240–6
31. [Kolte](#) AP, [Kolte](#) RA, [Laddha](#) RK
(2012) Effect of smoking on salivary composition and periodontal status
Indian Soc Periodontol. Jul-Sep; 16(3): 350–353.
32. <https://clinicaltrials.gov/ct2/show/NCT02123511?term=GRIX&rank=1>
<https://clinicaltrials.gov/ct2/show/NCT01823445?term=GRIX&rank=2>
Accessed October 2014
33. MS Tiwana, MK Mahajan, B Uppal, G Koshy , J Sachdeva, HN Lee, SD Talole
(2011) Whole saliva physico-biochemical changes and quality of life in head and neck cancer patients following conventional radiation therapy: a prospective longitudinal study
Indian journal of cancer, 48(3), 289-295
34. Meirovitz A, Murdoch-Kinch CA, Schipper M, C Pan, A Eisbruch
(2006) Grading xerostomia by physicians or by patients after intensity-modulated radiotherapy of head-and-neck cancer.
Int J Radiat Oncol Biol Phys 66(2):445–453.
35. TL Lovelace, NF Fox, AJ Sood et al
(2014) Management of radiotherapy-induced salivary hypofunction and consequent xerostomia in patients with oral or head and neck cancer: meta-analysis and literature review
Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology 117(5); 595-607
36. G Chaushu, M Bercovici, S Dori, A Waller, S Taicher, J Kronenberg, YP Talmi
(2000) Salivary flow and its relation with oral symptoms in terminally ill patients
Cancer, 88(5) 984-7

37. CM Nutting, JP Morden, KJ Harrington et al
(2011) Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial
Lancet Oncol; 12: 127–36
38. J Setton, N Caria, J Romanyshyn et al
(2012) Intensity-modulated radiotherapy in the treatment of oropharyngeal cancer: An update of the Memorial Sloan-Kettering Cancer Centre Experience
Int. J. Radiation Oncology Biol. Phys., Vol. 82, No. 1, 291–298,
39. M Kam, SF Leung, B Zee
(2007) Prospective Randomized Study of Intensity-Modulated Radiotherapy on Salivary Gland Function in Early-Stage Nasopharyngeal Carcinoma Patients
Journal Clinical Oncology Volume 25 (31); 4873-9
40. Online document
(2013) The Radiotherapy Innovation Fund. An evaluation of the prime minister's £23 million fund
http://www.cancerresearchuk.org/prod_consump/groups/cr_common/@nre/@pol/documents/generalcontent/rifevalreport.pdf
Accessed October 2014
41. A Trotti, A Eisbruch
(2011) Reducing xerostomia through advanced technology
The Lancet Oncology, Volume 12, 110
42. MASCC Oral care study group
Summary of the Oral Complications Systematic Reviews
<http://www.mascc.org/oral-care>
Accessed April 2015

Figure



Figure

