Optimising radiotherapy outcomes for patients with head and neck cancer

Claire Paterson

MB ChB, MRCP, FRCR

Submitted in fulfilment of the requirements for the
Degree of Doctor of Medicine in Cancer Studies (Research),
Institute of Cancer Studies,
College of Medical, Veterinary and Life Sciences,
University of Glasgow

October 2023

Table of Contents

	Page
List of tables	8
List of figures	10
Acknowledgements	11
Author's declaration	12
Abbreviations	13
Chapter 1. Introduction to head and neck cancer and head and neck cancer radiotherapy	16
1.1 Head and neck cancer epidemiology	16
1.2 HNC treatment - general concepts	16
1.3 Staging	17
1.4 Human Papillomavirus, p16 and prognosis	22
1.5 Radiotherapy for HNSCC	23
1.5.1 Thermoplastic mask	24
1.5.2 Simulation CT	24
1.5.3 Target volume delineation	25
1.5.4 Organs at risk delineation	25
1.5.5 Peer review	26
1.5.6 Treatment planning and delivery	26
1.5.7 Treatment verification	26
1.6 Technological advances in RT	27
1.6.1. Intensity modulated radiation therapy (IMRT)	27
1.6.2 Image-guided radiation therapy (IGRT)	28
1.7 Toxicities from RT for HNC	28
1.8 Good prognosis HNSCC	29
1.9 Poor prognosis HNSCC	30
1.9.1 Intensification of treatment with systemic anti-cancer treatment (SACT)	30
1.9.2 Intensification of treatment with RT	31
1.10 Introduction to thesis	32

Chapter 2. Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection 33
2.1 Abstract 33
2.1.1 Background and Aim 33
2.1.2 Material and Methods 33
2.1.3 Results 33
2.1.4 Conclusion 34
2.2 Background 34
2.3 Methods 35
2.3.1 Ethical consideration 35
2.3.2 Patients 35
2.3.3 Treatment 35
2.3.4 Follow up assessment and outcomes 36
2.3.5 Analysis 37
2.4 Results 38
2.4.1 Patient, treatment and tumour characteristics 38
2.4.2 Neck dissections and clinical outcomes 39
2.4.3 Overall survival 42
2.5 Discussion 43
2.6 Conclusions 46
2.7 Post viva additions to chapter 46
2.8 References 48

Chapter 3. Comparative cohort study of volumetric modulated arc therapy for squamous cell cancer of unknown primary in the head and neck—Involved neck only versus mucosal irradiation 51
3.1 Abstract 51
3.1.1 Objectives 51
3.1.2 Design 51
3.1.3 Results 51
3.1.4 Conclusion 52
3.2 Introduction 52

3.3 Methods 53
3.3.1 Ethical considerations 53
3.3.2 Participants 53
3.3.3 Treatment 53
3.3.4 Toxicity 54
3.3.5 Statistical analysis 54
3.4 Results 55
3.4.1 Patient demographics and diagnostic investigations 55
3.4.2 Neck dissections 55
3.4.3 Radiotherapy 55
3.4.4 Chemotherapy 56
3.4.5 Dose to organs at risk 56
3.4.6 Acute toxicity 57
3.4.7 Late toxicity 58
3.4.8 Disease control and overall survival 58
3.5 Discussion 60
3.5.1 Synopsis of key findings 60
3.5.2 Strengths and limitations 60
3.5.3 Comparison with other studies 60
3.5.4 Clinical applicability 61
3.6 Conclusions 61
3.7 Post viva additions to chapter 62
3.8 References 63

Chapter 4. Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study. 66
4.1 Abstract 66
4.1.1 Introduction and background 66
4.1.2 Methods/design 66
4.1.3 Discussion 67
4.2 Introduction and background 67
4.3 Methods/design 69
4.3.1 Study organisation/funding 69

4.3.2 Study design and patient population 69
4.3.3 Study objectives and endpoints 71
4.3.4 Chemoradiotherapy 71
4.3.5 Diffusion weighted MRI 72
4.3.6 Quality assurance (QA) 73
4.3.7 Clinical follow up 73
4.3.8 Statistical analysis 74
4.4 Discussion 75
4.4.1 The need for a predictive biomarker 75
4.4.2 Potential future translational studies 76
4.5 References 77

Chapter 5. Feasibility of DW-MRI analysis of salivary glands during head and neck radiotherapy. 81
5.1 Abstract 81
5.1.1 Introduction 81
5.1.2 Methods 81
5.1.3 Results 81
5.1.4 Conclusion 82
5.2 Introduction 82
5.2.1 Aim 83
5.3 Methods 83
5.3.1 Patient selection 83
5.3.2 MRI scan acquisitions 84
5.3.3 Delineation and quantification of ROI on MR 84
5.3.4 Chemo-radiotherapy 84
5.3.5 Toxicity assessment 85
5.3.6 MRI quality assurance (QA) 85
5.3.7 Statistical method 86
5.4 Results 86
5.4.1 Feasibility of outlining salivary glands 87
5.4.2 Salivary gland volumes, ADC at baseline 87
5.4.3 Changes in measurements between scans 88
5.4.4 Toxicity groups 88
5.5 Discussion 90

5.6 Conclusions 93
5.7 Post viva additions to chapter 93
5.8 References 94

Chapter 6. Radiotherapy-induced xerostomia, pre-clinical promise of LMS-611 99
6.1 Abstract 99
6.1.1 Purpose 99
6.1.2 Methods 99
6.1.3 Results 99
6.1.4 Conclusions 100
6.2 Introduction 100
6.3 Materials and methods 101
6.3.1 Participants 101
6.3.2 Procedures 101
6.3.3 Statistical analysis 103
6.3.4 Role of the funding source 103
6.4 Results 103
6.4.1 Demographics 104
6.4.2 Saliva adhesiveness and viscosity tests 104
6.4.2.1 Inclined plane test 104
6.4.2.2 Surface tension and contact angle 106
6.4.2.3 Inclined plane tests with the addition of LMS-611 107
6.4.3 Patient reported xerostomia 109
6.4.4 Correlation of saliva adhesiveness/viscosity with xerostomia scores 110
6.5 Discussion 110
6.6 Conclusions 114
6.7 Post viva additions to chapter 114
6.8 References 115

Chapter 7. Radiotherapy induced xerostomia: a randomised, double-blind, controlled trial of Visco-ease™ oral spray compared with placebo in patients with cancer of the head 119

and neck
7.1 Abstract 119
7.2 Introduction 119
7.3 Materials and methods 120
7.3.1 Participants 120
7.3.2 Randomisation and blinding 121
7.3.3 Procedures 121
7.3.4 Statistical analysis 121
7.3.5 Ethics approval and consent to participate 122
7.4 Results 122
7.4.1 Participants 122
7.4.2 Baseline demographics 123
7.4.3 Patient reported xerostomia scores 124
7.4.4 Frequency of use of oral spray 125
7.4.5 Safety endpoints 126
7.5 Discussion 126
7.6 Post viva additions to chapter 129
7.7 References 129

Chapter 8. Summary – Optimising radiotherapy outcomes for patients with head and neck cancer 132
8.1 Introduction 132
8.2 De-intensification of treatment 133
8.2.1 Omission of surgery 133
8.2.2 Reduction in RT target volumes 135
8.3 Individualised escalation of treatment 137
8.4 Predicting and managing toxicity – xerostomia 140
8.4.1 Predicting xerostomia 140
8.4.2 Managing xerostomia 141
8.5 Conclusion 145

References 145

List of tables

Table 1-1	Page 18	Clinical staging, p16 positive OPSCC, TNM 8th Edition, UICC, 2017
Table 1-2	Page 19	Clinical staging, p16 negative OPSCC, TNM 8th Edition, UICC, 2017
Table 1-3	Page 20	Clinical staging, unknown primary cervical nodes, p16/HPV positive, TNM 8th Edition, UICC, 2017
Table 1-4	Page 21	Clinical staging, unknown primary cervical nodes, p16/HPV negative or unknown. TNM 8th Edition, UICC, 2017
Table 1-5	Page 23	Risk of death in OPSCC.
Table 2-1	Page 39	Baseline patient demographics, tumour characteristics and treatments received
Table 2-2	Page 40	Neck dissection data
Table 2-3	Page 41	EQR subgroup – neck dissections and clinical outcomes
Table 2-1	Page 37	Baseline patient demographics, tumour characteristics and treatments received (amended post viva)
Table 3-1	Page 55	Patient demographics
Table 3-2	Page 57	Doses to organs at risk
Table 3-3	Page 58	Acute toxicity
Table 3-4	Page 59	Patients with recurrent disease
Table 3-1	Page 63	Patient demographics (amended post viva)
Table 4-1	Page 68	Risk of death in OPSCC
Table 5-1	Page 87	Summary of disease characteristics
Table 5-2	Page 88	Summary of gland volumes, mean ADC at baseline
Table 5-3	Page 88	Mean change in absolute volume, mean ADC and percentage change after 2 weeks of treatment, by gland type
Table 5-4	Page 89	Association with percentage change in mean volume and percent change in ADC with grade of toxicity at 12 months
Table 6-1	Page 104	Patient demographics
Table 6-2	Page 105	Inclined plane test results
Table 6-3	Page 108	Inclined plane test results with the addition of saline

		or LMS-611
Table 6-4	Page 109	All inclined plane test results with addition of LMS-611 or saline, adjusted for week of radiotherapy. Cox proportional hazards model
Table 7-1	Page 124	Baseline demographics for APT
Table 7-2	Page 125	Weekly Groningen radiotherapy—induced xerostomia (GRIX) scores
Table 7-3	Page 126	Comparison between patients who had at least one adverse event (AE) or serious adverse event (SAE) (APT)
Table 8-1	Page 139	Dosimetry for OARS with standard and dose-escalated RT plans

List of figures

Figure 2-1	Page 43	Kaplan-meier estimates for overall survival
Figure 2-2	Page 46	Decision making in cases with ICR or EQR on post-treatment FDG PET-CT.
Figure 4-1	Page 70	Trial pathway
Figure 5-1	Page 90	Percent change in mean ADC of parotid glands after 2 weeks of treatment when categorised by severity of toxicity (Grade 0-1 versus 2-3) at 12 months
Figure 6-1	Page 106	Violin plots of the time taken to descend the IP against treatment duration
Figure 6-2	Page 110	Violin plots of GRIX scores by treatment duration
Figure 7-1	Page 123	Trial profile, CONSORT flow diagram
Figure 7-2	Page 125	Weekly GRIX clinic scores
Figure 7-3	Page 126	Number of sprays used per day

Acknowledgements

I dedicate this thesis to my daughters, Ava and Darcie. Girls, please believe we can do anything we set our minds to.
To mum and dad, thank you for your love and support (and to mum for proof reading help).

I would like to thank Professor Anthony Chalmers for his supervision, support and review of this thesis.

I am grateful to my co-investigators and co-authors, to my clinical colleagues and most importantly to the patients who have participated in the studies described.

Financial support has been gratefully received from CRUK RadNet Glasgow, the Beatson Cancer Charity and NHS Greater Glasgow and Clyde via a NRS Senior Fellowship and their endowment fund.

Author’s declaration

Please see separately submitted declaration document for each published paper.

I declare that this thesis is the result of my own work unless otherwise acknowledged and has not been previously submitted for a higher degree.

Abbreviations

AAA	Analytical Anisotropic Algorithm
ADC	Apparent diffusion coefficient
AE	Adverse event
AJCC	American Joint Committee on Cancer
AUC	Area under the curve
CBCT	Cone-beam CT
CI	Confidence interval
CR	Complete response
CRT	Chemoradiotherapy
CT	Computed tomography
CTCAE	Common terminology criteria for adverse events
CTV	Clinical target volume
CUP	Cancer of unknown primary
DFS	Disease free survival
Dmax	Maximum dose
DND	Delayed neck dissection
DW MRI	Diffusion weighted magnetic resonance imaging
ECS	Extracapsular spread
EQR	Equivocal response
EUA	Examination under anaesthetic
18 FDG-PET/CT	18F-fluorodeoxyglucose Positron Emission Tomography/Computed Tomography
GRIX	Groningen RIX
GTV	Gross tumour volume
Gy	Gray
H&N	Head and neck
HNC	Head and neck cancer
HNSCC	Head and neck squamous cell cancer
HNSCCUP	Head and neck squamous cell cancer unknown primary
HPV	Human papillomavirus
ICR	Incomplete response
IGRT	Image guided radiotherapy
IHC	Immunohistochemistry

IMRT	Intensity modulated radiotherapy
IND	Immediate neck dissection
INO	Involved neck only
IPT	Inclined plane test
IQR	Interquartile range
ISH	in situ hybridization
LRC	Locoregional control
MDADI	MD Anderson Dysphagia Inventory
MDT	Multi-disciplinary team
MRI	Magnetic resonance imaging
MUC	Potential mucosal sites and contralateral neck
ND	Neck dissection
NOTCH	National Oncology Trainees Collaborative for Healthcare Research
NPV	Negative predictive value
OARs	Organs at risk
OPSCC	Oropharyngeal squamous cell cancer
OS	Overall survival
PCFS	Post contrast fat saturated
PCR	Polymerase chain reaction
PET	Positron Emission Tomography
PFS	Progression free survival
PG	Parotid gland
PPV	Positive predictive value
PRV	Planning risk volume
PTV	Planning target volume
PVP	Polyvinylpyrrolidone
QOL	Quality of life
RCR	Royal College of Radiologists
RIX	Radiotherapy-induced xerostomia
ROC	Receiver operator characteristic
RT	Radiotherapy
RTOG	Radiation therapy oncology group
SACT	Systemic anti-cancer therapy
SAE	Serious adverse event

SCC	Squamous cell cancer
SCCUP	Squamous cell cancer unknown primary
SD	Standard deviation
SLG	Sublingual gland
SMG	Submandibular gland
TPF	Taxotere platinum 5FU
TPS	Treatment planning system
US	Ultrasound
UICC	Union Internationale Contre le Cancer
VMAT	Volumetric arc therapy
WHO	World health organisation

1. Introduction to head and neck cancer and head and neck cancer radiotherapy

1.1 Head and neck cancer epidemiology

Head and neck cancer (HNC) is the 8th most common cancer in the UK, with around 12,400 new cases per annum, accounting for 3% of all new cancer cases. HNC incidence rates are significantly higher than the UK average in Scotland. The age standardised rate in Scotland is 24.0% per 100 000 population (95% CI 23.2- 24.7%) compared to 19.9% per 100 000 population (95% CI 19.7-20.1%) for the UK as a whole. The differences between the home countries largely reflect risk factor prevalence in previous years. Smoking, alcohol consumption and human papillomavirus (HPV) infection are the most common risk factors for developing HNC (Lambert 2011); it remains largely a disease associated with deprivation. Around 60% of HNC diagnosed in Scotland are managed in the west of Scotland clinical network. Since the early 1990s, HNC incidence rates have increased by around a third (34%) in the UK. HNC incidence rates are projected to rise by 3% in the UK between 2023-2025 and 2038-2040. There are around 4143 deaths from HNC per year in the UK, contributing to 2% of all cancer deaths (Head and neck cancers statistics | Cancer Research UK accessed July 2023). Mortality is strongly related to age with an association also demonstrated for deprivation.

1.2 HNC treatment - general concepts

Treatment is determined by the pathology of the tumour, the stage and sub-site of disease. Squamous cell carcinoma (SCC) accounts for over 90% of HNCs and is the focus of this work. Head and neck SCC (HNSCC) originates from the mucosal lining of the upper aero digestive tract including the oral cavity, oropharynx, hypopharynx and larynx sub-sites (Siegel 2023).

Treatment with curative intent (radical treatment) is investigated in this thesis, and may comprise surgery or radiotherapy (RT) or both. RT may be delivered with concurrent chemotherapy in both primary and adjuvant settings.

Patient suitability for radical treatment is determined by assessment of performance status and co-morbidities. Co-morbidities are prevalent in this population, often limiting feasible treatment options. The appropriateness of embarking on a radical treatment course must take account of the balance of

long term control/cure with morbidity from treatment, patient suitability, alternative non-curative options and the patient counselled regarding this. The functional impact and long term toxicities following treatment for HNSCC may be considerable and will be discussed in more depth in section 1.7.

1.3 Staging

Initial diagnosis and staging are based on clinical examination which may include nasendoscopy, examination under anaesthetic (EUA), and imaging. Computed tomography (CT), ultrasound (US) magnetic resonance imaging (MRI) and Positron Emission Tomography (PET) may all be useful and clinical guidelines recommend different imaging modalities for different sub-sites and stages of disease (Lewis-Jones 2016).

Staging is reported according to the International Staging System, published by the American Joint Committee on Cancer (AJCC) and the Union Internationale Contre le Cancer (UICC).[Brierly 2016] The TNM staging system refers to staging of the primary tumour (T), regional lymph nodes (N) and the presence or absence of distant metastases (M). Accurate cancer staging is important for treatment selection and prognostication. The TNM Classification of Malignant Tumours - 8th edition was published in December 2016, some of this work predates this with staging performed according to TNM 7th edition. The most relevant differences between the 7th and 8th editions with regards to this work are as follows:

i. a new classification for p16 positive oropharyngeal cancers, i.e tumours that have p16 immunohistochemistry overexpression.
ii. a new classification for cervical nodal involvement with unknown primary HNSCC (HNSCCUP)

The current staging systems for HNSCCUP and p16 positive and negative oropharynx SCC (OPSCC) are detailed below in tables 1-1to 1-4. These have been included here as are the sub-sites which feature prominently in this work. Separate staging systems exist for oral cavity, larynx and hypopharynx SCC sub-sites which follow similar principles.

Clinical staging, p16 positive OPSCC, TNM 8th Edition, UICC, 2017			
Primary tumour (T) T1 Tumours 2cm or less in greatest dimension T2 Tumours greater than 2cm but not greater than 4cm in greatest dimension T3 Tumour more than 4cm in dimension or extension into lingual surface of epiglottis T4 Tumour invades any of the following: larynx, deep/extrinsic muscles of the tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate, mandible, lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base or encases carotid artery. NB Mucosal extension to the lingual surface of the epiglottis from primary tumours of the base of tongue or vallecula does not constitute invasion of the larynx			
Regional Lymph Nodes (N) Nx Regional lymph nodes cannot be assessed N0 No regional lymph node metastasis N1 Unilateral metastasis, in lymph node(s), all 6cm or less in greatest dimension N2 Contralateral or bilateral metastasis in lymph node(s) all 6cm or less in greatest dimension N3 Metastasis in a node greater than 6cm in dimension NB Midline nodes are considered ipsilateral			
Distant Metastasis (M) M0 No distant metastasis M1 Distant metastasis			
Stage I	T1-2	N0-1	M0
Stage II	T1-2	N2	M0
	T3	N0-2	M0
Stage III	T1-4	N3	M0
	T4	Any N	M0
Stage IV	Any T	Any N	M1

Table 1-1. Clinical staging, p16 positive OPSCC, TNM 8th Edition, UICC, 2017

Clinical staging, p16 negative OPSCC, TNM 8th Edition, UICC, 2017

Primary tumour (T)

T1 Tumours 2cm or less in greatest dimension

T2 Tumours greater than 2cm but not greater than 4cm in greatest dimension

T3 Tumour more than 4cm in dimension or extension into lingual surface of epiglottis

T4a Advanced local disease with tumour invasion of any of the following: larynx, deep/extrinsic muscles of the tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate or mandible

T4b Advanced local disease with tumour invasion of any of the following: lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base or encases carotid artery

NB Mucosal extension to the lingual surface of the epiglottis from primary tumours of the base of tongue or vallecula does not constitute invasion of the larynx

Regional Lymph Nodes (N)

Nx Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis in a single ipsilateral lymph node, ≤3 cm and no extranodal extension (ENE)

N2a Metastasis in a single ipsilateral lymph node, >3cm but ≤6cm, no ENE

N2b Metastasis in multiple ipsilateral lymph nodes, all ≤6cm and no ENE

N2c Metastasis in bilateral or contralateral lymph nodes, ≤6cm and no ENE

N3a Metastasis in any lymph node >6cm with no ENE

N3b Metastasis in any lymph node(s) with clinical ENE *

Notes: * The presence of skin involvement or soft tissue invasion with deep fixation/tethering to underlying muscle or adjacent structures or clinical signs of nerve involvement is classified as clinical extranodal extension.

Midline nodes are considered ipsilateral nodes.

Distant Metastasis (M)

M0 No distant metastasis

M1 Distant metastasis

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1-3	N1	M0
Stage IVa	T1-3	N2	M0
	T4a	N0-2	M0
Stage IVb	T4b	Nany	M0
	Tany	N3	M0
Stage IVc	Tany	Nany	M1

Table 1-2. Clinical staging, p16 negative OPSCC, TNM 8th Edition, UICC, 2017

Clinical staging, unknown primary - cervical nodes, p16/HPV positive. TNM 8th Edition, UICC, 2017			
Primary tumour (T) T0 No evidence of primary tumour			
Regional Lymph Nodes (N) N1 Unilateral metastasis, in lymph node(s), all 6cm or less in greatest dimension N2 Contralateral or bilateral metastasis in lymph node(s) all 6cm or less in greatest dimension N3 Metastasis in a node greater than 6cm in dimension			
Distant Metastasis (M) M0 No distant metastasis M1 Distant metastasis			
Stage I	T0	N1	M0
Stage II	T0	N2	M0
Stage III	T0	N3	M0
Stage IV	T0	N1-3	M1

Table 1-3. Clinical staging, unknown primary - cervical nodes, p16/HPV positive. TNM 8th Edition, UICC, 2017

Clinical staging, unknown primary - cervical nodes, p16/HPV negative or unknown. TNM 8th Edition, UICC, 2017			
Primary tumour (T) T0 No evidence of primary tumour			
Regional Lymph Nodes (N) N1 Metastasis in a single ipsilateral lymph node, ≤3 cm and no extranodal extension (ENE) N2a Metastasis in a single ipsilateral lymph node, >3cm but ≤6cm, no ENE N2b Metastasis in multiple ipsilateral lymph nodes, all ≤6cm and no ENE N2c Metastasis in bilateral or contralateral lymph nodes, ≤6cm and no ENE N3a Metastasis in any lymph node >6cm with no ENE N3b Metastasis in any lymph node(s) with clinical ENE * Notes: * The presence of skin involvement or soft tissue invasion with deep fixation/tethering to underlying muscle or adjacent structures or clinical signs of nerve involvement is classified as clinical extranodal extension.			
Distant Metastasis (M) M0 No distant metastasis M1 Distant metastasis			
Stage III	T0	N1	M0
Stage IVa	T0	N2	M0
Stage IVb	T0	N3	M0
Stage IVc	T0	N1-3	M1

Table 1-4. Clinical staging, unknown primary - cervical nodes, p16/HPV negative or unknown. TNM 8th Edition, UICC, 2017

Generally, stage I and II disease is considered early stage and may be treated radically with single modality treatment (surgery or RT). Stage III and IV (excluding metastatic disease) is considered loco-regionally advanced and requires multi-modality treatment (surgery plus RT, RT plus chemotherapy, surgery plus chemoRT) for best chance of cure/long term control.
While the current staging system (TNM 8th edition) reflects prognosis for p16 positive OPSCC, treatment options should be considered in the context of TNM 7th edition for node positive disease, i.e. N1 disease should still generally be considered loco-regionally advanced and receive multi-modality treatment.

1.4 Human Papillomavirus, p16 and prognosis

The rise in overall incidence of HNSCC is as a result of a significantly increased incidence of OPSCC, particularly in the developed world. In turn this has been attributed to HPV driven disease (Mehanna 2010).

HPV status in tumors can be determined by several assays including HPV DNA detection by in situ hybridization (ISH) or polymerase chain reaction (PCR), and/or p16 protein expression by immunohistochemistry (IHC) staining as a surrogate marker of oncogenic HPV infection. Increased expression of p16 is highly correlated with HPV mediated OPSCC, as the HPV E7 oncoprotein inactivates pRb, which induces upregulation of the cyclin-depended-kinase, p16. The concordance rate between HPV ISH and p16 IHC is approximately 90% in OPSCC (Shi 2009, Ang 2010, Rischin 2010, Jordan 2012, Lingen 2013, Rietbergen 2013).

There has been a 57% increase in incidence in OPSCC between 1975 and 2014 in the USA and in the UK an even more striking rise of 290% between 1988 and 20062 with clinicians internationally describing it as an epidemic. OPSCC is now the most common HPV associated cancer in the USA with 20,000 cases per year, this number is expected to continue to rise until at least 2060 despite HPV vaccination programs (Junor 2010, Van Dyne 2018).

HPV-positive OPSCCs exhibit significantly improved sensitivity to treatment than their HPV negative counterparts, with the risk of death being approximately 50% lower with 2-year overall survival rates of 87.5-95% (Fakhry 2008, Ang 2010). HPV-positive OPSCC is now considered a distinct disease entity and as such a new staging system, as described in section 1.3, and prognostic guidance has been implemented internationally (Huang 2015). In contrast, Ang's pivotal study showed patients with high-risk features including heavy smoking history and HPV-negative disease had a 3-year overall survival of only 46.2% (Ang 2010). He proposed 3 'risk groups' for OPSCC, using tumour stage, HPV status and smoking history to classify patients into low, intermediate or high risk of death, Table 1-5.

Risk category	OS at 3 years	Demographics
Low risk	93%	HPV+, <10 pack years HPV+, >10 pack years, N0-2a
Intermediate risk	70.8%	HPV+, >10 pack years, N2b-3 HPV−, <10 pack years
High risk	46.2%	HPV−, >10 pack years HPV−, <10 pack years, T4

Table 1-5. Risk of death in OPSCC.

In parallel with the sharp increase in the incidence of OPSCC, there has been an escalation in numbers of patients with HNSCCUP which is also reported to be linked to the significant increase in HPV-driven disease (Garnaes 2015, Garnaes 2015, Motz 2016, Zamani 2020) Outcomes in HPV-positive HNSCCUP are comparable with those where a HPV-positive primary is detected in the oropharynx, with overall survival 75-90% at 2-5 years in recent literature (Perkins 2012, Tribius 2012, Keller 2014, Demiroz 2014, Sivars 2014, Straetmans 2015, O'Sullivan 2016, Tiong 2018) . Again, in keeping with OPSCC, survival outcomes in HPV-positive HNSCCUP are better compared with HPV-negative disease.

1.5 Radiotherapy for HNSCC

Radiotherapy may be used as a single, radical treatment modality for Stage I and II HNSCC, and in combination with either surgery or chemotherapy for loco-regionally advanced disease. High doses of radiation are required to achieve tumour control, with standard regimens 60-70 Gray (Gy) in 30-35 fractions (#) delivered over 6-7 weeks, subject to regional variation globally and depending on whether used as primary or adjuvant treatment.

The addition of concurrent chemotherapy to radical RT for locally advanced HNSCC has been shown to improve outcomes. High dose cisplatin based chemoRT is considered standard of care, with 100mg/m2 every 3 weeks the most widely used regimen. A meta-analysis of around 19000 patients demonstrated an overall survival advantage of 6.5% at 5 years compared to RT alone (Lacas 2021). In the adjuvant setting, concurrent cisplatin also offers a disease control benefit to those patients with the highest risk disease, generally accepted to be that with

extra-nodal extension (ENE) and/or positive resection margin (Bernier 2004, Bernier 2005, Cooper 2004, Cooper 2012).
Cetuximab is a monoclonal antibody that binds to and inhibits the epidermal growth factor receptor (EGFR). EGFR is over expressed in HNSCC and associated with poor prognosis. In loco-regionally advanced disease cetuximab-RT has been shown to improve overall survival compared with RT alone (Bonner 2006, Bonner 2010). More recent studies have shown cetuximab-RT to be inferior to cisplatin-RT (Mehanna 2019, Gillison 2019, Gebre-Medhin 2021).
The accurate and precise delivery of radical RT for HNSCC depends on robust planning and treatment processes, detailed below.

1.5.1 Thermoplastic mask

A 5-point fixation thermoplastic mask is created for each patient in the mould room by technicians. The patient lies on the couch, equivalent to those used in the CT simulator and treatment machine, with a head rest. The thermoplastic material softens when placed in hot water and is then custom moulded round the face, neck and shoulders of the supine patient. Once solid, the mask prevents the patients from moving during the process of scanning and radiotherapy treatment with an average set-up error <3mm and is fixed to the table of the CT simulator and radiotherapy machines.

1.5.2 Simulation CT

The CT simulator is a conventional diagnostic CT scanner with radiotherapy planning software and lasers to aid patient set-up. The immobilisation mask is used to ensure that the patient is in the same position as they will be during their treatment and that what is planned using the simulator is consistent with what will be treated. CT contrast is administered to patients without contraindications to aid target delineation. Markers are attached to the mask for laser alignment purposes and to facilitate reproducible set-up between simulator and the treatment room.
While MRI simulation has recently been introduced in my institution and is now part of the HNSCC RT planning process, my work pre-dates its introduction.

1.5.3 Target volume delineation

Target volume delineation is carried out by the treating oncologist, using all available clinical and radiological information from diagnosis and staging and with reference to international guidelines (Gregoire 2000, Gregoire 2003, Gregoire 2006, Gregoire 2013, Gregoire 2017).

Initially the gross tumour volume (GTV) is delineated; this is the visible extent of the malignant tumour and may be separated into GTVp (primary tumour) and GTVn (involved lymph nodes).

A margin for microscopic spread of the disease is added to each GTV to create the clinical target volume(s) (CTV). The CTV to receive a radical dose of RT may be identified by CTV1. The margin required to create a CTV from GTV has been a topic of debate over the years but national and international consensus guidelines now generally suggest a 5mm GTV to CTV expansion is appropriate for CTV1 (with some caveats) (Gregoire 2017, Head and neck cancer - RCR consensus statements | The Royal College of Radiologists, accessed August 2023).

CTV2 encompasses the volume that should receive an elective dose of RT, generally 50Gy equivalent. An elective, or prophylactic, dose is used for clinically & radiologically uninvolved regions where there is concern about the presence of microscopic disease. This is based on recognised patterns of tumour spread. In keeping with recent guidelines this should include a 10mm expansion around the GTV(s) if using the '5+5' approach. Areas of the neck at risk of containing microscopic disease should also be included in CTV2. These areas are defined in international consensus guidelines, with descriptions of their main anatomic boundaries. The areas of the neck at risk are dependent on the sub-site and stage of HNSCC (Gregoire 2000, Gregoire 2003, Gregoire 2006, Gregoire 2013).

Finally, a margin for set-up error encompassing both systematic errors, due to any discrepancy between the CT simulator and treatment machine, and random set-up errors (that can occur on a day to day basis during treatment) is created around each CTV to create PTV1 and PTV2.

1.5.4 Organs at risk delineation

The organs at risk (OARs) also require to be delineated. These are normal tissues, adjacent to the target volumes that are sensitive to radiation and may

require the dose received by them to be restricted, depending on their RT tolerance.

Standard OARs for HNSCC RT are brainstem, spinal cord, paired parotid glands, larynx, trachea/oesophagus. Critical OARs (spinal cord and brainstem) may require an isotropic margin of 3-5mm (depending on institutional set-up errors) to create a Planning Risk Volume (PRV_spinal cord and PRV_brainstem respectively.) Additional OARs may be added if desired.

1.5.5 Peer review

The Royal College of Radiologists (RCR) recommend that 'radiotherapy target volume contours should be subject to systematic review by appropriately trained and experienced peer professionals' (radiotherapy-peer-review-2022.pdf (SECURED) (rcr.ac.uk), accessed August 2023). Therefore peer review, which includes discussion of clinical information, pathology and diagnostic imaging for each case, and review of target volumes is mandatory, and standard practice in my centre for all H&N cancers being planned for radical RT. All RT described in my thesis was subject to peer review of target volumes.

1.5.6 Treatment planning and delivery

Treatment planning is carried out by medical physics experts using specialised software. In my centre this is with the Eclipse™ treatment planning system (TPS) (Varian Medical System, Palo Alto, CA). Plans are optimised using the inverse planning Analytical Anisotropic Algorithm (AAA). Dose constraints are applied to target volumes and OARs.

RT is delivered by a linear accelerator, or linac. Truebeam® linacs (Varian Medical System, Palo Alto, CA) are used in my centre. All HNSCC RT is planned and delivered with volumetric arc therapy (VMAT).

1.5.7 Treatment verification

Image guided RT (IGRT) allows set-up errors to be corrected prior to treatment delivery and ensures the planned dose is consistently delivered throughout treatment (Bell 2018, Leech 2016). The modality and scheduling of verification images for IGRT are not standardised. Repeat planning CT or cone-beam CT (CBCT) are often applied although protocols are dependent on available clinical resources rather than established best practice.

In my centre, daily kilovoltage (KV) images are acquired for verification with corrections based on bony matching applied before treatment. A repeat planning CT is acquired at fraction 16 for volumetric and dosimetric assessment.

1.6 Technological advances in RT

1.6.1. Intensity modulated radiation therapy (IMRT)

Over the past few decades, significant technical advances have been made in RT for HNSCC. Intensity-modulated radiation therapy (IMRT) is a highly conformal RT technique which uses multiple beams of varying intensity to create dose distributions that can be shaped around target volumes or OARs. Sharp dose gradients can be achieved between target volumes and healthy tissue. Volumetric-modulated arc therapy (VMAT) is a special type of IMRT technique where treatment is delivered with an intensity-modulated beam that rotates around the patient. The beam is modulated by dynamic multi-leaf collimation, variable dose rate and variable gantry speed in optimised arc/arcs around the patient to generate even more conformal dose distributions than traditional IMRT.

Both conventional IMRT and VMAT plans are based on the inverse planning system where expected dose distributions are given first then a mathematical equation is solved to find appropriate beam intensities needed to provide the dose (see section 1.5). VMAT is used as standard of care in my institution for HNC, and all RT described in this thesis was delivered with VMAT.

The conformal dose distributions and steep dose gradients generated by IMRT and VMAT allows OARs to be spared from high doses of radiation, thereby not only reducing the toxicity of RT but also making dose escalation to the tumour and subsequent improved loco-regional control a realistic possibility.

The PARSPORT trial was the first multicentre randomised controlled trial to assess parotid-sparing IMRT in patients with HNSCC. 94 patients were recruited between 2003 and 2007. At 12 months grade 2 or worse xerostomia was significantly lower in the IMRT group than in the conventional radiotherapy group (25 [74%; 95% CI 56–87] of 34 patients given conventional radiotherapy vs 15 [38%; 23–55] of 39 given IMRT, p=0·0027). At 24 months, grade 2 or worse xerostomia was significantly less common with IMRT than with conventional radiotherapy (20 [83%; 95% CI 63–95] of 24 patients given conventional

radiotherapy vs nine [29%; 14–48] of 31 given IMRT; p<0·0001). The study concluded that ‘sparing the parotid glands with IMRT significantly reduces the incidence of xerostomia and leads to recovery of saliva secretion and improvements in associated quality of life, and thus strongly supports a role for IMRT in squamous-cell carcinoma of the head and neck’ (Nutting 2011).
A more recent study led by the same group has evaluated dysphagia-optimised IMRT (Do-IMRT). The DARS study (Dysphagia-optimised intensity-modulated radiotherapy versus standard intensity-modulated radiotherapy in patients with head and neck cancer: a phase 3, multicentre, randomised, controlled trial) randomised 112 patients between 2016 and 2018. Patients in the DO-IMRT group had significantly higher MD Anderson Dysphagia Inventory (MDADI) composite scores at 12 months than patients in the standard IMRT group (mean score 77·7 [SD 16·1] vs 70·6 [17·3]; mean difference 7·2 [95% CI 0·4-13·9]; p=0·037). The study concluded that ‘our findings suggest that DO-IMRT improves patient-reported swallowing function compared with standard IMRT. DO-IMRT should be considered a new standard of care for patients receiving radiotherapy for pharyngeal cancers’ (Nutting 2023).
IMRT is now considered standard of care for HNC in high income countries.

1.6.2 Image-guided radiation therapy (IGRT)

Image-guided radiation therapy (IGRT) is complementary to IMRT to ensure the safe delivery of this highly conformal treatment through frequent imaging throughout the RT course. The tumour volume and anatomy may change during the 6-7 week treatment course, which in turn may change dosimetry to target volumes and OARs. IGRT allows these potential undesirable changes to be monitored and re-planning to be instigated if the changes are felt to be clinically significant. Re-planning during treatment is known as adaptive RT.

1.7 Toxicities from RT for HNC

While technological advances such as IMRT have resulted in modest improvements in observer-rated and patient-reported xerostomia (Nutting 2011), clinically significant xerostomia remains a problem for many patients (Little 2012, Vissink 2010).

Modern RT therefore, unfortunately, continues to be associated with significant permanent toxicities including pain, dry mouth, and speech and swallowing difficulties (Wilson 2007, Goldstein 2007). These often result in significant psychological harm and impaired quality of life after treatment. Furthermore the addition of chemotherapy or surgery to RT has been shown to increase the rates of severe late toxicity (Machtay 2008). Indeed, a recent study found that survivors of head and neck cancer were almost twice as likely to die from suicide as survivors of other cancers (Osazuwa-Peters 2018). This is a stark reminder of the long-term psychosocial issues faced by our patients living with the permanent effects of current treatments.

Chapters 5, 6 and 7 of this thesis investigate the most common RT side effect, xerostomia (dry mouth).

1.8 Good prognosis HNSCC

As described in section 1.4, outcomes in HPV-positive HNSCC are much better than in HPV-negative disease with patients having excellent survival prospects. The issue of long-term toxicity from RT becomes even more relevant in this sub-group. Furthermore, HPV-positive HNSCC occurs predominantly in younger patients (typically aged 30-60). Patients are usually employed, and many have young families (Herrero 2003). The long-term side effects described above prevent a significant proportion of patients from returning to work and leading a normal life. Patients must live with the sequelae of their treatment for many years and many require long-term rehabilitation at considerable cost to the health care system and society in general (Semple 2001).

It is internationally accepted that less toxic treatment is required for patients with HPV driven OPSCC. Treatment de-intensification for this group is the focus of significant attention internationally and several clinical trials investigating different strategies are underway (Mirghani 2015, Mehanna 2017, Mirghani 2018, Adelstein 2019). These fall into 4 broad categories:

1. Replacement of cisplatin with cetuximab. However, results from De-Escalate (Mehanna 2019) and RTOG 1016 (Gillison 2019) studies have demonstrated that cetuximab-RT results in inferior OS to cisplatin-RT with no reduction in toxicity.

2. Less aggressive RT/CRT regimes e.g induction chemotherapy followed by reduced dose or volume RT in good responders, CRT with reduced doses of RT or chemotherapy, RT alone
3. Alternative to photon RT e.g proton therapy
4. Less invasive surgery, e.g. Trans-Oral Surgery (TOS)

While one or more of these novel treatment strategies may ultimately result in toxicity reduction, it is vital that this does not come at the cost of reduced cancer control. The negative cetuximab studies are a reminder of the perils of modifying proven treatment; both reported reduced OS in the cetuximab-RT arms compared to standard of care. Published work evaluating patients' attitudes towards de-intensification of treatment indicate most were unwilling to accept a significant reduction in survival probability in favour of reduced toxicity (Brotherston 2013), suggesting therefore that tumour control must remain the priority when considering reduced intensity treatment strategies. With this in mind, lower risk strategies may also play a part in de-intensifying treatment for HPV-driven HNSCC. Chapters 2 and 3 of this thesis reports on 2 such approaches, the omission of surgery after (chemo) RT and a reduction in RT target volumes.

1.9 Poor prognosis HNSCC

In contrast, with the good prognosis population described in section 1.8, Ang's pivotal study showed patients with high-risk features including heavy smoking history and HPV-negative disease had a 3-year overall survival of only 46.2% (Ang 2010). The analysis also showed that the differences in survival between the good and poor prognosis groups were largely due to differences in loco-regional control (LRC), indicating treatment intensification to improve LRC (i.e. control of the primary and involved lymph nodes) may improve outcomes significantly.

1.9.1 Intensification of treatment with systemic anti-cancer treatment (SACT)

Meta-analysis shows an absolute survival benefit of 6.5% at 5 years with concomitant, platinum based, chemotherapy with RT in patients <71 years of age with locally advanced HNSCC (Pignon 2009). CRT is therefore considered standard of care for locally advanced OPSCC. However, patients with poor

prognosis OPSCC tend to be older and often have a significant smoking and/or alcohol history (Herrero 2003). Co-morbidities resulting from these factors such as cardiac, kidney and lung disease are frequently present and in general this patient population is less fit than their HPV+OPSCC counterparts (Habbous 2014). A significant proportion of patients in the west of Scotland have contra-indications to concurrent cisplatin and receive radiotherapy alone (Mentel 2020, Leong 2021). Uniform intensification of a morbid treatment across a group who are already unsuitable for standard of care CRT is therefore an unattractive option. The addition of other systemic treatments to RT in a population with co-morbidities where the unmet need is LRC also seems counter-intuitive with several studies failing to show improved outcomes with new drugs added to standard of care (Yu 2019).

1.9.2 Intensification of treatment with RT

Intensification of RT is an appropriate avenue to explore; there is an established dose–response relationship in HNSCC, therefore escalating the radiation dose may improve tumour control and treatment outcomes (Guerrero Urbano 2007).

Dose escalation was previously difficult to implement in HNSCC with conventional radiotherapy techniques because of the proximity of OARs to target volumes and increased risk of radiation-induced toxicity. However, with advances in radiotherapy technologies such as IMRT and VMAT described in section 1.6, highly conformal escalated dose distributions and improved sparing of OARs can be achieved.

Higher rates of LRC have been demonstrated in early-stage clinical trials of uniform dose-escalation with acceptable toxicity profiles (Madani 2007, Leclerc 2013, Miah 2012). This is yet to translate into improved disease outcomes in larger phase 3 trials e.g., ART-DECO in larynx and hypopharynx SCC showed no improvement in LRC with dose-escalated IMRT but encouragingly severe toxicity was also no different between the treatment arms (Nutting 2021). This uniform approach to dose-escalation has only been applied to entire poor prognostic groups as described above. An individualised dose-escalation strategy using a predictive biomarker of response may allow us to realise the full potential of RT dose-escalation.

Chapter 4 investigates the use of diffusion weighted MRI (DW-MRI) as a predictive biomarker of response to RT, with a view to MR guided response adaptive RT.

1.10 Introduction to thesis

The overall aim of my research programme is to optimise radiotherapy outcomes for patients with head and neck cancer. Chapters 2 and 3 of this thesis report on two approaches to de-intensity treatment for HPV-driven HNSCC. Chapter 4 investigates the use of diffusion weighted MRI (DW-MRI) as a predictive biomarker of response to RT, with a view to MR guided response adaptive RT for poor prognosis HNSCC. Chapters 5, 6 and 7 of this thesis investigate the most common RT side effect, xerostomia (dry mouth).

2. Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection

Zhou S, Chan C, Rulach R, Dyab H, Hendry F, Maxfield C, Dempsey MF, James A, Grose D, Lamb C, Schipani S, Wilson C, Cheng Lau Y, **Paterson C**.
Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection. Oral Oncol. 2022 May;128:105870. doi: 10.1016/j.oraloncology.2022.105870. Epub 2022 Apr 18. PMID: 35447564.

2.1 ABSTRACT

2.1.1 Background and Aim:

The aim of this study was to evaluate the long-term safety of the omission of immediate neck dissections (IND) in patients with human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) achieving a less than complete nodal response on 12-week FDG PET-CT.

2.1.2 Material and Methods:

Patients with HPV-positive, node-positive HNSCC that were treated with radical (chemo) radiotherapy (RT) between January 2013 and September 2019 were identified. PET-CT responses were classified as complete (CR), incomplete (ICR) or equivocal (EQR) nodal responses. Clinical outcomes were obtained.

2.1.3 Results:

347 patients were identified. Median follow-up was 43.9 (IQR, 30.8-61.2) months.
62.8% (218/347) achieved a CR, 23.4% (81/347) EQR and 13.8% (48/347) ICR nodal response. 70 of 81 (86.4%) patients with an EQR and 25 of 48 (52.1%) with an ICR had no residual disease during follow up (a pathologically negative ND if surgery undertaken or no subsequent neck or distant relapse clinically/radiologically).
Median survival of the EQR and CR groups were not reached, and despite the omission of IND in 95% of the EQR group there was no statistically significant

differences in overall survival (OS) between the groups, p=1.0. Median survival of ICR was not reached. However, OS for ICR group was significantly worse than that of CR, and EQR, both p<0.001.

2.1.4 Conclusion

The omission of IND in those achieving an EQR nodal response does not compromise long-term survival. This supports the safety of extended surveillance in patients with HPV-positive disease and an EQR on 12-week FDG PET-CT.

2.2 BACKGROUND

The landmark PET-NECK study established the non-inferiority of 12-week FDG PET-CT surveillance compared to planned neck dissection (ND) in patients who achieve a complete nodal response (CR) after primary chemoradiation (CRT) [1]. This resulted in a change in standard of care for this subgroup of patients, who are now spared an immediate neck dissection (IND). However, as there is a lack of robust evidence supporting the safety of extended surveillance in those achieving less than a CR, many centres continue to advocate IND for this group [2], regardless of Human Papilloma Virus (HPV) status. Meanwhile, it is now accepted that HPV-related tumours can take more than 12 weeks to involute completely and that 12-week FDG PET-CT has a low positive predictive value (PPV) for residual disease in those who achieve a less than CR [3-8], especially in those with an equivocal response (EQR) [3]. Mehanna et al. acknowledged that the PET-Neck study population was not stratified for HPV status, and therefore, FDG PET-CT at 12 weeks may overestimate the risk of residual disease for the group with HPV-related disease [1]. Evidence has shown that an extended period of surveillance is a safe alternative to IND with acceptable short-term outcomes for patients who have HPV-related head and neck squamous cell carcinomas (HNSCC) [3, 8, 9]. However, late relapses are well recognised in HPV-positive disease and should also be assessed [10, 11]. This is the first study to evaluate this, with the aim to examine the long-term safety of the omission of an IND in patients with HPV-positive HNSCC achieving less than a CR on 12-week FDG PET-CT.

2.3 METHODS

2.3.1 Ethical consideration

Anonymised patient data were obtained and reviewed retrospectively by members of the treating clinical team, therefore, a review by the regional ethical committee was not required for this study.

2.3.2 Patients

This was a retrospective cohort study of patients from a tertiary level oncology institution. All patients with HPV positive, histologically proven oropharyngeal squamous cell cancer (OPSCC) and cancers of unknown primary (CUP) with regional nodal involvement who were treated with radical radiotherapy (RT) or chemo-radiotherapy (CRT) between January 2013 and September 2019, and underwent 12-week FDG PET-CT surveillance were identified from the PET centre database. Patient demographics, treatment and clinical outcomes were extracted from electronic clinical records. Patients were staged according to the American Joint Committee on Cancer TNM Staging System 8th edition (AJCC TNM 8th edition). HPV status was determined by polymerase chain reaction-based fluorescent Luminex assay for HPV DNA and/or by diffuse immunohistochemical nuclear and cytoplasmic staining (>70%) for the presence of p16 protein.

2.3.3 Treatment

All patients completed radical RT or CRT. RT was delivered with volumetric arc therapy (VMAT) to a dose of 65Gy in 30 fractions to gross disease and entirety of the involved nodal level(s); areas at risk of microscopic metastases were prophylactically treated to a dose of 54Gy in 30 fractions. Delineation was as per international guidelines [12, 13]. Concurrent chemotherapy (cisplatin 100mg/m2, given on days 1 and 22 of radiotherapy) was standard of care for those eligible for concurrent chemoradiotherapy. If Cisplatin was contra-indicated but patient was suitable for concurrent therapy then carboplatin AUC5, day 1 and 22 of radiotherapy, or cetuximab administered weekly at 250mg/m2 throughout radiotherapy, with a loading dose of 400mg/m2 commencing the week prior was administered.

2.3.4 Follow up assessment and outcomes

Clinical review with examination and, if indicated, flexible naso-endoscopy were performed on completion of treatment at week 6, week 12, then 3 monthly for the first 2 years and 3-6 monthly from years 3-5 to assess for recurrences in keeping with national and international guidelines [14, 15]. Beyond 5 years, clinical outcomes were retrieved from electronic clinical records of patient interaction with National Health Services and investigation outcomes (if any). All patients underwent a surveillance FDG PET-CT approximately 12 weeks following radiotherapy completion. Radionucleotide radiologists at the regional PET centre provided qualitative interpretations and categorised treatment responses into complete nodal response (CR), incomplete nodal response (ICR) or equivocal nodal response (EQR) in accordance with the definitions from the PET-Neck study [1]. Intense FDG uptake at the regional cervical nodes (SUVmax above the reference hepatic uptake level) were deemed ICR, regardless of nodal size. Nodes demonstrating uptake between reference blood pool and hepatic uptake values were classified as EQRs, regardless of nodal size. Enlarged nodes demonstrating uptake less than the reference blood pool value were also classified as EQRs. CRs were those with normal sized nodes and uptake less than the reference blood pool value. As per the PET Neck protocol, patients who achieved a CR underwent clinical follow-up only. However, patients with EQR/ICR were discussed in the multi-disciplinary team meetings (MDT) and individualised treatment decisions were made relating to the need for further investigations (e.g biopsy) and the role of ongoing surveillance versus an IND (see additional figure 2.2). In the event that the MDT concurred there was a significant risk of residual nodal disease (avid FDG uptake or an increase in tumour burden), then either a biopsy or an IND was recommended to the patient, provided the disease was deemed operable and that the patient was a suitable candidate to undergo surgery. However, when there was no clear indication of active nodal disease (e.g. a significant treatment response with considerable reduction in tumour size or FDG avidity, negative biopsy, or normal nodal architecture), the MDT advocated active surveillance with interval scanning 3 months later. Imaging modalities (PET-CT, CT, MRI, and ultrasound) were used at the discretion of the treating clinician. All decisions regarding delayed neck dissection (DND) were reached by MDT consensus guided by clinical findings coupled with suspicious or progressive features concerning of active

disease on imaging +/- histological confirmation. Residual/recurrent disease was defined as either primary, neck or distant metastases detected on serial imaging, or disease confirmed histologically. To reflect the timing of surveillance investigations, IND are defined as those performed 3-6 months after RT, and DND those beyond 6 months.

2.3.5 Analysis

Overall survival was the primary endpoint. This was measured from the date of diagnosis to death from any cause. Survival between the different response groups, CR, EQR and ICR, were calculated using the Kaplan- Meier method and compared using a log-rank test. Deaths are categorised as either HNSCC-related or non-HNSCC related.

Time to disease recurrence or neck dissection was determined by the date of confirmed recurrence on imaging/histopathology or date of surgery respectively, from completion of RT.

The negative predictive value (NPV) and PPV of the PET-CT was calculated with 95% confidence intervals. Conventionally these test statistics would be calculated on the basis of pathological outcome compared to that predicted by imaging. As not all patients underwent NDs, any neck or distal relapse (in the absence of active disease at primary site) have been used as a surrogate for residual neck disease. This accounts for any microscopic residual nodal disease that may have ultimately led to distal metastases. It is acknowledged that this approach may under estimate the NPV and overestimate the PPV of the PET-CT for residual neck disease.

Statistical analysis was performed using Minitab 20 software (State College, PA) and SPSS version 23 (IBM, Armonk, NY). (Acknowledgement S Zhou and Y Cheng Lau). Continuously variable data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR) dependent on normal or non-normal distribution. Categorical variables were compared by Chi-Square and Fisher's exact test. Level of statistical significance was set at <0.05.

2.4 RESULTS

2.4.1 Patient, treatment and tumour characteristics

347 patients were eligible for analysis, having received radical (chemo)-radiotherapy for HPV-positive, node-positive head and neck squamous cell cancer. HPV status was ascertained by HPV DNA in 88.8% and p16 expression as a surrogate marker in 11.2% of the cohort. Table 2-1 illustrates the baseline patient demographics, tumour characteristics and treatment details. 89.0% of patients had OPSCC and 11.0% had CUP. Mean age was 57 years. 79.3% had N1, 16.7% N2 and 4.0% had N3 disease (based on AJCC TNM 8th Ed). 85.3% received chemo-radiotherapy. All patients underwent a post-RT surveillance FDG PET-CT to assess response to treatment, with a median time from RT to PET-CT of 94 days (IQR,
88 – 103 days). All patients had a minimum of 24 months follow up and were followed-up for up to 8 years; with a median follow-up of 43.9 months (IQR, 30.8-61.2 months).

Table 2-1. Baseline patient demographics, tumour characteristics and treatments received.	
Total number of patients	347
Mean age, years (SD)	57 (+/- 8.4)
Gender, Male – no. (%)	276 (79.5)
Smoking status –no. (%) Never Current/Former Unknown	 149 (42.9) 196 (56.5) 2 (0.6)
Tumour site – no. (%) Oropharynx SCC Unknown primary SCC	 309 (89.0) 38 (11.0)
Tumour classification[a] – no. (%) T0 T1 T2 T3 T4	 38 (10.9) 86 (24.8) 105 (30.3) 40 (11.5) 78 (22.5)
Nodal classification[a] – no. (%) N1 N2 N3	 275 (79.3) 58 (16.7) 14 (4.0)
HPV detected by DNA P16	 308 (88.8) 39 (11.2)
Types of concurrent SACT[b] Cisplatin Carboplatin Cetuximab	 265 (89.5) 10 (3.4) 21 (7.1)
Radiotherapy only	51 (14.7)
a Based on AJCC 8th Ed. b SACT denotes systemic anti-cancer treatment	

2.4.2 Neck Dissections and clinical outcomes

The median time from RT to IND was 4.8 (IQR: 4.2 – 5.8) months, whilst the median time from RT to a DND was 9.7 (IQR: 7.2 – 26.7) months. Neck dissection data is detailed in Table 2-2.

Table 2-2. Neck dissection data					
Nodal response	n (%)	Immediate ND	IND residual disease (%)	Delayed ND	DND residual disease (%)
CR	218 (62.8)	1	0	5	2 (40)
EQR	81 (23.4)	4	2 (50)	9	5 (55.6)
ICR	48 (13.8)	8	6 (75)	3	3 (100)
Median time from RT to ND months (IQR)		4.8 (4.2-5.8)		9.7 (7.2 - 26.7)	

Complete nodal response (CR)

218 patients achieved a CR; 1 patient whom had CR was found to have residual disease at the primary site with distant metastasis at their 12-week PET-CT. One IND was performed (at 5 months) as the patient clinically had a residual neck lump; ND was pathologically negative. 5 delayed ND (DND) were performed in total; 3 were performed (at 10, 31 & 45 months) due to relapsed disease at the primary site and surgical salvage - all 3 DNDs were pathologically negative. 2 DND were performed at 18 & 36 months due to concerns of nodal relapse, both were pathologically positive. The former remains disease free and alive, whilst the latter is deceased following further nodal relapse.

Of the 211 patients who underwent surveillance only with neither IND nor DND, 16 recurrences occurred (1 neck only, 3 neck and distant metastasis, 9 with distant metastasis only, 2 primary site only and 1 primary site with distant metastasis).

The NPV of a nodal CR for residual neck disease alone was 97.2% (95% CI, 94.1-99.0), and 93.1% (95% CI, 88.9-96.1) if distal relapses included.

Equivocal nodal response (EQR)

81 patients achieved an EQR.

1 patient with EQR had residual disease at the primary site with distant metastasis at their posttreatment 12-week PET-CT, and therefore, was not eligible for salvage surgery.

4/81 of the EQR group had an IND performed between 4-6 months post-RT. Of these, 2 (50%) were pathologically positive; one patient remains disease free and alive, whilst the other has deceased from a new primary cancer (non-HNSCC). Both patients who had negative NDs remain disease free and alive. Equivocal nodal response data is detailed in Table 2-3.

Table 2-3: EQR subgroup – neck dissections and clinical outcomes

	IND	Active surveillance group		P-Value
		DNDs	No NDs	
No. (pathologically positive ND)	4 (2)	9 (5)	67	-
Pathological ND – n (%)	2 (50%)	5 (55.6)	-	-
Nodal failures	0	0	0	NA
Distant Failures	0	2	2	0.07
Accumulative HNSCC related deaths – n	0	2	2	0.07

Exclude 1 patient who had distant metastasis on 12-week surveillance PET-CT.
NA denotes not applicable.

76 patients underwent extended surveillance. 9 DNDs were carried out; 5 (55.6%) were positive (NDs performed at 6, 7, 21, 22 & 31 months) – 4 patients remain disease free, 1 patient developed distant metastasis. 4 of the DNDs (performed between 6-8 months) were pathologically negative, 3 patients remain disease free, and 1 patient developed distant metastasis at 7 months. The remaining 67 patients underwent extended surveillance only. 1 patient was offered an IND, however, it was the patient's wish to undergo surveillance in first instance but unfortunately developed distant metastasis on subsequent imaging at 6 months post-treatment, despite continued improvement of the neck disease. Another patient had an EQR in the neck on initial surveillance imaging, but later went on to develop an enlarged neck node which stabilised in size. This was found to be pathologically negative on biopsy, but they developed distant metastasis at 53 months posttreatment.

The remaining 65 (of 67, 97.0%) patients who underwent surveillance without a ND have remained disease free. Overall, 70 of 81 (86.4%) patients with an EQR on 12-week PET-CT had no residual disease during follow up (either a pathologically negative ND if surgery undertaken or no subsequent neck or

distant relapse clinically/radiologically). The PPV of an EQR was 9.9% (95% CI, 4.4-18.5) for predicting nodal relapse alone, and 13.6% (95% CI, 7.0-23.0) for predicting nodal or distant relapse.

Incomplete nodal response (ICR)

48 patients achieved an ICR; 10 patients had inoperable disease (locoregionally advanced disease with/without distant metastasis) on surveillance FDG PET-CT, and 1 patient declined salvage surgery and subsequently developed distant metastasis. 8 patients had an IND (between 4-6 months); 6 (75.0%) were pathologically positive - 2 patients developed further relapse at 23 and 31 months post-RT; 1 relapsed with neck disease only and 1 patient relapsed at the primary site with distant metastasis. 2 INDs were pathologically negative; 1 patient developed distant metastasis at 20 months post-RT, the other patient remains disease free. As 29 patients showed significant improvement in tumour burden on 12-week PET-CT it was felt that extended surveillance rather than IND was appropriate. 3 of these patients had a DND between 7-10 months post-RT, 2 of whom also had simultaneous salvage surgery for relapsed disease at the primary sites. All 3 (100%) DNDs were pathologically positive. Of these 3 patients, 1 patient chose surveillance over IND and had a DND performed when residual disease was evident at 10 months of surveillance, but unfortunately developed distant metastasis at 13 months post-RT. 1 patient had positive surgical margins, and subsequently developed further neck relapse at 15 months; the third patient relapsed in the neck with distant metastasis at 13 months. 26 patients who underwent surveillance did not have a ND; 24 patients did not experience disease relapse at the neck or distant metastasis during follow-up. Of the 48 patients with an ICR on 12-week PET-CT, 25 (52.1%) had no residual disease during follow up (either a pathologically negative ND if surgery undertaken or no subsequent neck or distant relapse clinically/radiologically). The PPV of an ICR for nodal relapse alone was 43.8% (95% CI, 29.5-58.8), and 47.9% (95% CI, 33.3- 62.8) for nodal relapse or distant metastasis.

2.4.3 Overall survival (OS)

Overall, 53 (15.3%) of 347 patients died; 36 (10.4%) died of HNSCC and 17 (4.9%) of non-HNSCC causes. 2-year OS for the whole cohort was 94.1% (95% CI 91.1-

96.4). 2-year OS for the subgroups were 98.1% (95% CI 95.3-99.5), 95.0% (95% CI 87.7-98.6) and 74.5% (95% CI 59.7-86.1) for CR, EQR and ICR groups respectively. Median survival of the EQR and CR groups were not reached with no statistically significant differences in overall survival (OS) between the groups, p=1.0 (Figure 2-1). Median survival of the ICR group was also not reached. However, OS for the ICR group was significantly worse than that of CR, and EQR, log-rank test for both p<0.001.

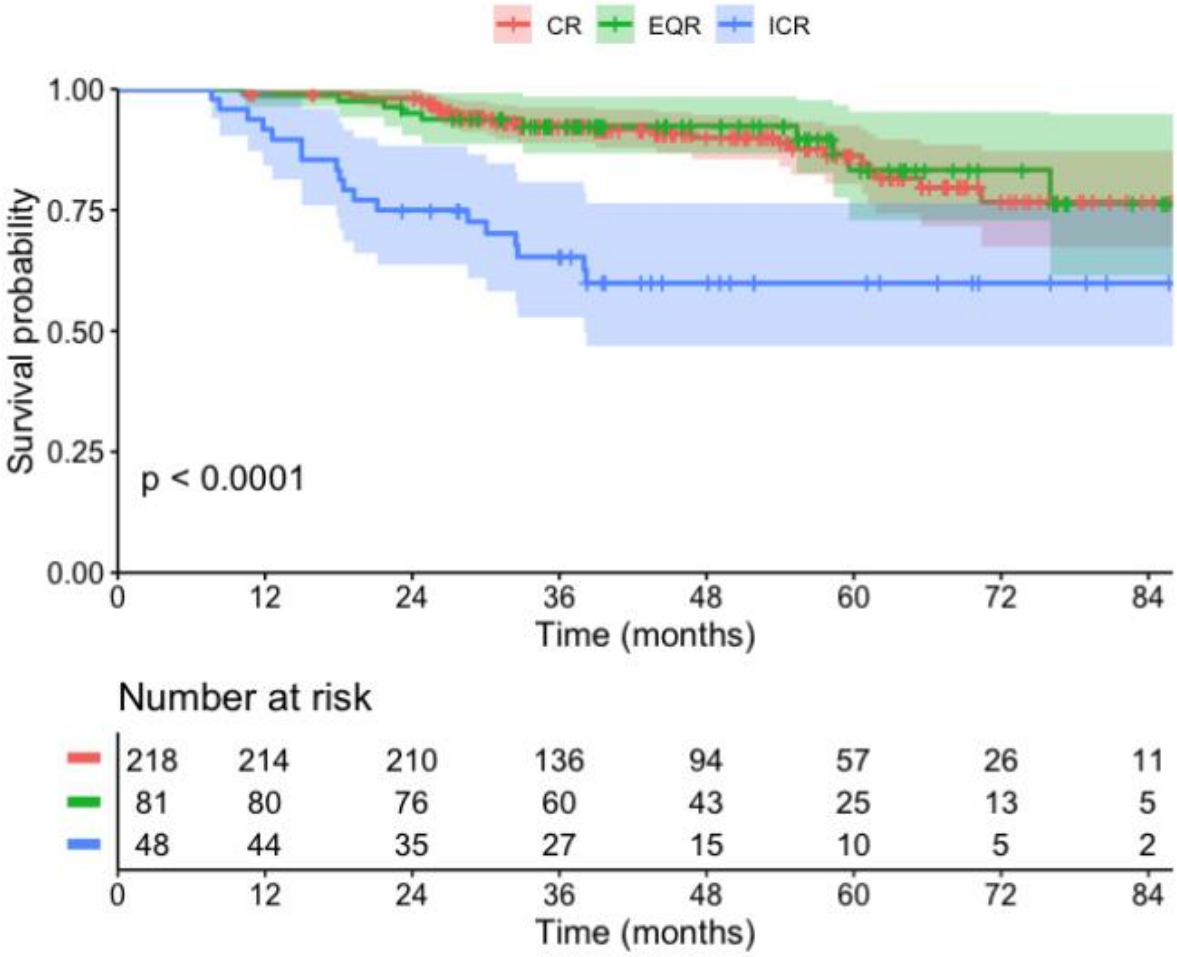

Figure 2-1: Kaplan-Meier estimates for overall survival (acknowledgement Suyun Zhou)

2.5 DISCUSSION

This data demonstrates that a less than complete nodal response on 12-week FDG PET-CT post (chemo) RT is a flawed assessment of residual disease and the need for salvage ND in HPV-positive HNSCC. From this cohort, 86% of those who achieved an equivocal nodal response and 52% of those who achieved an incomplete nodal response were in fact disease free during follow up (no neck or

distant relapse, including those who had a neck dissection that were pathologically negative). While the NPV of a nodal CR is high (97%) the PPV of an EQR is only 10%. This is consistent with other studies that has ascertained a high NPV and poor PPV of PET-CT when performed at 12 weeks posttreatment for HPV-positive HNSCC [3, 8, 16]. The omission of IND for those who achieve an incomplete nodal response remains controversial but the combination of novel biomarkers such as circulating HPV DNA [13] with FDG PET-CT may allow this strategy to be refined in the future.

Extended surveillance rather than IND for equivocal responders has been adopted in clinical practice by many centres due to the poor positive predictive value of 12-week PET-CT and the well-recognised good prognosis of HPV-positive HNSCC. However, valid concerns remain about compromising long term outcomes for these patients, either due to missed opportunities to successfully resect residual nodal disease, or the opportunity for the dissemination of distant metastases from active neck disease. In our cohort of EQR nodal responders, there were no patients who were subject to extended surveillance who developed inoperable neck recurrence. One patient who had a delayed positive neck dissection went on to develop distant metastases and in retrospect may have benefitted from an immediate neck dissection. Two further patients had EQRs and clinically resolving neck disease but subsequently developed distant metastases. There was no pathological evidence of active neck disease at that time, thus a neck dissection may or may not have been beneficial. These 3 (of 76, 3.9%) patients may represent the only cases with an EQR where extended surveillance may have been detrimental to outcome. By comparison, 15 (of 216, 6.9%) patients in the CR group subsequently developed recurrent disease in the neck and/or distant metastases (without evidence of relapse in the primary site). The low rate of subsequent disease relapse with extended surveillance in the EQR group, which is no higher than that in the CR group, means it is increasingly difficult to justify the morbidity of INDs for all patients with an EQR and HPV positive HNSCC. This paradigm is of course already accepted as standard of care, regardless of HPV status, for those with a CR.

Median survival was not reached across all response groups, which is compatible with the favourable prognosis associated with HPV-positive HNSCC [17]. The long-term overall survival of the equivocal responders was comparable to that of the complete responders with no statistical difference between the two groups.

Immediate neck dissections were carried out in only 5% of equivocal responders. This is therefore unlikely to be the main driver of the positive survival outcomes of the group. Late disease relapses, particularly distant metastases, are well recognised in HPV-positive HNSCC [10, 11]. The omission of an IND may, in theory, contribute to the development of late distant metastases. This is the first study to evaluate extended surveillance with follow-up to 8 years in HPV positive HNSCC, allowing capture of these late events. We demonstrated that extended surveillance in patients who achieve an equivocal nodal response does not result in excess late relapses or compromise long term survival. Practice in many centres has transitioned to extended surveillance rather than IND for this group of patients with increasing evidence to support this approach [3, 5, 7, 8, 18]. However, surveillance imaging protocols remain ill-defined beyond the 12-week PET-CT, with modality and scheduling of subsequent imaging at the discretion of the local MDT.

There is evidence that the majority (70-75%) of those who have HPV-positive HNSCC and achieve a less than complete response on 12 week PET-CT go on to convert to a complete response on subsequent PET-CT at 16 weeks or later [8, 18]. This data coupled with the reassuring survival outcomes from this cohort has allowed us to refine our imaging strategy. In our centre patients with HPV-positive HNSCC now undergo PET-CT at 16 weeks post-treatment instead of 12 weeks. Those with less than a complete response and no additional clinical or radiological concerns of active disease then undergo '2nd look' PET-CT at 6 months. The impact of this change is under evaluation. Recommendations for optimal imaging protocols are beyond the scope of the current work and further data is needed.

Limitations of the study

This was a retrospective review with the inherent limitations of such. When categorised into complete, equivocal, and incomplete nodal responses, sample sizes are modest. However, the 2-year overall survival of 94.1% demonstrated in our cohort is comparable to that of the De-ESCALate Study population [19], this suggests our data is reflective of contemporary practice and outcomes.

A prospective randomised clinical trial to assess long term outcomes with extended surveillance versus immediate neck dissections for those achieving an equivocal nodal response is desirable but unlikely to be feasible as extended surveillance has already been implemented into clinical practice in many

centres. Without the prospect of being able to generate level one evidence, multi-centre validation of this strategy may be useful.

2.6 CONCLUSION

Despite the omission of IND in 95% of those who achieved an EQR nodal response, there was no difference in late survival between the EQR and CR groups. Our study suggests extended surveillance does not compromise long-term survival in patients with HPV-positive HNSCC who achieve an EQR on 12-week FDG PET-CT.

2.7 POST VIVA ADDITIONS TO CHAPTER

The following section was added as correction to the thesis post viva, taking into account examiners' comments.

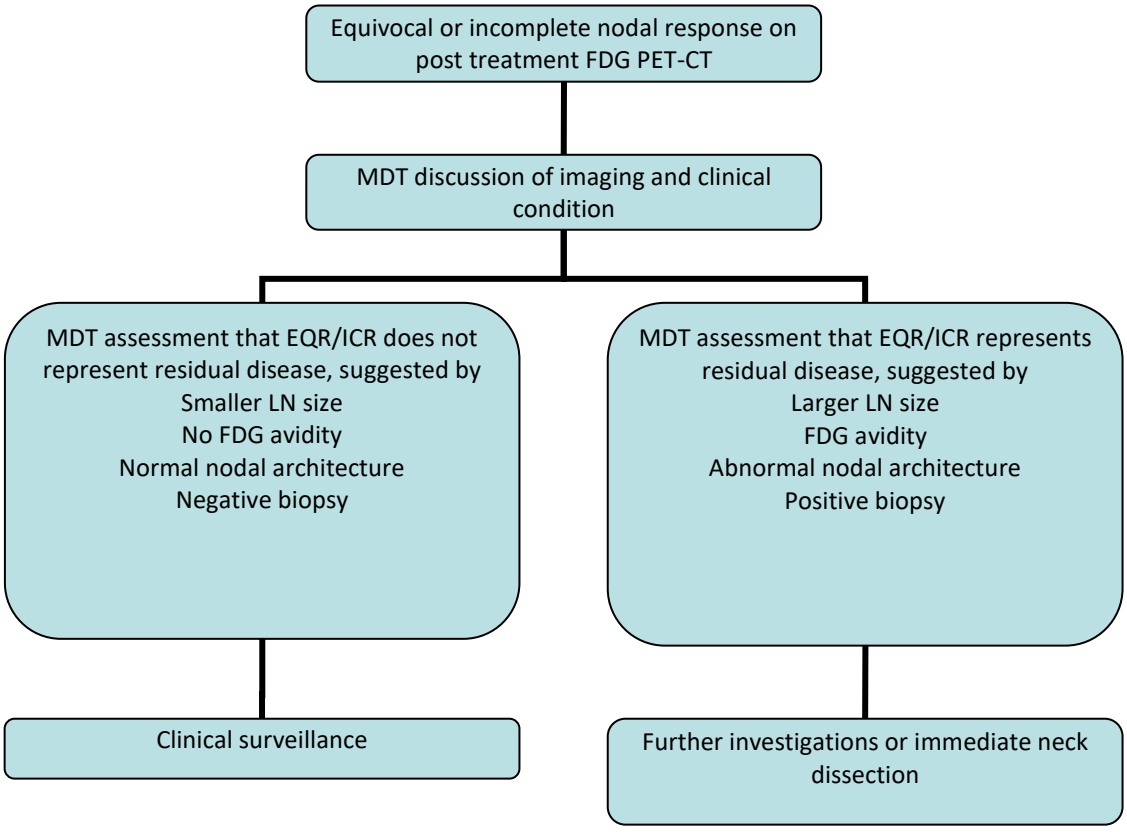

Figure 2-2: Decision making in cases with ICR or EQR on post-treatment FDG PET-CT.

OS analysis was stratified by CR, EQR and ICR groups. While the aim of the work was to examine whether OS was affected by IND in patients with a <CR, the number of INDs in this sub-group was small and unlikely to allow any meaningful

analysis. Of 81 patients with an EQR, 4 underwent IND. Of 48 patients with an ICR, 8 underwent IND. Therefore OS of the sub-groups (without further stratification by IND) were examined. There was no significant difference in OS between the CR and EQR groups. 2 positive INDs of 81 cases in the EQR group was felt, on balance, unlikely to contribute to the good OS seen. It is acknowledged however that a formal sample size calculation to assess the power of the analyses was not carried out.

Table 2-1 amended

Table 2-1. Baseline patient demographics, tumour characteristics and treatments received.

	Whole cohort	Sub-group undergoing IND	Sub-group not undergoing IND
Total number of patients	347	12 (3.5)	335 (96.5)
Mean age, years	57	59.5	58
Gender, Male – no. (%)	276 (79.5)	10 (83.3)	266 (79.4)
Smoking status –no. (%) Never Current/Former Unknown	 149 (42.9) 196 (56.5) 2 (0.6)	 4 (33.3) 8 (67.7) 0 (0)	 145 (43.3) 188 (56.1) 2 (0.6)
Tumour site – no. (%) Oropharynx SCC Unknown primary SCC	 309 (89.0) 38 (11.0)	 11 (91.7) 1 (8.3)	 298 (89.0) 37 (11.0)
Tumour classification[a] – no. (%) T0 T1 T2 T3 T4	 38 (10.9) 86 (24.8) 105 (30.3) 40 (11.5) 78 (22.5)	 1 (8.3) 4 (33.3) 2 (16.7) 3 (25) 2 (16.7)	 37 (11.0) 82 (24.5) 102 (30.5) 37 (11.0) 76 (22.7)
Nodal classification[a] – no. (%) N1 N2 N3	 275 (79.3) 58 (16.7) 14 (4.0)	 11 (91.7) 1 (8.3) 0 (0)	 264 (78.8) 57 (17.0) 14 (4.2)
HPV detected by DNA P16	 308 (88.8) 39 (11.2)	 12 (100) 0 (0)	 296 (88.4) 39 (11.6)
Types of concurrent SACT[b] Cisplatin Carboplatin Cetuximab	 265 (89.5) 10 (3.4) 21 (7.1)	 8 (66.7) 0 (0) 2 (16.7)	 257 (76.7) 10 (3.0) 19 (5.7)
Radiotherapy only	51 (14.7)	2 (16.7)	49 (14.6)
a Based on AJCC 8th Ed. b SACT denotes systemic anti-cancer treatment			

Formal comparison of baseline patient and tumour features and treatment received was not carried out but additional detail in the table above has been added. Imbalances in these baseline features may affect results but as described above with only 8 cases of pathologically positive IND (2 from EQR and 6 from ICR group) Vs 339 cases with either negative IND or no IND, any baseline differences in the 8 cases are not felt likely to have confounded outcomes. While the aim of the study was to examine the long-term safety of the omission of an IND in patients with HPV-positive HNSCC achieving less than a CR on 12-week FDG PET-CT when the <CR group were split into EQR and ICR (as is routine in clinical practice) we found survival differences between the EQR and ICR sub-groups. Hence the conclusion that the current practice of extended surveillance, rather than IND, does not compromise long-term survival in the EQR sub-group only.

2.8 REFERENCES

1. Mehanna H, Wong WL, McConkey CC, Rahman JK, Robinson M, Hartley AG, et al. PET-CT Surveillance versus neck dissection in advanced head and neck cancer. N Engl J Med. 2016; 374(15): 1444-1454.
2. Mehanna H, Evans M, Beasley M, Chatterjee S, Dilkes M, Homer J, et al. Oropharyngeal cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016; 130(Suppl 2): S90–S96.
3. Rulach R, Zhou S, Hendry F, Stobo D, James A, Dempsey MF, et al. 12 week PET-CT has low positive predictive value for nodal residual disease in human papillomavirus-positive oropharyngeal cancers. Oral Oncol. 2019; 97:76-81.
4. Bird T, Barrington S, Thavaraj S, Jeannon JP, Lyons A, Oakley R, et al. 18F-FDG PET/CT to assess response and guide risk-stratified follow-up after chemoradiotherapy for oropharyngeal squamous cell carcinoma. Eur J Nucl Med Mol Imaging. 2016; 43(7): 1239-47.
5. Huang SH, O'Sullivan BO, Xu W, Zhao H, Chen D, Ringash J, et al. Temporal nodal regression and regional control after primary radiation therapy for N2-N3 head-and-neck cancer stratified by HPV status. RadiatOncolBiol Elsevier Inc. 2013; 87(5): 1078-85.

6. Mirghani H, Amen F, Tao Y, Deutsch E, Levy A. Increased radiosensitivity of HPV-positive head and neck cancers: molecular basis and therapeutic perspectives. Cancer Treat Rev. 2015; 41(10): 844-52.
7. Urban R, Godoy T, Olson R, Wu J, Berthelet E, Tran E, et al. FDG-PET/CT Scan assessment of response 12 weeks post radical radiotherapy in orpharyngeax head and neck cancer: The impact of p16 status. Radiother Oncol. 2020; 148: 14-20.
8. Liu HY, Milne R, Lock G, Panizza BJ, Bernard A, Foote M, et al. Utility of a repeat PET/CT scan in HPV-associated Oropharyngeal Cancer following incomplete nodal response from (chemo)radiotherapy. Oral Oncol. 2019;88(September 218): 153-159.
9. Zhou S, Rulach R, Hendry F, Stobo D, James A, Dempsey MF, et al. Positron Emission Tomography-Computed Tomography Surveillance after (Chemo)Radiotherapy in Advanced Head and Neck Squamous Cell Cancer: Beyond the PET-NECK Protocol. Clin. Oncol. 2020; 32: 665-673.
10. Huang SH, Perez-Ordonez B, Weinreb I, Hope A, Massey C, Waldron JN, et al. Natural course of distant metastases following radiotherapy or chemoradiotherapy in HPV-related oropharyngeal cancer. Oral Oncol. 2013;49(1):79-85.
11. Trosman SJ, Koyfman SA, Ward MC, Al-Khudari S, Nwizu T, Greskovich JF, et al. Effect of human papillomavirus on patterns of distant metastatic failure in oropharyngeal squamous cell carcinoma treated with chemoradiotherapy [published correction appears in JAMA Otolaryngol Head Neck Surg. 2015 May 1;141(5):428]. JAMA Otolaryngol Head Neck Surg. 2015;141(5):457-462.
12. Gregoire V, Coche E, Cosnard G, Hamoir M, Reychler 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. RadiotherOncol; 56 (2): 135–50.
13. Lee JY, Garcia-Murillas I, Cutts RJ, De Castro DG, Grove L, Hurley T, et al. Predicting response to radical (chemo) radiotherapy with circulating HPV DNA in locally advanced head and neck squamous carcinoma. Br J Cancer. 2017; 117: 876-883.
14. Simo R, Homer J, Clarke P, Mackenzie K, Paleri V, Pracy P, et al. Follow-up after treatment for head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;130(S2):S208-S211.

15. Machiels JP, René Leemans C, Golusinski W, Grau C, Licitra L, Gregoire V. Squamous cell carcinoma of the oral cavity, larynx, oropharynx and hypopharynx: EHNS-ESMO-ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020; 31(11):1462-1475.
16. Helsen N, Van den Wyngaert T, Carp L, Stroobants S. FDG-PET/CT for treatment response assessment in head and neck squamous cell carcinoma: a systematic review and meta-analysis of diagnostic performance. Eur J Nucl Med Mol Imaging. 2018; 45(6): 1063-71.
17. Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis. 2019; 10(8):540.
18. Prestwich RJD, Arunsingh M, Zhong J, Dyker KE, Vaidyanathan S, Scarsbrook AF. Second-look PET-CT following an initial incomplete PET-CT response to (chemo) radiotherapy for head and neck squamous cell carcinoma. Eur Radiol. 2020 Feb; 30(2):1212-1220.
19. Mehanna H, Robinson M, Hartley A, Kong A, Foran B, Fulton-Lieuw T, et al; De-ESCALaTE HPV Trial Group. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial. Lancet. 2019; 393(10166):51-60.

3. Comparative cohort study of volumetric modulated arc therapy for squamous cell cancer of unknown primary in the head and neck—Involved neck only versus mucosal irradiation

Poon WY, Thomson M, McLoone P, Wilson C, Crosbie R, Schipani S, Grose D, James A, Lamb C, Rizwanullah M, Campbell F, Easton F, **Paterson C.** Comparative cohort study of volumetric modulated arc therapy for squamous cell cancer of unknown primary in the head and neck-Involved neck only versus mucosal irradiation. Clin Otolaryngol. 2020 Nov;45(6):847-852. doi: 10.1111/coa.13593. Epub 2020 Sep 17. PMID: 32501648.

3.1 ABSTRACT

3.1.1 Objectives

Target volumes for irradiation remain ill-defined for squamous cell cancer of unknown primary in the head and neck (SCCUP). The aim of this study was to compare involved neck only (INO) radiotherapy (RT) with irradiating involved neck plus potential mucosal primary sites and contralateral neck (MUC) in patients diagnosed and treated with modern diagnostics and techniques.

3.1.2 Design

This is a retrospective cohort study. Patients with a diagnosis of SCCUP with unilateral neck disease were included.

3.1.3 Results

Thirty patients were identified. All underwent FDG PET-CT. 47% of patients had HPV-positive SCC. 20 patients received RT to INO, 10 patients to MUC, all with volumetric modulated arc therapy (VMAT). A significantly lower dose for each organ at risk was delivered in INO-treated patients, with mean dose to contralateral parotid gland 57% less. The proportion of patients with late grade 2 or worse xerostomia was higher in MUC patients. The incidence of grade 2-3 mucositis (89% vs 45%) and grade 3 or worse dysphagia (50% vs 10%) was higher in MUC patients. Median follow-up was 31 months. No mucosal primaries emerged. Progression-free survival at 2 years was 74.7% for INO patients, 70% in the MUC

group. Overall survival at 2 years was 79.7% in the INO group and 70% in the MUC patients.

3.1.4 Conclusion

INO radiotherapy for patients with SCCUP of the head and neck is a safe treatment strategy resulting in clinically significant lower RT doses to OARS. Acute and late toxicities are reduced without detriment to patient survival.

3.2 INTRODUCTION

The diagnosis of squamous cell cancer of unknown primary in the head and neck (SCCUP) is rare, [1, 2] and therefore no randomised trials [3] regarding optimal management have been completed. [4] Practice is guided by retrospective studies, which are often limited by institutional bias and lengthy duration of data collection resulting in heterogeneous diagnostic techniques and treatment over that time.

For radiation oncologists, the most important questions in the treatment of SCCUP remain unanswered: what volume to treat and to what dose? [5]Advances in radiation technology with intensity-modulated radiation therapy (IMRT) mean that it is now possible to treat different target volumes to different doses and to spare “organs at risk” (OARS). [4, 6]There has been renewed interest in “mucosal irradiation,” focussing on delivering a radical dose of radiotherapy to areas of known disease only [5] and an elective dose of irradiation to areas at risk of microscopic disease (potential primary sites and contralateral neck). [7] The rate of mucosal primary emergence is low [3, 8] with this approach, but a significant length of pharyngeal mucosa is treated to a dose of at least 50Gy meaning toxicity can be significant. [9]

There may be an opportunity to spare toxicity by avoiding elective irradiation of potential primary sites. As SCCUP in the head and neck has a much better prognosis than unknown primary cancers below the clavicles, [5, 10] the avoidance of late and permanent toxicity is relevant.

We present a series of patients with SCCUP treated in our tertiary cancer centre between August 2012 and December 2016, all of whom have undergone FDG PET-CT as part of their work up—the first series to do this. Our aim was to evaluate disease-related outcomes; dosimetry to OARS and toxicities in patients

investigated, diagnosed and treated for SCCUP with modern diagnostic and radiotherapy techniques.

3.3 METHODS

Eligible patients were identified from our radiotherapy database. Those with histologically confirmed squamous cell cancer cervical lymph nodes and no primary tumour site were selected for further evaluation. Patients with bilateral neck involvement were excluded. Data including patient demographics, treatment details, toxicities and disease control were extracted from case records.

3.3.1 Ethical considerations

This study used anonymised patient information, gathered retrospectively and therefore was exempt from the regional ethical committee review.

3.3.2 Participants

Pathological diagnosis was made by either biopsy or fine-needle aspiration from the lymph node(s). Potential primary sites were assessed by fibre-optic nasendoscopy of upper aero digestive tract. Cross-sectional imaging was undertaken with CT. Assuming no primary site was found, fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) was obtained. EUA and either imaging-directed or blind biopsies were then undertaken. SCCUP was a diagnosis of exclusion, made on the basis of these negative investigations. HPV status was assessed routinely from 2013 onwards, and HPV status was determined using either HPV DNA or p16 staining (where staining of >70% was considered positive). All patients were assessed by a consultant head and neck oncologist.

3.3.3 Treatment

All patients underwent radiotherapy with volumetric modulated arc therapy (VMAT). Patients receiving radiotherapy as primary treatment received 65Gy in 30 fractions to areas of gross disease and the entirety of that involved nodal level and 54Gy/30# to areas at risk of microscopic disease. Patients who received adjuvant radiotherapy following neck dissection received either

65Gy/30# if there was extracapsular spread (ECS) or 60Gy/30# (if no ECS) to involved nodal levels and 54Gy/30# to areas at risk of microscopic disease. Two approaches to target volume selection were utilised. One entailed treating involved neck only (INO—radical dose to involved nodal levels and elective dose to uninvolved nodal levels in ipsilateral neck). The second approach was to treat involved neck as described as well as potential mucosal sites and contralateral neck with an elective dose of radiotherapy (MUC). The decision to employ INO or MUC was at the discretion of the treating clinician. Mucosal RT with HPV-positive disease tended to include oropharynx only and HPV-negative disease more extensive mucosal irradiation. All radiotherapy outlining was subject to peer review. Therefore, despite differing treatment philosophies between clinicians, quality assurance of target delineation was consistent.
Standard chemotherapy protocol was cisplatin 100 mg/m^2 on day 1 and 22 of RT.

3.3.4 Toxicity

Patients were reviewed weekly during radiotherapy and, the presence of acute skin toxicity, dysphagia and mucositis was graded using Radiation Therapy Oncology Group (RTOG) and WHO toxicity criteria.
Late toxicity (xerostomia and dysphagia) was graded using the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) and was assessed at 6-, 12- and 24-months post-radiotherapy.

3.3.5 Statistical analysis

Follow-up and survival statistics were calculated from the start of radiotherapy treatment until death or last follow-up. Actuarial 1- and 2-year rates for overall and progression-free survival (PFS) were estimated using the Kaplan-Meier method. PFS was defined as survival until recurrence or death from any cause. Comparison of survival between INO- and MUC-treated patients were performed using the log rank test.
Differences between INO- and MUC-treated patients were tested using two-sample *t* tests for dosimetry measures and Fisher's exact test for toxicity categories. All tests were two-sided, and *P*-values < .05 were considered statistically significant.

3.4 RESULTS

3.4.1 Patient demographics and diagnostic investigations

A total of 30 eligible patients were treated with radiotherapy in our centre between 1 August 2012 and 30 April 2016. Demographics are shown in Table 3-1. 86.7% of patients had level 2 involvement.

		Whole cohort n = 30 (%)
Gender	Male: female	20 (66.7%): 10 (33.3%)
Age	Median (IQR)	58.1 (50-66.6)
Smoking history	Current/former	22 (73.3)
	Never	6 (20)
	Unknown	2 (6.7)
N stage	N1	4 (13.3)
	N2a	4 (13.3)
	N2b	18 (60.0)
	N3	4 (13.3)
HPV status	Positive	14 (46.7)
	Negative	9 (29.9)
	Unknown	7 (23.3)

Table 3-1. Table of patient demographics

3.4.2 Neck dissections

Seventeen of thirty (56.7%) of patients underwent neck dissection (ND) prior to radiotherapy with 9 modified radical NDs, 4 selective NDs, 3 radical NDs and 1 extended radical ND. ECS was seen in 12/17 (70.6%) patients undergoing primary neck dissection.

3.4.3 Radiotherapy

Twenty patients (66.7%) received RT to INO, 10 patients (33.3%) to MUC, all with VMAT. Radiotherapy was delivered over a median of 42 days (IQR 41-43) with no difference between INO and MUC groups.

Of the patients receiving mucosal RT, 6 had oropharyngeal mucosa only treated, 2 total mucosal irradiation (nasopharynx to larynx) and 2 nasopharynx and oropharynx mucosa only. 7 of the 13 HPV-positive patients were treated with

INO RT, and 6 underwent elective oropharyngeal mucosal and contralateral neck RT.

3.4.4 Chemotherapy

One patient received induction chemotherapy with 2 cycles of the TPF regime. 17 patients (56.7%) received concurrent chemotherapy: 16 with cisplatin and 1 with carboplatin. 13 of 16 patients received cisplatin 100 mg/m^2 for 2 cycles. 50% of the patients in the MUC group received concurrent chemotherapy, and 60% of patients in the INO group received concurrent chemotherapy.

3.4.5 Dose to organs at risk

The doses delivered to OARs are shown in Table 3-2. A statistically significant lower dose for each OAR was delivered in INO-treated patients. The largest dose difference was seen in the mean dose to the contralateral parotid (57% less). The maximum dose to the brain stem and mean upper midline mucosal dose were over 20% less.

	Involved neck only (INO) n = 20	Involved neck with mucosal sites and contralateral neck (MUC) n = 10	
	Mean dose cGy (SD)	Mean dose cGy (SD)	*p*-value
Mean dose contralateral parotid	1526 (866)	3574 (1042)	<.001
Maximum dose brain stem	3220 (901)	4105 (245)	.005
Maximum dose spinal cord	3636 (455)	3971 (228)	.038
Mean dose upper midline mucosa (hyoid-cricoid)	3954 (564)	5068 (506)	<.0001
Mean dose lower midline mucosa (cricoid-sternum)	2613 (943)	3324 (603)	.039

Table 3-2. Table of doses to organs at risk

3.4.6 Acute toxicity

There was no statistically significant difference between the skin toxicity in the INO and MUC patients. However, the incidence of grade 2 and 3 mucositis and grade 3 or worse dysphagia was higher in MUC patients (Table 3-3).

		All n = 30 (%)	INO n = 20 (%)	MUC n = 10 (%)	Fisher's exact test
Skin* (RTOG)	Grade 0-1	4 (13.8)	2 (10)	2 (22.2)	*P* = .57
	Grade 2a-b	25 (86.2)	18 (90)	7 (77.8)	
Mucositis* (WHO)	Grade 0-1	12 (41.4)	11 (55.0)	1 (11.1)	*P* = .043
	Grade 2-3	17 (58.6)	9 (45.0)	8 (88.9)	
Dysphagia (RTOG)	Grade 0-2	23 (76.7)	18 (90.0)	5 (50.0)	*P* = .026
	Grade 3 or >	7 (23.3)	2 (10.0)	5 (50.0)	

Table 3-3. Table of acute toxicity

**Acute skin and mucositis toxicity data missing for 1 MUC patient.*

3.4.7 Late toxicity

Only 14 of 30 patients were evaluable at 24 months for late toxicity outcomes. No patients experienced late grade 3 dysphagia during follow-up. Only one INO patient experienced grade 2 + xerostomia at 6, 12 and 24 months. The proportion of patients with grade 2 + xerostomia was higher in MUC patients during the first year of follow-up (55.5% (5/9) vs 7.7% (1/13) at 6 months (*P* = .023) and 28.6% (2/7) vs 7.7% (1/13) at 12 months (*P* = .27)). At 24 months, no MUC (0/6) patients experienced grade 2 + xerostomia.

3.4.8 Disease control and overall survival

Median follow-up for the whole group was 31 months (range 5.5 to 69.5 months). No mucosal primaries emerged during follow-up. In total, 10 patients died, 3 from causes unrelated to their SCCUP diagnosis. One patient suffered an out of hospital cardiac arrest, while another developed an adenocarcinoma of the oesophagus. The cause of death was unclear for the third patient. However, all 3 patients were disease-free prior to death. Seven patients died from recurrent SCCUP: 4 of 20 patients in the INO group, 3 of 10 patients in the MUC group.

Table 3-4 details further the patients with recurrent disease. Recurrences in the involved neck all occurred in the high-dose (65Gy/30#) radiotherapy region. The contralateral neck recurrence occurred in the elective (54Gy/30#) volume. The patient with involved neck only recurrence with no distal disease underwent salvage surgery but experienced further disease recurrence in the same area and subsequently died of progressive disease.

	Patient	**Stage**	**HPV**	**Primary Treatment**	**Concurrent chemotherapy**	**Site recurrence**
Involved neck only RT group	1	T0N2b	Positive	CRT	Carboplatin AUC5 × 1 cycle	Involved neck, mediastinal LNs
	2	T0pN3	Not done	ND	Cisplatin 100 mg/m^2 ×2	Lung, adrenal, bone mets
	3	T0pN2b	Negative	ND	Cisplatin 100 mg/m^2 ×2	Lung mets
	4	T0pN2b	Not done	ND	No chemotx	Lung mets
Mucosal and bilateral neck RT group	1	T0N2b	Negative	RT	No chemotx	Involved neck, contralateral neck, lung mets
	2	T0N3	Negative	CRT	Cisplatin 100 mg/m^2 ×2	Involved neck only
	3	T0pN2a	Positive	Neck dissection	No chemotx	Bone, liver, lung mets

Table 3-4. Table of patients with recurrent disease

Progression-free survival (PFS) at 2 years was 74.7% in the INO patients (95% CI 49.4-88.6%) and 70% in MUC patients (95% CI 49.4-88.6%). Overall survival (OS) at 2 years was 79.7% (95% CI 54.5-91.9%) in the INO group and 70% (95% CI 32.9-89.2%) in the MUC group.

3.5 DISCUSSION

3.5.1 Synopsis of key findings

This is the only series of SCCUP head and neck where all patients have undergone a FDG PET-CT as part of their diagnostic work up. It is the first series to directly compare outcomes from unilateral neck radiotherapy with VMAT to irradiating potential mucosal primary sites with VMAT. A statistically significant lower dose for each OAR was delivered to patients treated with an INO approach, with clinically meaningful dose reductions to the contralateral parotid gland and midline mucosa. Significantly reduced rates of acute mucositis and dysphagia were experienced by this group and lower rates of late xerostomia during the first 12 months. Improved toxicity was not achieved at the expense of disease control. We found that the rate of mucosal emergence of the primary was not different between the two treatments. PFS and OS at 2 years were comparable between the 2 groups.

3.5.2 Strengths and limitations

Our study suffers from several limitations. It is retrospective and involves a small number of patients, although this is in keeping with previous reports in this area. We also found significant attrition in the recording of toxicity during follow-up, meaning few patients were evaluable for late toxicity outcomes. However, its strength is in the homogeneity of the overall study cohort. Patients were accrued over a short time meaning uniformity of investigations and treatment, with many other published series taking several years, with changes in diagnostic techniques and treatment. Median follow-up was 35 months with most work in this area reporting no recurrences beyond 2 years. It is likely therefore that this series has captured the majority of relapses.

3.5.3 Comparison with other studies

Most publications concerning diagnosis, treatment and subsequent outcomes in SCCUP head and neck pre-date recent advances in both diagnostic work up (eg FDG PET-CT and HPV testing) and contemporary treatment techniques (eg IMRT/VMAT). This series is the first where PET-CT was carried out for 100% of the patients. HPV status was available for 77%, and all were treated with highly conformal radiotherapy (VMAT). This series therefore makes a significant

contribution to the literature regarding the contemporary management of SCCUP head and neck.

A recent UK phase II study focussed on limiting dose to OARs while delivering total mucosal irradiation with IMRT. Toxicity was “moderate” despite this, and 1 mucosal primary emerged in the 36 patients. Only 44% underwent FDG PET-CT during diagnostic work up. [11] In contrast, another recent series found no emergence of putative primaries and no isolated contralateral neck failures with unilateral neck only radiotherapy HPV-positive SCCUP, suggesting this may be an “underutilised management strategy” for this group.[12]

Our series includes patients with HPV-positive and HPV-negative disease. INO radiotherapy for both groups results in reduced dose delivered to OARs, improved toxicity and no isolated contralateral neck recurrences or primary site emergence, suggesting this strategy may be valid for both HPV-positive and HPV-negative sub-groups.

3.5.4 Clinical applicability

For patients with a diagnosis of SCCUP head and neck with unilateral neck disease, the most appropriate target volume for radiotherapy remains unclear and is unlikely to be confirmed via a randomised control trial. Given that SCCUP head and neck has a relatively good prognosis, particularly in the HPV- positive sub-group, the avoidance of late toxicity is important.

Some modest gains have been made using modern radiotherapy techniques to spare dose to OARs while delivering elective mucosal radiation. The use of molecular biology to test for HPV and target mucosal radiation to most likely primary sites has also reduced the length of mucosa irradiated for some patients with improved toxicity.[1, 8]

FDG PET-CT identifies the primary in approximately 40% of patients presenting with a head and neck cancer of unknown primary despite comprehensive evaluation with cross-sectional imaging and endoscopy.[6] A true SCCUP in the PET-CT era is therefore a different entity to what was previously described, and the need to irradiate potential mucosal sites must be questioned again.[5]

3.6 CONCLUSIONS

Patients with a diagnosis of SCCUP head and neck may be safely treated with INO radiotherapy. This strategy results in clinically significant lower doses to the

contralateral parotid gland and midline mucosa. Rates of severe acute mucositis and dysphagia are reduced. Late xerostomia rates are lower at 12 months post-treatment. Confirmation in a larger cohort is required to demonstrate on-going benefit beyond this time. No isolated contralateral neck relapses or primaries have emerged in the group treated with INO radiotherapy.

3.7 POST VIVA ADDITIONS TO CHAPTER

The following section was added as correction to the thesis post viva, taking into account examiners' comments.

Clinician decision to employ INO or MUC is subject to personal bias. The National Comprehensive Cancer Network (NCCN) Guidelines class the issue as category 3, where 'no general agreement can be made'. A narrative review suggested that MUC is more likely to be employed in cases with more advanced nodal disease. [Additional ref 13]. This does not seem to be true in this cohort however, if anything more advanced nodal disease is seen in the INO sub-group.

Survival analysis was carried out from the 1st day of RT treatment to allow inclusion of any on-treatment deaths. Duration of treatment is the same between INO and MUC groups. Primary endpoint was local control (i.e. primary site emergence) compared between the 2 groups. It is acknowledged however that a formal sample size calculation to assess the power of the analyses was not carried out.

Regression analysis may have been a useful additional analysis to control for confounders but the small sample size and even smaller sub-groups after stratification by variables may have limited its utility.

		Whole cohort n = 30 (%)	INO n=20 (%)	MUC n=10 (%)
Gender	Male: female	20 (66.7%): 10 (33.3%)	13 (65%):7 (35%)	7 (70%):3 (30%)
Age	Median (IQR)	58.1 (50-66.6)	61 (IQR 53-66)	Not available
Smoking history	Current/former	22 (73.3)	16 (80)	6 (60)
	Never	6 (20)	4 (20)	2 (20)
	Unknown	2 (6.7)	0 (0)	2 (20)
N stage	N1	4 (13.3)	3 (15)	1 (10)
	N2a	4 (13.3)	1 (5)	3 (30)
	N2b	18 (60.0)	13 (65)	5 (50)
	N3	4 (13.3)	3 (15)	1 (10)
HPV status	Positive	7 (23.3)	3 (15)	4 (40)
	Negative	9 (29.9)	3 (15)	6 (60)
	Unknown	14 (46.7)	14 (70)	0 (0)
Treatment	Radiotherapy alone	13 (43.3)	8 (40)	5 (50)
	Chemoradiotherapy	17 (56.7)	12 (60)	5 (50)
	Neck dissection	17 (56.7)	14 (70)	3 (30)

Amended Table 3-1. Table of patient and treatment demographics

Limitations – Data available does not allow retrospective construction of KM plots, nor PFS or OS survival curves. The means the impact of censoring or patients being lost to follow up cannot be assessed.

3.8 REFERENCES

1. Axelsson L, Nyman J, Haugen-Cange H, et al. Prognostic factors for head and neck cancer of unknown primary including the impact of human papilloma virus infection. J Otolaryngol Head Neck Surg. 2017; 46:45.

2. Cabrera Rodriguez J, Cacicedo J, Giralt J, et al. GEORCC recommendations on target volumes in radiotherapy for head neck cancer of unkown primary. Crit Rev Oncol Hematol. 2018; 130: 51-59.

3. Cerezo L, Raboso E, Ballesteros AI. Unknown primary cancer of the head and neck: a multidisciplinary approach. Clin Transl Oncol.

2011; 13(2):88-97.
4. Chen AM, Li BQ, Farwell DG, Marsano J, Vijayakumar S, Purdy JA. Improved dosimetric and clinical outcomes with intensity-modulated radiotherapy for head-and-neck cancer of unknown primary origin. Int J Radiat Oncol Biol Phys. 2011; 79(3):756-762.
5. Galloway TJ, Ridge JA. Management of squamous cancer metastatic to cervical nodes with an unknown primary site. J Clin Oncol. 2015; 33(29):3328-3337.
6. Mackenzie K, Watson M, Jankowska P, Bhide S, Simo R. Investigation and management of the unknown primary with metastatic neck disease: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016; 130(S2):S170-S175.
7. Grau C, Johansen LV, Jakobsen J, Geertsen P, Andersen E, Jensen BB. Cervical lymph node metastases from unknown primary tumours. Results from a national survey by the Danish Society for Head and Neck Oncology. Radiother Oncol. 2000; 55(2):121-129.
8. Strojan P, Ferlito A, Langendijk JA, et al. Contemporary management of lymph node metastases from an unknown primary to the neck: II. A review of therapeutic options. Head Neck. 2013; 35(2):286-293.
9. Strojan P, Kokalj M, Zadnik V, et al. Squamous cell carcinoma of unknown primary tumor metastatic to neck nodes: role of elective irradiation. Eur Arch Otorhinolaryngol. 2016; 273(12):4561-4569.
10. Nieder C, Gregoire V, Ang KK. Cervical lymph node metastases from occult squamous cell carcinoma: cut down a tree to get an apple? Int J Radiat Oncol Biol Phys. 2001; 50(3):727-733.
11. Richards TM, Bhide SA, Miah SA, et al. Total mucosal irradiation with intensity-modulated radiotherapy in patients with head and neck carcinoma of unknown primary: a pooled analysis of two prospective studies. Clin Oncol (R Coll Radiol). 2016; 28(9):e77-e84.
12. Tiong A, Rischin D, Young RJ, et al. Unilateral radiotherapy treatment for p16/human papillomavirus-positive squamous cell carcinoma of unknown primary in the head and neck. Laryngoscope. 2018; 128(9):2076-2083.
13. Iqbal MS, Jackson M, Paterson C. Radiotherapy to the neck after neck dissection for head and neck squamous cell carcinoma from an unknown primary:

A narrative review. *Clinical Otolaryngology*. 2024; 49(4): 376–383. https://doi.org/10.1111/coa.14155

4. Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study.

Paterson C, Allwood-Spiers S, McCrea I, et al. Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study. Clin Transl Radiat Oncol. 2017; 2:13-18. Published 2017 Jan 10. doi:10.1016/j.ctro.2016.12.003

4.1 ABSTRACT

4.1.1 Introduction and background

A significant proportion of patients with intermediate and high risk squamous cell cancer of the oropharynx (OPSCC) continue to relapse locally despite radical chemoradiotherapy (CRT). The toxicity of the current combination of intensified dose per fraction radiotherapy and platinum based chemotherapy limits further uniform intensification. If a predictive biomarker for outcomes from CRT can be identified during treatment then individualised and adaptive treatment strategies may be employed.

4.1.2 Methods/design

The MeRInO study is a prospective observational imaging study of patients with intermediate and high risk, locally advanced OPSCC receiving radical RT or concurrent CRT Patients undergo diffusion weighted MRI prior to treatment (MRI_1) and during the third week of RT (MRI_2). Apparent diffusion coefficient (ADC) measurements will be made on each scan for previously specified target lesions (primary and lymph nodes) and change in ADC calculated. Patients will be followed up and disease status for each target lesion noted. The primary aim of the MeRInO study is to determine the threshold change in ADC from baseline to week 3 of RT that may identify the sub-group of non-responders during treatment.

4.1.3 Discussion

The use of DW-MRI as a predictive biomarker during RT for SCC H&N is in its infancy but studies to date have found that response to treatment may indeed be predicted by comparison of DW-MRI carried out before and during treatment. However, previous studies have included all sub-sites and biological sub-types. Establishing ADC thresholds that predict for local failure is an essential step towards using DW-MRI to improve the therapeutic ratio in treating SCC H&N. This would be done most robustly in a specific H&N sub-site and in sub-types with similar biological behaviour. The MeRInO study will help establish these thresholds in OPSCC.

4.2 INTRODUCTION AND BACKGROUND

The incidence of oropharyngeal squamous cell cancer (OPSCC) has increased greatly in the developed world in recent years [1]. Radiotherapy (RT) or chemo-radiotherapy (CRT) is an organ-preserving alternative to surgery with at least equivalent loco-regional control and disease-free survival (DFS) [2, 3].
Smoking and alcohol are well established risk factors. The recent increase in incidence, however, is attributed to a rise in Human Papilloma Virus (HPV) driven OPSCC [4]. It has been shown that these HPV + OPSCC are more responsive to treatments and patients have better overall survival (OS) rates than their HPV negative counterparts [5-7]. However, it has also been demonstrated that smoking remains a significant factor in disease control with the risk of death increasing directly as a function of tobacco exposure in all OPSCC patients [8]. Ang et al. [7] suggested that the biological behaviour of HPV + OPSCC may be altered by tobacco use, rendering them less responsive to therapy. He proposed 3 ‘risk groups’ for OPSCC, using tumour stage, HPV status and smoking history to classify patients into low, intermediate or high risk of death, (Table 4-1). Current strategies in the low risk group focus on de-escalation of therapy and clinical trials are ongoing [9]. Conversely, intensification of treatment should be considered for the intermediate and high risk groups, which are the focus of the proposed study.

Risk category	OS at 3 years	Demographics
Low risk	93%	HPV+, <10 pack years HPV+, >10 pack years, N0-2a
Intermediate risk	70.8%	HPV+, >10 pack years, N2b-3 HPV−, <10 pack years
High risk	46.2%	HPV−, >10 pack years HPV−, <10 pack years, T4

Table 4-1. Risk of death in OPSCC. Ang et al. [7]

Patients with HPV-OPSCC tend to be older with significant smoking and/or alcohol history [10], resulting in more co-morbidities than their HPV + OPSCC counterparts. Uniform treatment intensification of an already morbid treatment across this group is therefore unattractive. If, however, a predictive biomarker could be established to select patients who respond poorly to RT, an individualised treatment intensification strategy could be used for those who require it.

The role of imaging in early response detection for SCC H&N is currently ill-defined. Volumetric assessment during RT using CT or MRI based anatomical imaging has shown conflicting results [11-17]. PET-CT with FDG and other tracers continues to be investigated. There is some evidence that changes in FDG PET uptake early during the course of RT correlates with ultimate tumour response [18-21]. However, difficulties in delineating target volumes using PET-CT during treatment have been reported [22, 23].

DW-MRI enables us to detect the Brownian motion of water molecules in biological tissues [24]. The apparent diffusion co-efficient (ADC) value is the parameter that is used to quantify DW-MRI, estimating the diffusion rate of water molecules in tissue. [25].

MRI is attractive as an imaging biomarker as it is non-invasive and does not involve additional radiation exposure. It has better tissue contrast resolution when compared to CT and is the imaging modality of choice to accurately define the extent of OPSCC [3, 26, 27].

The use of DW-MRI as a predictive biomarker during RT for SCC H&N is in its infancy but studies have found that response to treatment may indeed be predicted by DW-MRI by acquiring images before and during treatment. It has been suggested that ‘for DW imaging to be of clinical value, ADC thresholds need

to be established that can help predict local failure' [28]. This is the primary aim of the MeRInO study – to establish the threshold change in ADC from baseline to week 3 of RT that can differentiate responders from non-responders to treatment. This may then allow an individualised and adaptive approach to treatment based on the biological behaviour of a tumour during RT.

4.3 METHODS/DESIGN

4.3.1 Study organisation/funding

The MeRInO study was designed by a multi-disciplinary collaboration from The Beatson West of Scotland Cancer Centre, the Department of Clinical Physics and Bioengineering, NHS Greater Glasgow and Clyde, and the University of Glasgow. The study sponsor is NHS Greater Glasgow and Clyde (Sponsor reference number GN15ON249). The study received in-house approval by the Clinical Trials Executive Committee (CTEC) and regional Research Ethics Committee approval (REC number 15/WS/0159), part of the national ethics service. The study is registered on the publically accessible database Clinicaltrials.gov (NCT02497573).

Funding for the study is provided by the Beatson Cancer Charity (Funding application number 14-15-109). Some participating investigators are already funded or part funded by the Beatson Cancer Charity and NHS Research Scotland.

4.3.2 Study design and patient population

The study is a prospective, longitudinal, single centre, observational imaging study of patients with intermediate and high risk, locally advanced OPSCC receiving primary radical RT or concurrent CRT.

Two DW-MRI scans will be carried out on participants in addition to all standard procedures. The information gained from the MRI scans will not be used to change standard treatment. The first DW-MRI (MRI_1) will be obtained on the same day RT commences. The second DW-MRI (MRI_2) will be carried out during the third week of RT treatment.

The DW-MRI scans will be used to measure ADC in each target lesion (primary and lymph nodes) and to calculate change in ADC between the 2 scans. After

completion of RT, patients will attend for follow up visits at 3, 6, 12, 18 and 24 months post treatment. Figure 4-1 shows this schematically.

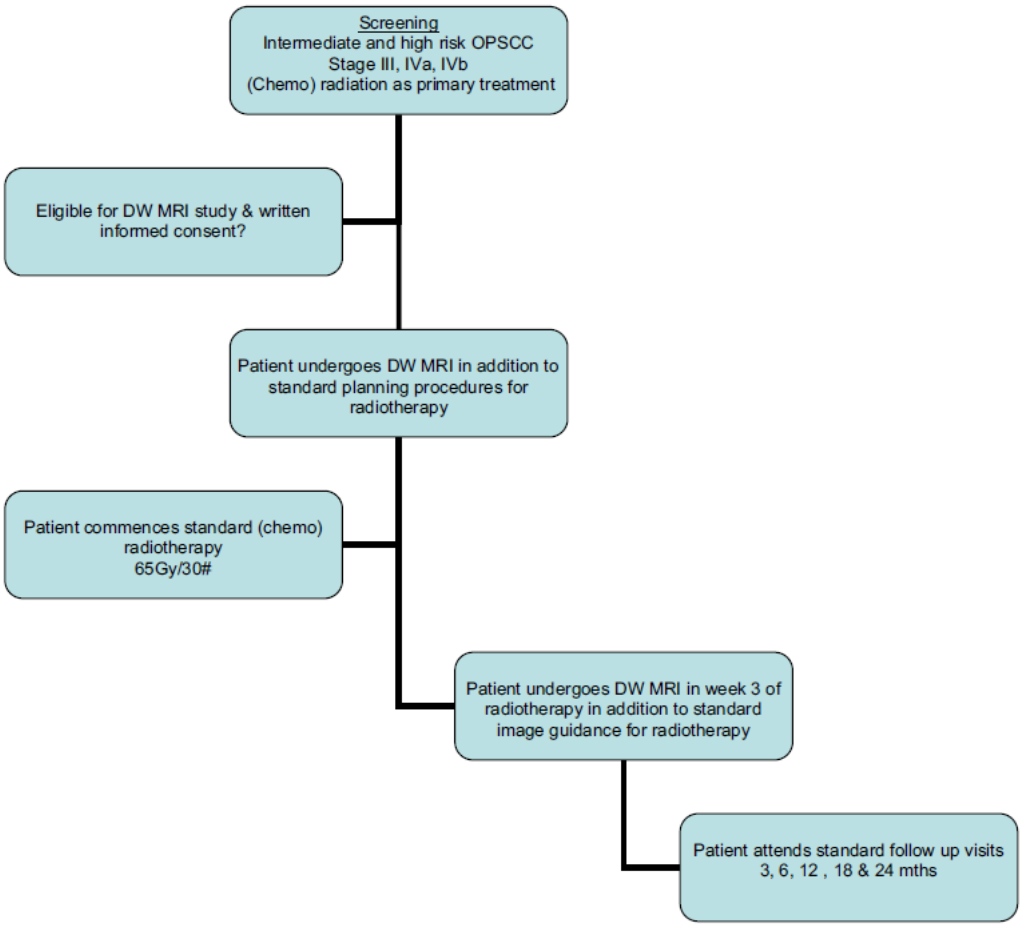

Fig. 4-1. Trial pathway

Inclusion criteria:

•Histologically confirmed HPV negative OPSCC or HPV positive OPSCC and a significant smoking history (>10 pack years).

•Stage III or IVa or IVb disease.

•Scheduled to undergo radical RT or CRT as primary treatment.

•18 years of age or older.

HPV status: As defined by the Scottish HPV reference laboratory, multiplex assay on Luminex technology.

Diagnosis and staging will be carried out as per standard regional guidelines [29].

Exclusion criteria:

•Sub sites other than oropharynx.

•Low risk OPSCC.

•Patients receiving cetuximab-RT.

•Confirmed distal metastatic disease (stage IVc).
•Patients who have undergone primary surgery for SCC H&N.
•Patients who have received induction chemotherapy.
•Patients with contra-indications to MRI scanning (cardiac pacemaker, surgery within 8 weeks, aneurysm clipped/treated, metal fragments in eye, previous cranial surgery, any ferrous metal in the body, pregnancy).

4.3.3 Study objectives and end-points

The primary objective of the MeRInO study is to determine the threshold change in ADC from baseline to week 3 of RT that can differentiate responders from non-responders to treatment. This will be achieved by measuring ADC on MRI_1 and MRI_2 for all target lesions. Change in ADC (ΔADC) and % change in ADC (% ΔADC) will be calculated for each lesion and recorded.
Loco-regional failures at 24 months post-treatment will be recorded and pattern of relapse noted. Relapse status for each target lesion at 24 months post-treatment will be recorded. Progression free survival at 24 months will also be noted to account for distal metastases.
Secondary objectives are to assess

- feasibility of measuring ADC at baseline and week 3 of RT,
- time to relapse for each target lesion,
- correlation of ΔADC with pattern relapse.

4.3.4 Chemoradiotherapy

Patients may receive concurrent CRT or RT alone as definitive primary treatment.
For RT planning and treatment patients are immobilised with a custom made thermoplastic mould with 5 point fixation (Klarity Medical Products, Newark, Ohio). A contrast enhanced planning scan is obtained on either a Discovery CT590 RT (GE Medical Systems, Amersham, UK) or a Philips Brilliance Big Bore (Philips Medical Systems B.V, The Netherlands).
Target delineation is carried out by the treating oncologist, using all available clinical and radiological information from diagnosis and staging and with reference to international guidelines [30]. Peer review and approval of the

planning target volumes is mandatory and standard practice in our centre for all H&N cancers.

A treatment plan is created using the Eclipse™ planning system (Varian medical systems, Palo Alto, CA) and approved by the treating clinician.

All patients receive RT delivered on a Varian Clinac® 600 linear accelerator using Rapid Arc® (Varian medical systems, Palo Alto, CA) volumetric-modulated arc therapy (VMAT) with 6 MV photons. Gross tumour and the entirety of involved nodal levels receive 65 Gy/30# over 6 weeks. Prophylactic dose to areas considered at high risk of occult disease is 54 Gy/30# over 6 weeks.

Cisplatin is delivered at 100 mg/m2 on days 1 and 22 of treatment for those receiving concurrent chemotherapy as per local protocols [31]. Concurrent cetuximab is not permitted, nor is induction chemotherapy to keep the cohort as homogeneous as possible.

4.3.5 Diffusion weighted MRI

All MRI images will be acquired on a Signa 1.5T HDxt (GE, Crawley, UK) scanner with patients in a supine position with neutral neck position. The decision to not employ immobilisation was taken to enable the use of the neurovascular coil and to facilitate recruitment and retention of patients to the study. A measurement from supra-sternal notch to mandible will ensure intra-subject consistency of head position at repeat scan. The position of the hard palate will also be checked on the sagittal localiser to verify axial alignment. A 16 channel neurovascular coil (HNS NV full, GE, 2012) provides coverage of the H&N area using the head coils, anterior and posterior neck coils and anterior chest coils. MRI sequences will be acquired for anatomical identification of each target region. T1 weighted, T1 weighted fat saturated (fat sat), and T2 weighted fat sat and post-gadolinium contrast images will be obtained. The diffusion weighted images will be acquired using a single shot echo planar imaging (EPI) sequence with several b-values between 0 and 1000 s/mm2. The ADC map will be calculated automatically using inline post-processing (Optima Edition 23, GE, Milwaukee, 2012) from the acquired b value images using a mono-exponential fit.

All anatomical and DW images will be imported into the Eclipse™ treatment planning system (TPS) (Varian medical systems, Palo Alto, CA). Target lesions will be delineated on each axial slice of the ADC maps by expert clinical

oncologist (CP) and radiologist (IMcC) with over 10 years' experience. The anatomical MR sequences will be used to aid delineation generally and in particular identification and exclusion of necrotic areas on the ADC maps. Necrotic/cystic areas will be excluded from ADC analysis.

4.3.6 Quality assurance (QA)

To verify the accuracy of ADC measurement by the scanner software, a phantom will be scanned monthly. The phantom comprises four vials containing different concentrations of polyvinylpyrrolidone (PVP) solution and one vial of distilled water. The range of ADC values covered by the different concentrations of PVP encompasses the clinical range of interest [32]. The ADC measurements will be recorded using the scanner software and the Eclipse™ TPS (Varian medical systems, Palo Alto, CA).

Daily QA is performed on the MRI scanner to check fundamental parameters of the system.

RT treatment QA features throughout the process. Peer review of target volumes created by the treating clinician is required prior to planning. RT plans are produced and checked by two operators prior to treating clinician review and approval. Daily on-line KV-KV imaging is carried out prior to treatment to ensure accuracy of set-up. Our centre is an active participant in several UK multi-centre RT studies (e.g. recently NIMRAD [33], ART DECO [34]) and therefore subject to scrutiny by the NCRI RTQA group as well as meeting individual study QA requirements.

4.3.7 Clinical follow up

Following completion of RT, patients will attend for evaluation at 3, 6-, 12-, 18- and 24-months post treatment. At each visit, disease status will be recorded. This assessment will be based on clinical examination and any available imaging as per standard regional practice [35].

Local control is defined as:

•Absence of any new mass.

•Static or reduction of residual mass during follow up (FU) $\geqslant$ 24 months.

•Histological confirmation of absence (based on surgical resection, not biopsy due to potential sampling error).

Local failure or relapse/recurrence is defined as:

•Biopsy proven recurrence.
•Development of new mass or serial increase in size of residual mass during FU ⩾ 24 months.

4.3.8 Statistical analysis

Sample size

Around 80 OPSCC patients per year are treated in our centre with RT or CRT. Analysis of a local database found an overall relapse rate of 30% for intermediate and high risk OPSCC patients (unpublished work). This is consistent with previously published outcomes for locally advanced SCC H&N which had loco-regional failure of 26.7% in our centre [36].

We estimate that recruiting a sample of 80 patients will provide 24 patients who relapse and 56 patients who do not relapse assuming a 30% relapse rate. A sample of 24 relapsed patients will differentiate a test sensitivity of >80% from a sensitivity of <60% at 80% power and 10% 1-sided level of statistical significance. It is expected that there will be 56 patients who do not relapse. This number of patients will provide 94% power to distinguish a specificity of <60% from a specificity of >80% (assuming a 5% 1-sided level of statistical significance). We anticipate sensitivity and specificity of over 80% as reported by Kim et al. who found that the normalised ADC values after the first week of treatment had the highest accuracy to separate complete from partial responders, with a sensitivity of 86% and a specificity of 83% [37].

Primary analysis

The distribution of baseline demographic and clinical characteristics of patients will be described. We will report the percentage of patients with measurements of ADC at baseline and week 3 of RT. The proportion of patients experiencing loco-regional failure and progression free survival at the end of the study will be reported. We will determine the sensitivity and specificity (with 95% CIs) for loco-regional failure of different cut-off values of the change in ADC from baseline to week 3. A receiver operator characteristic (ROC) curve will be used to illustrate the performance of change in ADC as its discrimination threshold is varied.

Secondary analyses

We will determine whether the association between change in ADC and relapse is different for primary and lymph node lesions. We will employ survival analysis

techniques to determine whether associations between baseline characteristics (for example initial ADC value, age, or sex), change in ADC value and time to relapse exist. We will describe factors associated with drop out or discontinuation/ interruption of treatment.

Interim data analysis

The feasibility of measuring ADC at baseline and week 3 of RT will be assessed after the first 40 patients enter the study. The percentage of patients where it is feasible to measure ADC for at baseline and week 3 of RT will be calculated and the study will be discontinued if this is <50%. This secondary endpoint was included on the recommendation of the Research Ethics Committee during their review. It was included as a secondary rather than primary endpoint as prior studies of this sort in H&N cancer had already demonstrated the strategy was feasible.

4.4 DISCUSSION

4.4.1 The need for a predictive biomarker

Historically, prognostic, rather than predictive, information in the form of the tumour, nodes, metastases (TNM) staging system has been used to inform therapeutic decision-making in the management of OPSCC. A standard approach to treatment of locally advanced disease has resulted in treatment failure in a significant proportion and perhaps unnecessary toxicity and functional impairment in others who may have achieved disease control with less intensive treatment [38].

The identification of a predictive biomarker is the first step towards an individualised cancer treatment approach and may allow an improved therapeutic ratio.

DW-MRI and ΔADC have been shown to correlate with response to treatment in prospective and retrospective studies in SCC H&N.

Kim et al. performed DW-MRI before, 1 week into and approximately 2 weeks after CRT in 33 patients. They found that the normalised ADC values after the first week of treatment had the highest accuracy to separate complete from partial responders (sensitivity 86%, specificity 83%) [37].

In a similar study, Vandecaveye et al. found that the ΔADC values at week 2 and 4 of CRT were significantly correlated with 2-year LRC and DFS [[29]]. In patients with recurrence, the ΔADC at 2 and 4 weeks was significantly lower than in patients with a complete response in both adenopathies and primary tumour [28].

King et al. corroborated these results, reporting that changes in serial ADC values were associated with treatment response [[39]]. They calculated that a fall in ADC during treatment identified patients who developed treatment failure with 90% accuracy.

A further study by King et al. [40] investigated ΔADC 2 weeks into CRT. Tumours that responded to treatment displayed a significantly higher percentage increase in ADC value at 2 weeks compared to those that failed treatment.

These results suggest an insufficient rise in ADC during treatment correlates with a poor response to RT. Less evidence is available for a threshold rise in ADC that could be used to select non-responding tumours for treatment intensification with only 2 studies assessing this [28, 40]. Vandecaveye et al. [28] reported a threshold rise of 14% for primary lesions and 14.61% for lymph node metastases. The threshold rise to predict local control in primary lesions was identified as 15.5% in the study by King et al. [40].

These studies have included all H&N sub-sites with no differentiation between biological sub-types. Establishing a threshold rise in ADC to predict responders from non-responders would be done most robustly in a specific H&N sub-site and in sub-types with similar biological behaviour. This study will therefore validate the use of DW-MRI as a predictive biomarker specifically in the intermediate and high risk groups of OPSCC. If a discriminatory threshold rise in ADC can be identified early in treatment to discriminate non-responders an adaptive, dose-escalated radiotherapy treatment plan could be delivered for the remainder of the course in a safe but meaningful fashion. This would form the basis of subsequent clinical trials.

4.4.2 Potential future translational studies

All patients that are recruited for the MeRInO study will be given information about an exploratory biomarker study collecting blood samples of patients with malignant disease run by the Glasgow experimental cancer medicine centre [[41]]. Furthermore, histology samples taken at the time of diagnosis will be

stored by the Greater Glasgow & Clyde Bio-repository. It is intended that the bank of clinical and radiological data that is acquired from the MeRInO study may be examined in conjunction with these blood and tissue samples in the future and potential biomarkers identified.

4.5 REFERENCES

1. Mehanna H, Jones TM, Gregoire V. Ang KK. Oropharyngeal carcinoma related to human papilloma virus BMJ 2010; 340:c1439.
2. Parsons JT, Mendenhall WM, Stringer SP, Amdur RJ, Hinerman RW, Villaret DB, et al. Squamous cell carcinoma of the oropharynx: Surgery, radiation therapy or both. Cancer 2002; 94:2967–80.
3. Mehanna H, Evans M, Beasley M, Chatterjee S, Dilkes M, Homer J, et al. Oropharyngeal cancer: United Kingdom national multidisciplinary guidelines. J Laryngol Otol 2016; 130(S2):S90–6.
4. Tran N, Rose BR, O'Brien CJ. Role of human papillomavirus in the etiology of head and neck cancer. Head Neck 2007; 29(1):64–70.
5. Worden FP, Kumar B, Lee JS, Wolf GT, Cordell KG, Taylor JM, et al. Chemoselection as a strategy for organ preservation in advanced oropharynx cancer: response and survival positively associated with HPV16 copy number. J Clin Oncol 2008; 26:3138–46.
6. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100:261–9.
7. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal D, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Eng J Med 2010; 363:24–35.
8. Gillison ML, Harris J, Westra W, Chung C, Jordan R, Rosenthal D, et al. Survival outcomes by tumor human papillomavirus (HPV) status in stage III-IV oropharyngeal cancer (OPC) in RTOG 0129 ASCO Meeting Abstracts. J Clin Oncol 2009; 27(15S):6003.
9. Masterson L, Moualed D, Masood A. Cochrane ear, nose and throat disorders group De-escalation treatment protocols for human papillomavirus-associated oropharyngeal squamous cell carcinoma. Cochrane Library 2014.
10. Herrero R, Castellsagué X, Pawlita M, Lissowska J, Kee F, Balaram P, et al.

Human papillomavirus and oral cancer: the International Agency for research on cancer multicenter study. J Natl Cancer Inst 2003; 95(23):1772–83.
11. Klug C, Keszthelyi D, Ploder O, Sulzbacher I, Voracek M, Wagner A, et al. Neoadjuvant radiochemotherapy of oral cavity and oropharyngeal cancer: evaluation of tumor response by CT differs from histopathologic response assessment in a significant fraction of patients. Head Neck 2004; 26:224–31.
12. Denys D, Kumar P, Wong FS, Newman LA. Bobbin KT the predictive value of tumor regression rates during chemoradiation therapy in patients with advanced head and neck squamous cell carcinoma. Am J Surg 1997; 174:561–4.
13. Jaulerry C, Dubray B, Brunin F, Rodriguez J, Point D, Blaszka B, et al. Prognostic value of tumor regression during radiotherapy for head and neck cancer: a prospective study. Int J Radiat Oncol Biol Phys 1995; 33:271–9.
14. Lee H, Ahn YC, Oh D, Nam H, Kim YI. Park SY tumor volume reduction rate measured during adaptive definitive radiation therapy as a potential prognosticator of locoregional control in patients with oropharyngeal cancer. Head Neck 2014; 36:499–504.
15. Bhatia KSS, King AD, Yu K-H, Vlantis AC, Mk Tse G, Kf Mo F, et al. Does primary tumour volumetry performed early in the course of definitive concomitant chemoradiotherapy for head and neck squamous cell carcinoma improve prediction of primary site outcome? Br J Radiol 2010; 83:964–70.
16. Yang SN, Lian CY, Chen SW, Liang JA, Tsai MH, Hua CH, et al. Clinical implications of the tumor volume reduction rate in head-and-neck cancer during definitive intensity-modulated radiotherapy for organ preservation Int.
J Radiation Oncology Biol Phys 2011; 79(4):1096–103.
17. Tyagi N, Riaz N, Hunt M, Wengler K, Hatzoglou V, Young R, et al. Weekly response assessment of involved lymph nodes to radiotherapy using diffusion weighted MRI in oropharynx squamous cell carcinoma. Med Phys 2016;43:137. doi: http://dx.doi.org/10.1118/1.4937791.
18. Madani I, Duthoy W, Derie C, De Gersem W, Boterberg T, Saerens M, et al. Positron emission tomography-guided, focal-dose escalation using intensity modulated radiotherapy for head and neck cancer. Int J Radiat Oncol Biol Phys 2007; 68:126–35.
19. Geets X, Tomsej M, Lee JA, Duprez T, Coche E, Cosnard G, et al. Adaptive biological image-guided IMRT with anatomic and functional imaging in pharyngo–

laryngeal tumors: impact on target volume delineation and dose distribution using helical tomotherapy. Radiother Oncol 2007; 85:105–15.
20. Grégoire V, Haustermans K, Geets X, Roels S. Lonneux M PET-based treatment planning in radiotherapy: a new standard? J Nucl Med 2007; 48(Suppl. 1):68S–77S.
21. Kostakoglu L, Goldsmith SJ. PET in the assessment of therapy response in patients with carcinoma of the head and neck and of the esophagus. J Nucl Med 2004; 45:56–68.
22. Moule RN, Kayani I, Prior T, Lemon C, Goodchild K, Sanghera B. Adaptive 18fluoro-2-deoxyglucose positron emission tomography/computed tomography-based target volume delineation in radiotherapy planning of head and neck cancer. Clin Oncol 2011; 23(5):364–71.
23. Hentschel M, Appold S, Schreiber A, Abramyuk A, Abolmaali N, Kotzerke J, et al. Serial FDG-PET on patients with head and neck cancer: implications for radiation therapy. Int J Radiat Biol 2009; 85:796–804.
24. Koh DM, Collins DJ. Diffusion weighted MRI in the body: applications and challenges in oncology. AJR Am J Roentgenol 2007; 188:1622–35.
25. Thoeny HC, De Keyzer F, King AD. Diffusion-weighted MR imaging in the head and neck. Radiology 2012; 263:19–32.
26. Olliff, Richards P, Connor S, Wong WL, Beale T, Madani G. Head and neck cancers. In: Nicholson T, editor. Recommendations for Cross-Sectional Imaging in Cancer Management. London: The Royal College of Radiologists; 2014. P 3–19.
27. Lewis-Jones H, Colley S, Gibson D. Imaging in head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J. Laryngol 2016; 130(Suppl. S2):S28–31.
28. Vandecaveye V, Dirix P, De Keyzer F, Op de Beeck K, Vander Poorten V, Roebben I, et al. Predictive value of diffusion-weighted magnetic resonance imaging during chemoradiotherapy for head and neck squamous cell carcinoma Eur. Radiol 2010;20(7):1703–14.
29. http://www.intranet.woscan.scot.nhs.uk/wp-content/uploads/2013/11/Oropharyngeal-CMG-v3.1_14-May-20141.pdf> [accessed October 2016].
30. Gregoire V, Coche E, Cosnard G, Hamoir M, Reychler H. 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 2000;56(2):135–50.

31. <http://www.intranet.woscan.scot.nhs.uk/UserFiles/CisRT%20HNWOS003v1.pdf> [accessed October 2016].
32. Jerome NP, Papoutsaki M-V, Orton MR, Parkes HG, Winfield JM, Boss MA, et al. Development of a temperature-controlled phantom for magnetic resonance quality assurance of diffusion, dynamic, and relaxometry measurements. Med Phys 2016;43:2998. doi: http://dx.doi.org/10.1118/1.4948997.
33. Thomson D, Yang H, Baines H, Miles E, Bolton S, West C, et al. NIMRAD: a Phase III trial to investigate the use of nimorazole hypoxia modification with intensity-modulated radiotherapy in head and neck cancer. Clin Oncol 2014; 26:344e347.
34. http://www.isrctn.com/ISRCTN01483375> [accessed October 2016].
35. http://www.intranet.woscan.scot.nhs.uk/wp-content/uploads/2013/11/Final_published-Head-and-Neck-Cancer-Regional-Follow-Up-Guideline-v2.0-15-December-2015-1.pdf> [accessed October 2016].
36. Sanders I, Haslett K, Correa P, Paterson C, James A, Rizwanullah M, et al. Sequential TPF chemotherapy followed by concurrent chemoradiotherapy in locally advanced head and neck cancer – a retrospective analysis of toxicity and outcomes. Scott Med J 2014; 59(1):50–5.
37. Kim S, Loevner L, Quon H, Sherman E, Weinstein G, Kilger A, et al. Diffusion weighted magnetic resonance imaging for predicting and detecting early response to chemoradiation therapy of squamous cell carcinomas of the head and neck. Clin Cancer Res 2009; 15:986–94.
38. Quon H, Brizel D. Predictive and prognostic role of functional imaging of head and neck squamous cell carcinomas. Semin Radiat Oncol 2012 Jul; 22 (3):220–32.
39. King AD, Mo FK, Yu KH. Squamous cell carcinoma of the head and neck: diffusion-weighted MR imaging for prediction and monitoring of treatment response. Eur Radiol 2010; 20:2213–20.
40. King AD, Chow KK, Yu KH, Mo FK, Yeung DK, Yuan J, et al. Head and neck squamous cell carcinoma: diagnostic performance of diffusion-weighted MR imaging for the prediction of treatment response. Radiology 2013; 266:531–8.
41. http://www.cancerresearchuk.org/about-cancer/find-a-clinical-trial/astudy-looking-biomarkers-people-with-solid-tumours#undefined [accessed October 2016].

5. Feasibility of DW-MRI analysis of salivary glands during head and neck radiotherapy.

Duffton A, Kemp O, Devlin L, Hay L, McLoone P, **Paterson C**. Feasibility of DW-MRI analysis of salivary glands during head and neck radiotherapy. *Tech Innov Patient Support Radiat Oncol*. 2021;19:46-51. Published 2021 Sep 4. doi:10.1016/j.tipsro.2021.07.002

5.1 ABSTRACT

5.1.1 Introduction

With no effective treatment for xerostomia, there remains an unmet need to reduce radiation induced toxicity. Measuring physiological changes during RT in salivary glands using DW-MRI may predict which patients are most at risk of severe toxicity. This study evaluated the feasibility of measuring apparent diffusion coefficient (ADC) in the major salivary glands and describes the observed changes in volume and ADC during RT.

5.1.2 Methods

Scans were acquired at baseline (MR_base) and after 10 fractions (MR_rpt). Sequences included T1 post contrast fat saturated (T1PCFS) and DW-MRI (5b values, 0–1000 s/mm2). Ipsilateral and contralateral parotid (iPG/cPG), submandibular (iSMG/cSMG) and sublingual glands (iSLG/cSLG) were delineated on T1PCFS, modified on b0 and copied to the ADC map.

5.1.3 Results

31 patients with intermediate/high risk squamous cell carcinoma (SCC) of the oropharynx were evaluated. On 124 scans, SMG and SLG delineations were successful on all; parotids were fully contoured in 90.7%. Baseline mean ADC were significantly different between each gland type ($p < 0.0001$). IPG and cPG volume decreased during treatment by 6.7% and 11.2%. ISMG, cSMG, iSLG and cSLG volume increased by 6.9, 0.9, 60.8 and 60.3% respectively. All structures showed an increase in mean_ADC values. For each gland the increase in ADC was statistically significant $p < 0.0001$. A smaller mean percentage increase in ADC was observed in the group experiencing a higher grade (2 or >) of toxicity.

5.1.4 Conclusion

It is feasible to measure volume and ADC of the salivary glands prior to and during RT for HNC. Early data suggests a lower rise in ADC during treatment is associated with more severe late xerostomia.

5.2 INTRODUCTION

Radiotherapy (RT) or chemo-radiotherapy (CRT) is a potentially curative standard of care for head and neck squamous cell cancer (HNSCC), delivering doses of up to 70 Gy, over 6–7 weeks. While technological advances such as parotid-sparing intensity-modulated radiotherapy (IMRT) have resulted in modest improvements in observer-rated and patient-reported xerostomia, [1] clinically significant xerostomia remains a problem for many patients [2], [3].

Radiotherapy-induced xerostomia is the most commonly reported late and permanent side effect of RT to the head and neck (H&N) [4]. RT preferentially damages the fluid-secreting serous cells, rather than the mucin secreting cells, of the salivary glands (SG), so patients experience a build-up of thick, sticky mucus and a dry mouth [5]. This can cause discomfort, taste alteration, speech and swallowing difficulties, accelerating dental caries [6].

The changing epidemiology of H&N cancer, mainly due to a rise in oropharyngeal cancer caused by human papilloma virus (HPV), means that patients are often younger with little comorbidity [7]. This group has a significantly improved response to treatment and overall survival [8], [9], [10] and will therefore live much longer with the consequences of treatment [11].

With no effective treatment for xerostomia, there remains an unmet clinical need to further reduce toxicity where possible. Functional imaging with serial quantification of tumour characteristics to predict treatment response is an area of much promise and may ultimately allow biologically adaptive RT strategies [12]. Likewise, there may be an opportunity to measure physiological changes during RT in SG or other organs at risk (OARs), and use these parameters to predict which patients are most at risk of severe toxicity. Again, biologically adaptive strategies may allow modification of RT for those at highest risk of long term morbidity.

MRI is a non-invasive, non-ionising imaging method which gives superior soft tissue contrast and is the imaging modality of choice to accurately define

tumour extent in several head and neck cancer sub-sites [13], [14]. Diffusion weighted MRI (DW-MRI) is a form of functional MR imaging based upon measuring the random Brownian motion of water molecules within tissue. Molecular movement is restricted by cellular structures in high-density tissue. DW-MRI measures the indirect value of cellularity by applying the same gradient at continuous short time intervals [15]. An apparent diffusion coefficient map (ADC) is created by acquiring multiple conventional DWI images with different amounts of DWI weighting (differing b values) and the change in signal is proportional to the rate of diffusion. Different rates of diffusion are seen as areas of high or low signal on the images acquired, this is also quantifiable using ADC to measure diffusion in a region of interest (ROI). By measuring change in ADC at intervals, changes in tissue characteristics can be identified.

5.2.1 Aim

The aim of this study was to evaluate the feasibility of measuring ADC in the major SG and to describe the observed changes in volume and ADC following 2 weeks of radiotherapy.

5.3 METHODS

5.3.1 Patient selection

Eligible patients were those with locally advanced intermediate or high risk oropharyngeal SCC [16] scheduled for radical primary RT or CRT. This was a sub-group analysis of the MeRInO study (study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx) [17]. Staging was defined using the TNM classification of malignant tumours, 7th edition [18] and those selected for this sub-study were participants with 12 months of follow up who had completed baseline (MR_base) and repeat MRI (MR_rpt) scans. Research ethics committee approval was gained for the primary study (reference 15/WS/0159) and written informed consent was obtained for each patient with, specific permission requested to use their scans for additional research beyond the primary study.

5.3.2 MRI scan acquisition

All scans were acquired on a dedicated scanner, GE Signa 1.5 T HDxt (GE, Crawley, UK) using a 16 channel neurovascular coil (HNS NV full, GE 2012). Sequences included T1 post contrast fat saturated (T1PCFS) followed by DW-MRI, using five b- values (0–1000 s/mm2). These were acquired using a single shot EPI sequence. To reduce distortion, parallel imaging was used. ADC maps were generated using a mono exponential fit. Scans were obtained immediately prior to treatment (MR_base) and after 10 fractions (MR_rpt) i.e. 2167 centigray (cGy) of radiotherapy. An individualised headrest and standard kneerest were used to ensure reproducibility of set-up, and to allow the use of the NV coil. A patient specific measurement i.e. distance from mental protuberance to sternal notch was recorded and verified to ensure consistency between scans. These were imported to Eclipse v 15.5 treatment planning system (Varian medical systems, Palo Alto).

5.3.3 Delineation and quantification of region of interest (ROI) on MR

Paired major SG were delineated in full, this included ipsilateral (i) and contralateral (c) parotid (iPG/cPG), submandibular (iSMG/cSMG) and sublingual (iSLG/cSLG) glands as per guidelines [19]. Glands were contoured on all slices by a medical student and verified by a HN clinical oncologist on each MRI scan. Structures were initially contoured on the T1PCFS sequence and copied onto the b0 images. Modification of each structure was made to account for motion and artefact between the sequences. Final volumes on the b0 sequence were copied to the ADC map (without further adjustments) to be used as the ROI.

All measurements were made on the ADC map where mean (and standard deviation of the mean) ADC (mm^2/s) and volume (cm3) were obtained and recorded for each ROI. To verify whether the full structure was included in the imaging datasets, coverage of each SG was checked on the T1PCFS and ADC map on each baseline and repeat image. Any incomplete structures were recorded.

5.3.4 Chemo-radiotherapy

Patients were immobilised for RT with a 5-point thermoplastic head and shoulder mask (Klarity Medical Products, Newark, Ohio) and an individualised headrest. A planning CT (slice thickness 2 mm) was acquired on a Phillips Brilliance Big Bore scanner (Philips Medical Systems B.V, The Netherlands).

Gross tumour volume (GTV) was delineated for primary tumour and involved lymph nodes. A 10–15 mm margin was added, then the outline further edited to exclude natural barriers to spread e.g. bone and air cavities, and extended to include the whole involved nodal level(s) to create the clinical target volume (CTV_65). CTV_54 included nodal areas considered at risk of containing microscopic disease as per international guidelines [20]. A 3–5 mm geometric expansion created planning target volumes (PTV_65 and PTV_54). A dose of 6500 cGy in 30 fractions over 6 weeks was prescribed to gross disease and 5400 cGy to elective areas.

The following planning criteria were applied D95% ≥ 95% to PTV_65. Critical organ at risk doses were maximum dose (Dmax) ≤ 4800 cGy, for planning risk volumes (PRV) PRV_spinal cord and Dmax < 5400 cGy for PRV_brainstem and were prioritized over PTV coverage. Ideally cPG, mean dose (Dmean) should be minimised to < 2400 cGy but PTV coverage was not compromised to achieve this. Mean planned dose was recorded for cPG and iPG from routine planning parameters. Paired SMG and SLG were not routinely delineated at CT planning, hence dose was not available for these.

Treatment plans were created on Eclipse ™ treatment planning system (TPS) (Varian Medical System, Palo Alto, CA). These were optimised using the inverse planning Analytical Anisotropic Algorithm (AAA Version 13.6.23).

Treatment was delivered on a Truebeam® linear accelerator (Varian Medical System, Palo Alto, CA). Daily kV-kV images were acquired for verification with corrections based on bony matching applied before treatment. A planning CT was acquired at fraction 16 for volumetric and dosimetric assessment.

For eligible patients, cisplatin was delivered at 100 mg/m^2 on day 1 and 22.

5.3.5 Toxicity assessment

Toxicity was recorded using Radiation Therapy Oncology Group (RTOG) scoring criteria [21] to measure xerostomia at 12 months following treatment. Investigators were blinded to toxicity outcomes during ADC measurement and analysis.

5.3.6 MRI quality assurance (QA)

QA was carried out to ensure accuracy of ADC values and reliability of measurements. This was performed monthly by scanning a phantom comprising

of four vials of polyvinylpyrrolidone (PVP) with different concentrations and one containing distilled water [22]. Other routine parameters were checked as part of the daily scanner QA.

5.3.7 Statistical method

Patient characteristics were summarised as counts and percentages, or means with standard deviations (SD).

The percentage change in volume and in mean ADC for each gland was calculated after 2 weeks of treatment, differentiated by laterality to primary tumour. i.e. contralateral or ipsilateral.

At 12 months patients were categorised into two toxicity groups - those who had experienced grade 0–1 toxicity, and those with toxicity of grade 2 or higher. The mean percentage change in volume and in mean ADC were compared between the two toxicity groups for the SG, distinguished by treatment side. Two-sample t-tests were employed and a p-value < 0.025 was used as the threshold for statistical significance to minimise the chance of a type I error.

5.4 RESULTS

The analytical sample consisted of 31 patients with intermediate or high risk squamous cell cancer of the oropharynx (OPSCC). Median age was 57 years (range 37 to 70 years). Disease characteristics are included in table 5-1.

Cancer stage		n	(%)
T stage	1–2	11	35.5
	3–4	20	64.5
N stage	0	5	(16.2)
	1	9	(29.0)
	2	1	(3.2)
	2a	1	(3.2)
	2b	12	(38.7)
	2c	2	(6.5)
	3	1	(3.2)
Stage group	III	7	(22.6)
	IVA	23	(74.2)
	IVB	1	(3.2)

Table 5-1 Summary of disease characteristics.

5.4.1 Feasibility of outlining SG

Sixty-two scans (thirty-one each from baseline and #10) were acquired. Images at each time point included 2 sequences – T1PCFS and the ADC map, meaning 124 image sets were available for evaluation. Bilateral SMG and SLG delineations were successful on all 124 images. PGs were not fully included on 8 baseline image sets (3 on T1PCFS and 5 on ADC map) and PGs were incomplete on 15 repeat sequences (8 on T1PCFS and 7 on ADC map). Of the 248 PGs on T1PCFS and ADC maps on MRI_base and MRI_rpt, 201 (90.7%) were fully visualised and contoured.

5.4.2 SG volumes, ADC at baseline

The largest volumes were recorded for PG, and smallest for SLG (table 5-2). Ipsilateral and contralateral were of similar volumes for each gland at baseline. The mean planned dose for the iPG and cPG was 3665 cGy and 2461 cGy repectively, dosimetric information was not available for the other glands. The highest mean_ADC values were observed in SLG, then SMG and the lowest in PG. Mean_ADC values in the SLG displayed the greatest variation between patients. The baseline mean ADC were significantly different between each gland type ($p < 0.0001$).

Gland	Side	Mean (SD)			
		Volume (cm^3)		Mean ADC (mm^2/s)	
Parotid	contralateral	30.0	(10)	1224	(1 4 6)
	ipsilateral	29.7	(9)	1252	(1 4 0)
Submandibular	contralateral	9.1	(3)	1395	(1 9 6)
	ipsilateral	9.1	(3)	1404	(2 0 5)
Sublingual	contralateral	2.5	(1)	1522	(2 1 8)
	ipsilateral	2.7	(1)	1586	(2 4 7)

Table 5-2. Summary of gland volumes (cm^3), mean_ADC (mm^2/s) at baseline.

5.4.3 Change in measurements between scans

Parotid volume decreased during treatment by 6.7% and 11.2% for iPG and cPG respectively, with iSMG, cSMG, iSLG and cSLG volume increasing by 6.9, 0.9, 60.8 and 60.3% respectively (Table 5-3). All structures showed an increase in mean_ADC values at repeat scan. SMG showed the highest percentage change in ADC for all glands and least variation in measurements were seen in the PG. For each gland the increase in ADC was statistically significant $p < 0.0001$.

Gland	Side	Absolute change (SD)				Percent change (SD)			
		Volume (cm^3)		Mean ADC (mm^2/s)		Volume (%)		Mean ADC (%)	
Parotid	contralateral	−3.98	(7.22)	183.2	(134.0)	−11.2	(24.8)	15.8	(12.9)
	ipsilateral	−2.4	(8.87)	221.3	(137.5)	−6.7	(26.2)	18.3	(12.5)
Submandibular	contralateral	−0.46	(2.43)	237.1	(216.7)	0.9	(35.6)	18.6	(19.0)
	ipsilateral	0.27	(2.27)	282.1	(237.1)	6.9	(28.4)	21.9	(20.5)
Sublingual	contralateral	1.08	(1.28)	214.4	(242.7)	60.3	(75.0)	15.5	(17.8)
	ipsilateral	1.08	(1.52)	238.3	(312.8)	60.8	(84.7)	16.8	(21.7)

Table 5-3. Mean change in absolute volume (cm^3), mean ADC (mm^2/s) and percentage change (%) after 2 weeks of treatment, by gland type.

5.4.4 Toxicity groups

No statistically significant association was observed between toxicity groups and percent volume change (Table 5-4).

		Mean percent change (SD)					
		Volume percent change (SD)			Mean ADC percent change (SD)		
Gland	**Side**	**Grade 0-1**	**Grade 2+**	**p-value**	**Grade 0-1**	**Grade 2+**	**p-value**
Parotid	cPG	−13.0 (21)	−6.5 (31)	0.53	19.8 (14)	9.8 (11)	0.05
	iPG	−3.0 (26)	−8.3 (31)	0.64	23.5 (14)	10.9 (9)	0.013
Submandibular	cSMG	−9.5 (20)	14.9 (46)	0.08	22.1 (24)	15.5 (14)	0.41
	iSMG	−4.2 (23)	17.0 (29)	0.048	26.6 (27)	18.1 (14)	0.33
Sublingual	cSLG	39.8 (77)	98.3 (70)	0.06	16.6 (17)	14.8 (18)	0.8
	iSLG	43.3 (69)	94.9 (1 0 3)	0.14	11.3 (14)	19.6 (26)	0.31

Table 5-4. Association with percent change in mean volume and percent change in ADC, with grade of toxicity (RTOG) at 12 months.

A smaller mean percentage increase in ADC was observed in the group experiencing a higher grade (2 or >) of toxicity (Table 5-4). This was the case for bilateral PG (Fig. 5-1), bilateral SMG and cSLG but was only statistically significant in the iPG.

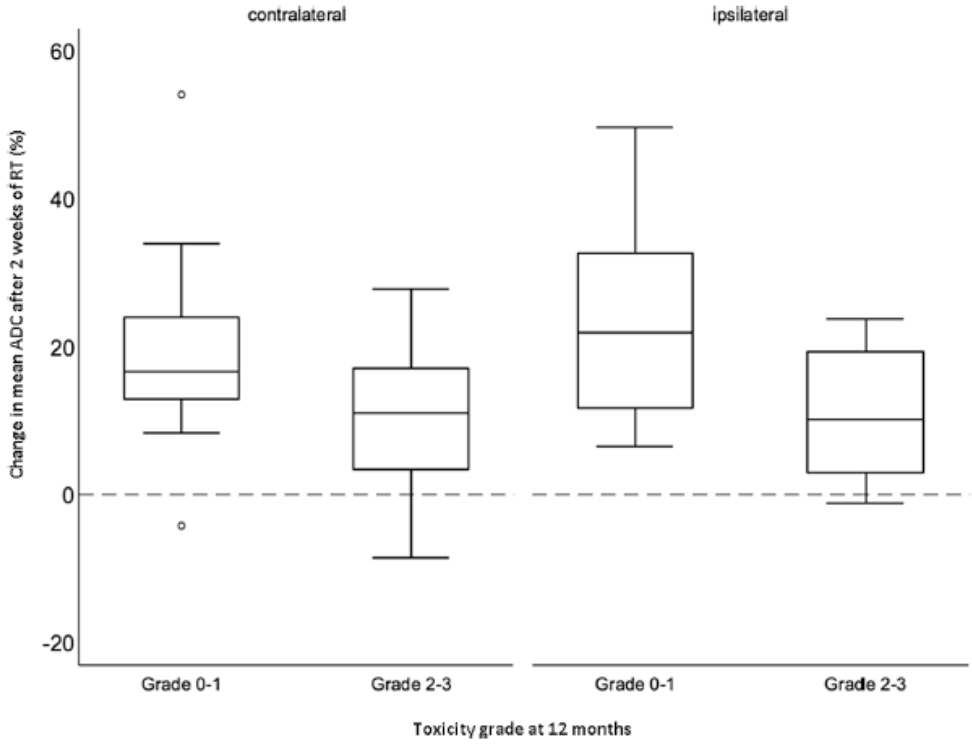

Fig. 5-1 Percent change in mean ADC of parotid glands after 2 weeks of treatment, when categorised by severity of toxicity (Grade 0–1 versus 2–3) at 12 months.

5.5 DISCUSSION

This is the largest DWI study of patients with a single sub-site of HNC to evaluate changes in SG during RT. It is also the first to include assessment of SLG during RT. A review by Stieb et al, [23] identified a number of publications that evaluated SG on serial MRI imaging, including baseline and mid treatment images. Of these studies, only 3 measured changes using DWI, and none assessed the SLG [24], [25], [26]. These studies included heterogeneous groups of patients with HNC. While differing disease characteristics may not directly impact assessment of changes in normal tissue with RT, it remains preferential to study a homogeneous group with similar characteristics and treatment. In this way we can ensure other variables that may confound results, e.g. dose received by SG are minimised and results are robust.

We have demonstrated the feasibility of outlining the paired major SG at baseline and during RT and measuring serial ADC on DW-MRI. On a small number of scans it was not possible to delineate full PG structures. This was due to the field of view being prioritised to include primary tumour and lymph nodes,

meaning PG contours were incomplete. This should be borne in mind when considering our results, although < 10% of PGs were affected in this way.

We report there is a significant difference in volume and ADC between each paired gland at baseline. Our finding that ADC was higher in SMG than PG at baseline (table 5-2) was consistent with previous work [24], [26], [27], [28]. The difference in ADC at baseline between each set of glands may indicate intrinsic differences in the microstructure and function of each. Alternatively, it may be that measured ADC is simply affected by the volume of the gland under study as discussed below.

Volumetrically the glands behaved differently during RT, with PG and cSMG absolute volumes decreasing, and iSMG and both SLG increasing. When assessing the percent change in volume, both PG volumes decreased and all SMG and SLG increased. Although absolute mean volume decreases, the variation is showing an overall mean percentage increase in cSMG and iSMG. This is due to large variability in percentage change among patients with small volumes. PG volume decreasing during RT is consistent with the findings of other groups on MRI, although they reported higher volume decrease during treatment, which continued following RT [25], [29], [30], [31]. A direct comparison is challenging, where different methodologies, timings and sequences are reported. SMG decreased following completion of RT in one study [32], but no groups so far have assessed volume change of SMG and SLG during RT. The differential volume change between iPG and cPG during RT (smaller reduction in iPG than cPG) may demonstrate that dose received influences this parameter and will be analysed in future work.

The SLG measured smallest of all glands at baseline; showed the largest increase in percentage volume after 2 weeks of treatment; and had most variation in measurements. This could be attributed to the gland being small, where a very small absolute change in volume is equivalent to a high percentage. The variation in ADC might be a consequence of the challenges in defining small ROIs, where ADC may be affected by inclusion of a small volume/large percentage of normal tissue at the periphery of the structure. This could also explain why least variation was seen in the parotid glands.

All glands showed an increase in ADC after 2 weeks of treatment as previously described [24], [25], [26]. This indicates a change in the cellularity, vascularity and function of SG tissue during RT, not just a change in the anatomy or volume

as is already well documented [23]. In this study, this increase in ADC could not be attributed to the dose received by the gland, as a similar change in ADC was seen in both ipsi- and contra- lateral PGs despite differences in planned dose to each. The influence of delivered dose on SG characteristics (change in volume and ADC) is beyond the scope of this work but is worthy of future consideration. A correlation in mean dose and change in ADC has been previously reported in pre and post RT scans but this was not analysed using images captured during RT [28].

Results from this sample indicate an association with change in ADC and toxicity at 12 months. Patients with higher grades of toxicity (2 +) demonstrated a smaller percentage increase in ADC compared to those with lower toxicity grades. This was consistent across all glands except for the iSLG, the reason for this is unclear and may simply relate to the small volume of the SLG as described above.

Some authors reported a significantly higher change in ADC values during or post RT in patients who experienced xerostomia [26], [28], [33]. Zhang et al. [26], collected RTOG toxicity at 6 months and described that ADC increase in the PG were associated with the level of toxicity. This 6-month duration of follow up is too short to capture late toxicity and others also recognise their lack of clinical data as a limitation [25]. While our cohort benefits from 12 month follow up post-RT it is established that severity of xerostomia may continue to improve for up to 18 months post treatment [1]. A later time point for toxicity assessment may allow more robust evaluation of the relationship between changes in ADC and late toxicity outcomes.

Differences in techniques, hardware and software have always been a limitation when using MRI for quantitative evaluation [34], [35]. These are further exasperated when repeated measurements are required. Here we have used a strict protocol to ensure reliable and valid results were obtained, mandating the same conditions throughout. Constants have included image acquisition on the same scanner, magnet strength, acquisition protocol (including high b values to reduce the effect of perfusion) and a protocolised methodology to define ROI. This ensures the process is reproducible. The reporting of percentage rather than absolute change in ADC also means results are more transferrable to other systems. A focus on standardising future work in a multi-centre setting should

aim to define the optimal follow up and scanning protocol to capture meaningful data.

Delineation was performed with observers blinded to toxicity, this was to reduce bias. Use of immobilisation may have improved set-up reproducibility and image registration. The decision not to use a thermoplastic shell in these patients was to allow the use of the neurovascular coil and to facilitate recruitment and retention of patients to the study. All contours were redefined at each timepoint therefore, any discrepancies in position are unlikely to affect results.

This interim analysis of 31 patients is a small sample from a larger study to determine whether DW-MR can predict response to RT by defining a threshold change in ADC [17]. Further analysis of SG and assessment of xerostomia at 24 months post RT will be undertaken for the full cohort to increase the robustness of our work and further explore the relationship between severities of late toxicity and change in ADC. As the primary study was not designed to predict SG toxicity using ADC, we found our field of view was a limitation. Our priority to capture primary tumour and lymph nodes resulted in a small number of patients having the superior aspect of their PG excluded from the scan. This does add uncertainty to our volumetric and ADC analysis; however, we still identified a trend in decreasing volume and increasing ADC. This feasibility work has proven invaluable to identify these factors requiring optimisation where the primary question is OAR analysis.

5.6 CONCLUSIONS

It is feasible to measure volume and ADC of the major SG prior to and during a course of radiotherapy for HNC. Image acquisition may need optimisation to ensure the field of view adequately allows tumour and OAR evaluation. Early data suggests that a lower rise in ADC during treatment is associated with more severe late xerostomia. Validation of these findings is required in a larger cohort with long term follow up.

5.7 POST VIVA ADDITIONS TO CHAPTER

The following section was added as correction to the thesis post viva, taking into account examiners' comments.

Limitations of work

As multiple testing was not accounted for in the pre-post paired observations there is therefore the chance of a type 1 error, and a larger study is required to confirm true differences.

'Parotid volume decreased during treatment by 6.7% and 11.2% for iPG and cPG respectively, with iSMG, cSMG, iSLG and cSLG volume increasing by 6.9, 0.9, 60.8 and 60.3% respectively (Table 5-3).' – raw data is no longer available to carry out statistical comparison of the volume changes.

While a statistically significant smaller mean percentage increase in ADC in iPG was observed in the group experiencing a higher grade (2 or >) of toxicity, it is acknowledged that patient and treatment factors which may confound this analysis were not accounted for. Furthermore, the overlap in % change in ADC between patients with G 0-1 and G2 or > toxicity may suggest that difference is not significant.

5.8 REFERENCES

1. Nutting C.M., Morden J.P., Harrington K.J., Urbano T.G., Bhide S.A., Clark C. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial. *Lancet Oncol*. 2011;12(2):127–136.

2. Little M., Schipper M., Feng F.Y., Vineberg K., Cornwall C., Murdoch-Kinch C.-A. Reducing xerostomia after chemo-IMRT for head-and-neck cancer: beyond sparing the parotid glands. *Int J Radiat Oncol Biol Phys*. 2012;83(3):1007–1014.

3. Vissink A., Mitchell J.B., Baum B.J., Limesand K.H., Jensen S.B., Fox P.C. Clinical management of salivary gland hypofunction and xerostomia in head-and neck cancer patients: successes and barriers. *Int J Radiat Oncol Biol Phys*. 2010;78(4):983–991.

4. Dirix P., Nuyts S., Van den Bogaert W. Radiation-induced xerostomia in patients with head and neck cancer: a literature review. *Cancer*. 2006;107(11):2525–2534.

5. Makkonen T.A., Tenovuo J., Vilja P., Heimdahl A. Changes in the protein composition of whole saliva during radiotherapy in patients with oral or pharyngeal cancer. *Oral Surg Oral Med Oral Pathol*. 1986;62(3):270–275.

6. Wijers O.B., Levendag P.C., Braaksma M.M.J., Boonzaaijer M., Visch L.L., Schmitz P.I.M. Patients with head and neck cancer cured by radiation therapy: a survey of the dry mouth syndrome in long-term survivors. *Head Neck*. 2002;24(8):737–747.
7. Mehanna H., Jones T.M., Gregoire V., Ang K.K. Oropharyngeal carcinoma related to human papilloma virus. *BMJ*. 2010;340
8. Gillison M.L., Harris J., Westra W. Survival outcomes by tumour human papilloma virus (HPV) status in stage III-IV oropharyngeal cancer (OPC) in RTOG 0129. *J Clin Oncol ASCO Meet Abstr*. 2009;27(15S):6003.
9. Fakhry C., Westra W.H., Li S., Cmelak A., Ridge J.A., Pinto H. Improved survival of patients with human papilloma virus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100(4):261–269.
10. 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.
11. Goldstein D.P., Hynds Karnell L., Christensen A.J., Funk G.F. Health-related quality of life profiles based on survivorship status for head and neck cancer patients. *Head Neck*. 2007;29(3):221–229.
12. Paterson C., Hargreaves S., Rumley C.N. Functional Imaging to Predict Treatment Response in Head and Neck Cancer: How Close are We to Biologically Adaptive Radiotherapy? *Clin Oncol (R Coll Radiol)*. 2020;32(12):861–873. doi: 10.1016/j.clon.2020.10.004.
13. Mehanna H., Evans M., Beasley M., Chatterjee S., Dilkes M., Homer J. Oropharyngeal cancer: United Kingdom National Multidisciplinary Guidelines. *The Journal of Laryngology & Otology*. 2016;130(S2):S90–S96. doi: 10.1017/S0022215116000505.
14. Lewis-Jones H., Colley S., Gibson D. Imaging in head and neck cancer: United Kingdom National Multidisciplinary Guidelines. *The Journal of Laryngology & Otology*. 2016;130(S2):S28–S31. doi: 10.1017/S0022215116000396.
15. Koh D.-M., Collins D.J. Diffusion-weighted MRI in the body: applications and challenges in oncology. *Am J Roentgenol*. 2007;188(6):1622–1635.
16. Ang K.K., Harris J., Wheeler R., Weber R., Rosenthal D.I., Nguyen-Tân P.F. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010;363(1):24–35. doi: 10.1056/NEJMoa0912217.

17. Paterson C., Allwood-Spiers S., McCrea I., Foster J., McJury M., Thomson M. Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study. *Clin Translational Radiation Oncol*. 2017;2:13–18.
18. Sobin, L. H., Gospodarowicz, M. K., & Wittekind, C. (Eds.). (2011). TNM classification of malignant tumours. John Wiley & Sons.
19. van de Water, T. A., Bijl, H. P., Westerlaan, H. E., & Langendijk, J. A. (2009). Delineation guidelines for organs at risk involved in radiation-induced salivary dysfunction and xerostomia. Radiotherapy and Oncology, 93(3), 545–552. https://doi.org/https://doi.org/10.1016/j.radonc.2009.09.008.
20. Grégoire V., Grau C., Lapeyre M., Maingon P. Target volume selection and delineation (T and N) for primary radiation treatment of oral cavity, oropharyngeal, hypopharyngeal and laryngeal squamous cell carcinoma. *Oral Oncol*. 2018;87:131–137. doi: 10.1016/j.oraloncology.2018.10.034.
21. Cox J.D., Stetz JoAnn, Pajak T.F. Toxicity criteria of the radiation therapy oncology group (RTOG) and the European organization for research and treatment of cancer (EORTC) *Int J Radiat Oncol Biol Phys*. 1995;31(5):1341–1346.
22. Jerome N.P., Papoutsaki M.-V., Orton M.R., Parkes H.G., Winfield J.M., Boss M.A. Development of a temperature-controlled phantom for magnetic resonance quality assurance of diffusion, dynamic, and relaxometry measurements. *Med Phys*. 2016;43(6Part1):2998–3007.
23. Stieb S., Elgohari B., Fuller C.D. Repetitive MRI of organs at risk in head and neck cancer patients undergoing radiotherapy. *Clin Translational Rad Oncol*. 2019;18:131–139.
24. Doornaert P., Dahele M., Ljumanovic R., de Bree R., Slotman B.J., Castelijns J.A. Use of diffusion-weighted magnetic resonance imaging (DW-MRI) to investigate the effect of chemoradiotherapy on the salivary glands. *Acta Oncol*. 2015;54(7):1068–1071. doi: 10.3109/0284186X.2014.987357.
25. Marzi S., Forina C., Marucci L., Giovinazzo G., Giordano C., Piludu F. Early radiation-induced changes evaluated by intravoxel incoherent motion in the major salivary glands. *J Magn Reson Imaging*. 2015;41(4):974–982. doi: 10.1002/jmri.24626.

26. Zhang Y., Ou D., Gu Y., He X., Peng W. Evaluation of salivary gland function using diffusion-weighted magnetic resonance imaging for follow-up of radiation-induced xerostomia. *Korean J Radiol*. 2018;19(4):758–766.
27. Loimu V., Seppälä T., Kapanen M., Tuomikoski L., Nurmi H., Mäkitie A. Diffusion-weighted magnetic resonance imaging for evaluation of salivary gland func- tion in head and neck cancer patients treated with intensity-modu- lated radiotherapy. *Radiother Oncol*. 2017;122(2):178–184.
28. Fan W.J., Teng F., Luo Y.R., Yu W., Zhang Q., Lu Y.P. Diffusion-weighted imaging as a follow-up modality for evaluation of major salivary gland function in nasopharyngeal carcinoma patients: a preliminary study. *Strahlenther Onkol*. 2020:1–12.
29. Zhou N et al. Early evaluation of radiation-induced parotid damage in patients with nasopharyngeal carcinoma by T2 mapping and mDIXON Quant imaging: initial findings. Radiat Oncol 2018;13(1):22.
30. Zhou N., Chu C., Dou X., Li M., Liu S., Guo T. Early changes of irradiated parotid glands evaluated by t1rho weighted imaging: a pilot study. *J Comput Assist Tomogr*. 2017;41(3):472–476.
31. Marzi S., Farneti A., Vidiri A., Di Giuliano F., Marucci L., Spasiano F. Radiation-induced parotid changes in oropharyngeal cancer patients: the role of early functional imaging and patient−/treatment-related factors. *Radiation Oncol*. 2018;13(1) doi: 10.1186/s13014-018-1137-4.
32. Houweling A.C., Schakel T., van den Berg C.A.T., Philippens M.E.P., Roesink J.M., Terhaard C.H.J. MRI to quantify early radiation-induced changes in thesalivary glands. *Radiother Oncol*. 2011;100(3):386–389.
33. Juan, C.-J., Cheng, C.-C., Chiu, S.-C., Jen, Y.-M., Liu, Y.-J., Chiu, H.-C. (2015). Temporal Evolution of Parotid Volume and Parotid Apparent Diffusion Coefficient in Nasopharyngeal Carcinoma Patients Treated by Intensity-Modulated Radiotherapy Investigated by Magnetic Resonance Imaging: A Pilot Study. PLOS ONE, 10(8), e0137073.
https://doi.org/10.1371/journal.pone.0137073.
34. Abramson R.G., Burton K.R., Yu J.-P., Scalzetti E.M., Yankeelov T.E., Rosenkrantz A.B. Methods and challenges in quantitative imaging biomarker development. *Acad Radiol*. 2015;22(1):25–32.
35. European Society of Radiology (ESR) communications@ myesr. org Angel Alberich-Bayarri Steven Sourbron Xavier Golay Nandita deSouza Marion Smits Aad

van der Lugt Ronald Boellard. (2020). ESR statement on the validation of imaging biomarkers. Insights into Imaging, 11, 1–5.

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

Paterson C, Caldwell B, Porteous S, McLean A, Messow CM, Thomson M. Radiotherapy-induced xerostomia, pre-clinical promise of LMS-611. Support Care Cancer. 2016 Feb; 24(2):629-636. doi: 10.1007/s00520-015-2823-5. Epub 2015 Jul 5. PMID: 26143037

6.1 ABSTRACT

6.1.1 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. The primary objective of this study was to assess saliva properties before and during RT to the H&N. The secondary objectives were to 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.

6.1.2 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 and weeks 2, 4 and 6 of RT. Saliva adhesiveness and viscosity were tested by measuring time taken to travel 5 cm down an inclined plane.

6.1.3 Results

Thirty patients were enrolled. The inclined plane test (IPT) results (s) were as follows: baseline 31.3, week 2 49.7, week 4 51.1 and 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, 0.25 at week 2, 0.12 at week 4 and 0.08 at week 6. LMS-611 concentrations of 10 and 20 mg/ml significantly reduced IPT times on saliva samples.

6.1.4 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.

6.2 INTRODUCTION

Radiotherapy (RT) or chemoradiotherapy (CRT) is well established as an alternative to surgery in squamous cell carcinoma (SCC) of the head and 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 papillomavirus, means that patients are often younger with little comorbidity [9]. This group has 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.

6.3 MATERIALS AND METHODS

6.3.1 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.

6.3.2 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 100 mg/m2 on days 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, 4 and 6 weeks into radiotherapy.

Saliva adhesiveness and viscosity were 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° 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 and 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 s.

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. The 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 interpatient differences in saliva properties, to correlate patient reported symptoms with laboratory measurements and to inform the design of the subsequent clinical study.

6.3.3 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 10,000 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 s 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].

6.3.4 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.

6.4 RESULTS

Thirty patients were recruited to the study between September 2013 and April 2014. Twenty-nine 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.

6.4.1 Demographics

Patient demographics are summarised in Table 6-1. 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.

Total number of patients (%)		30
Mean age (years)		54.8
Age range (years)		42-67
Gender	Male	24 (80%)
Stage	II	1 (3.3%)
	III	4 (13.3%)
	IV	25 (83.3%)
Treatment	Radiotherapy alone	3 (10%)
	Chemo radiotherapy	27 (90%)

Table 6-1 Patient demographics

6.4.2 Saliva adhesiveness and viscosity tests

6.4.2.1 Inclined plane test

The IPT results are summarised in Table 6-2 and Fig. 6-1.

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, these changes were not statistically significant. 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 s. 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.

	Time point during RT			
	Baseline	Week 2	Week 4	Week 6
Number samples assessable	28	26	22	22
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

Table 6-2 Inclined plane test results

Acknowledgement CM Messow

6.4.2.2 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.

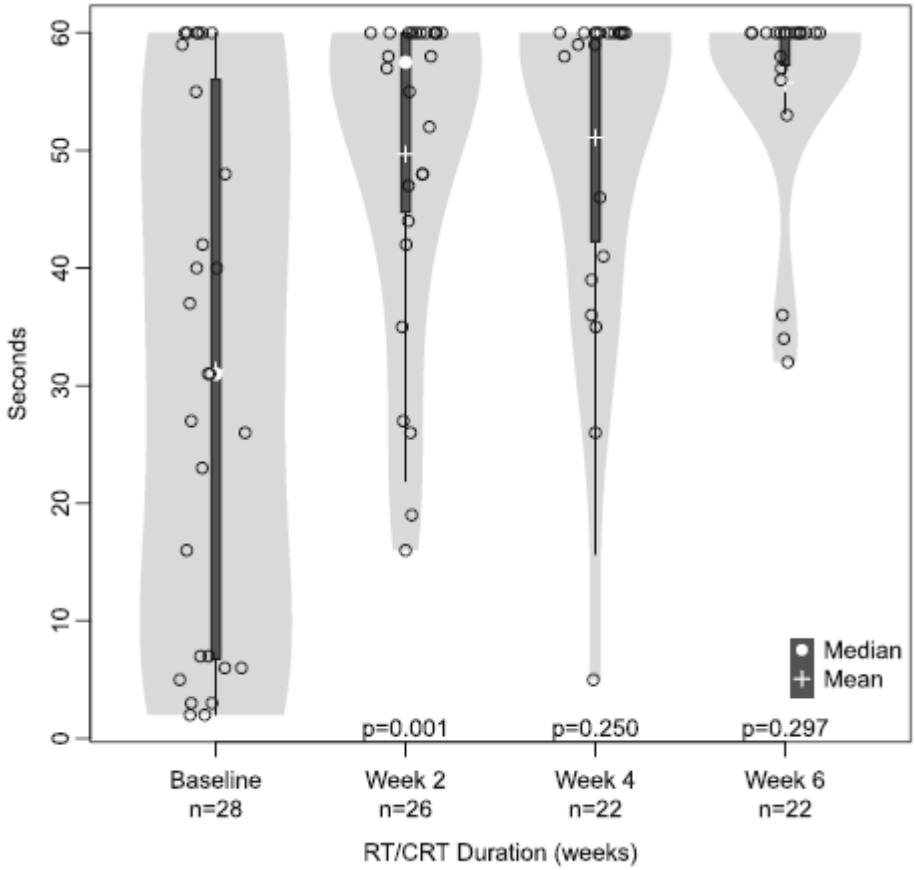

Figure 6-1 Violin plots of the time taken to descend the IP against treatment duration. p values refer to the comparison with previous visit by Wilcoxon test

Acknowledgement CM Messow

6.4.2.3 Inclined plane tests with addition of LMS-611

Table 6-3 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 2.5 and 5 mg/ml to the saliva samples did not reduce IPT times.

However, when LMS-611 at concentrations of 10 and 20 mg/ml was added, significant reductions were seen in the IPT at each time point.

			Timepoint during radiotherapy		
			Week 2	Week 4	Week 6
Inclined plane test	Untreated	N_{obs}	26	22	22
		Mean (SD)	49.7 (14.0)	51.1 (14.9)	55.7 (9.0)
		Median (IQR)	57.5 (44.8, 60.0)	60.0 (42.2, 60.0)	60.0 (57.2, 60.0)
	Saline	N_{obs}	23	18	22
		Mean (SD)	59.0 (4.4)	59.3 (2.4)	60.0 (0.0)
		Median (IQR)	60.0 (59.0, 60.0)	60 (60.0, 60.0)	60 (60.0, 60.0)
	LMS-611 2.5mg/ml	N_{obs}	23	17	18
		Mean (SD)	57.6 (5.3)	58.6 (4.9)	58.9 (4.7)
		Median (IQR)	60 (59.0, 60.0)	60.0 (60.0, 60.0)	60 (60.0, 60.0)
	LMS-611 5mg/ml	N_{obs}	23	16	19
		Mean (SD)	47.2 (18.4)	54.4 (10.3)	58.3 (7.3)
		Median (IQR)	60 (36.0, 60.0)	60 (53.2, 60.0)	60 (60.0, 60.0)
	LMS-611 10mg/ml	N_{obs}	23	18	22
		Mean (SD)	24.2 (20.2)	17.3 (14.6)	32.5 (16.7)
		Median (IQR)	16.0 (10.5, 37.0)	14.5 (10.0, 17.8)	29.0 (18.8, 44.5)
	LMS-611 20mg/ml	N_{obs}	23	18	22
		Mean (SD)	4.3 (2.9)	7.1 (8.0)	11.0 (9.6)
		Median (IQR)	3.0 (2.0, 6.0)	4.0 (2.0, 9.0)	8.5 (5.2, 12.8)

N_{obs} = Number of observations assessable

Table 6-3. Inclined plane test results with addition of saline or LMS-611

Acknowledgement CM Messow

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 6-4) demonstrates these statistically significant differences. The hazard ratio refers to the likelihood of saliva travelling the 5 cm; therefore, a small hazard ratio indicates stickier saliva. Interestingly, the addition of saline or LMS-611 2.5 mg/ml to saliva seems to produce significantly stickier saliva than no treatment. It is difficult to account for this effect.

	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

Table 6-4. All inclined plane test results with addition of LMS-611 or saline, adjusted for week of radiotherapy. Cox proportional hazards model.

Acknowledgement CM Messow

6.4.3 Patient-reported xerostomia

Patient-reported xerostomia scores collected using the GRIX questionnaire are summarised in Fig. 6-2. 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 the treatment.

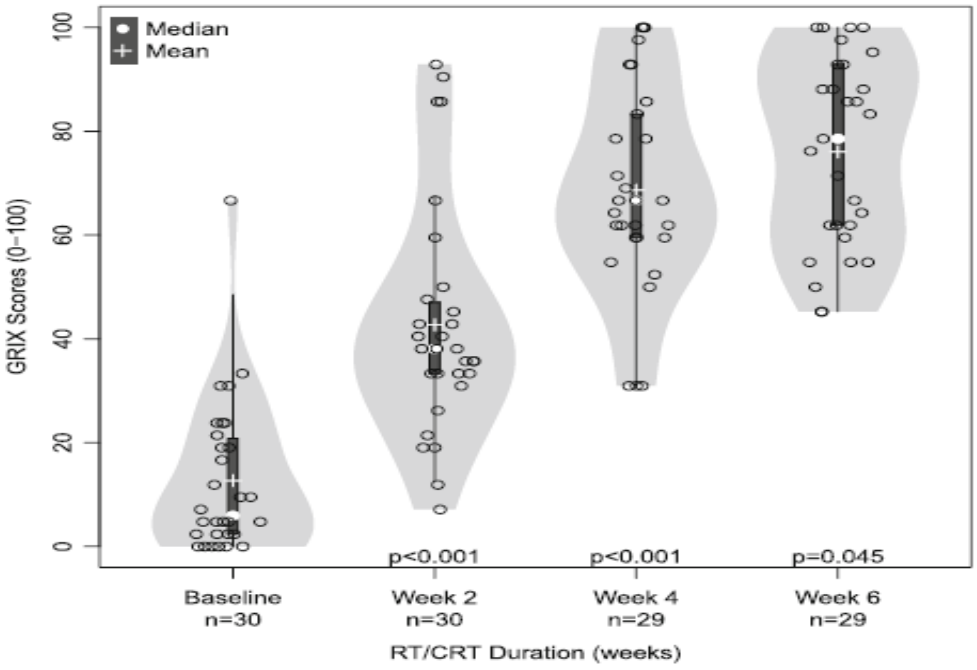

Figure 6-2. Violin plots of GRIX scores by treatment duration. p values refer to the comparison with the previous visit by Wilcoxon text.

Acknowledgement CM Messow

6.4.4 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 two measurements, with Spearman correlation coefficient 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).

6.5 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 the 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 2 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 during 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 s 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 weeks 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 the 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 10 and 20 mg/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 2.5 and 5 mg/ml had little or no impact on the saliva properties. The 10 and 20 mg/ml preparations demonstrated significant efficacy. As a result, the 2.5 and 5 mg/ml concentrations have been removed from the forthcoming clinical study. The effects of LMS-611 in concentrations of 10 and 20 mg/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 viscoadhesive properties.

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.

6.6 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 2 weeks of radiotherapy. Inter-patient variability in reported xerostomia remains constant throughout the treatment.

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

This data suggests that concentrations of 10 and 20 mg/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.

6.7 POST VIVA ADDITIONS TO CHAPTER

The following section was added as correction to the thesis post viva, taking into account examiners' comments.

6.4.2.1 During the IPT not all samples were analysed as they were unsuitable for testing; they were so viscous it was not possible for them to be handled in the laboratory. It is acknowledged that it would have been preferable for these samples to be included in the analysis as may have been derived from the most severely affected patients, but it was simply not possible. Likewise the truncation of the IPT after 60s was a pragmatic decision where samples did not

travel the full distance of the IPT in that time. Both of these strategies may have underestimated the true viscosity of the saliva samples. Given that this study was simply a preparatory step towards a clinical study of LMS-611 these 'workarounds' were not felt to be detrimental.

6.8 REFERENCES

1. Bhide SA, Nutting CM (2010) Advances in radiotherapy for head and neck cancer. Oral Oncol 46(6):439–441

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):487–491

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):592–597

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, Braaksma 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):737–747

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 (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, WestraW, 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 Meet Abstr 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):261–269
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, Funk GF (2007) Health-related quality of life profiles based on survivorship status for head and neck cancer patients. Head Neck 29(3):221–229
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 Radiat Oncol Biol Phys 83(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 Radiat Oncol Biol Phys 78(4):983–991
17. Gregoire V, Coche E, Cosnard G, Hamoir M, Reychler 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):135–150
18. Beetz I, Burlage FR, Henk P, et al (2010) The Groningen Radiotherapy-Induced Xerostomia Questionnaire: development and validation of a new questionnaire. Radiother Oncol 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-projectorg/. Accessed Sep 2014
20. Freitas DA, Caballero AD, Pereira MM, et al (2011) Oral sequelae of head and neck radiotherapy. CEFAC 13(6):1103–1108
21. Franzen L, Funegård U, Ericson T, Henriksson R (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. WescottWB, 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. Mira JG, Wescott WB, Starcke EN, Shannon IL (1981) Some factors influencing salivary function when treating with radiotherapy. Int J Radiat Oncol Biol Phys 7(4):535–541
26. Konings AWT, Coppes RP, Vissink A (2005) On the mechanism of salivary gland radio sensitivity. Int J Radiat Oncol Biol Phys 62(4): 1187–1194
27. Porter S, Scully C, Hegarty AM (2004) An update of the etiology and management of xerostomia. Oral Med Oral Surg Oral Pathol 97(1):28–46
28. Villa A, Abati S (2011) Risk factors and symptoms associated with xerostomia: a cross-sectional study. Aust Dent J 56:290–295
29. Thomson WM (2015) Dry mouth and older people. Aust Dent J 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 Periodontal 70:1240–1246
31. Kolte AP, Kolte RA, Laddha RK (2012) Effect of smoking on salivary composition and periodontal status. Indian Soc Periodontol 16(3):350–353
32. https://clinicaltrials.gov/ct2/show/NCT02123511?term=GRIX&rank=1. ht t ps : / / cli n ical t r ial s.gov/ct2/show/NCT01823445?term=GRIX&rank=2. Accessed Oct 2014
33. Tiwana MS, Mahajan MK, Uppal B, Koshy G, Sachdeva J, Lee HN, Talole SD (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 J Cancer 48(3):289–295
34. Meirovitz A, Murdoch-Kinch CA, Schipper M, Pan C, Eisbruch A (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. Lovelace TL, Fox NF, Sood AJ, 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 Surg Oral Med Oral Pathol Oral Radiol 117(5):595–607

36. Chaushu G, Bercovici M, Dori S,Waller A, Taicher S, Kronenberg J, Talmi YP (2000) Salivary flow and its relation with oral symptoms in terminally ill patients. Cancer 88(5):984–987
37. Nutting CM, Morden JP, Harrington KJ, 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 12:127–136
38. Setton J, Caria N, Romanyshyn J, et al (2012) Intensity-modulated radiotherapy in the treatment of oropharyngeal cancer: an update of the Memorial Sloan-Kettering Cancer Centre experience. Int J Radiat Oncol Biol Phys 82(1):291–298
39. Kam M, Leung SF, Zee B (2007) Prospective randomized study of intensity-modulated radiotherapy on salivary gland function in early-stage nasopharyngeal carcinoma patients. J Clin Oncol 25(31):4873–4879
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 Oct 2014
41. Trotti A, Eisbruch A (2011) Reducing xerostomia through advanced technology. Lancet Oncol 12:110
42. MASCC Oral care study group. Summary of the oral complications systematic reviews. http://www.mascc.org/oral-care. Accessed
Apr 2015

7. Radiotherapy induced xerostomia: a randomised, double-blind, controlled trial of Visco-ease™ oral spray compared with placebo in patients with cancer of the head and neck

Paterson C, Thomson MC, Caldwell B, Young R, McLean A, Porteous S, Clark S, Messow CM, Kean S, Grose D, Lamb C, Rizwannullah M, James A, Schipani S, Wilson C, Rulach R, Jones R.
Radiotherapy-induced xerostomia: a randomised, double-blind, controlled trial of Visco-ease™ oral spray compared with placebo in patients with cancer of the head and neck. Br J Oral Maxillofac Surg. 2019 Dec;57(10):1119-1125. doi: 10.1016/j.bjoms.2019.10.300. Epub 2019 Oct 29. PMID: 31672256.

7.1 ABSTRACT

Radiotherapy-induced xerostomia (RIX) is a common and untreatable side effect of radiotherapy to the head and neck. Visco-ease™ mouth spray (Lamellar Biomedical Ltd), a new product that is made from lamellar body mimetics, reduces the viscosity of saliva ex vivo. The purpose of this study was to evaluate its safety and effectiveness in the treatment of RIX in 43 patients with cancer of the head and neck. They were randomised into the Visco-ease ™ or placebo groups, and asked to complete the Groningen radiotherapy-induced xerostomia (GRIX) questionnaire each week. The primary endpoint was a change in GRIX score from baseline to end of treatment. There was no difference in scores between the two groups, and none of the patients had device-related serious adverse events. Visco-ease™ oral spray was safe and tolerable but no better than placebo in reducing RIX in this group of patients.

7.2 INTRODUCTION

Radiotherapy- induced xerostomia (RIX), the most commonly reported late and permanent side effect of radiotherapy for cancer of the head and neck,[1] impairs patients' quality of life (QoL),[2,3] and causes discomfort, altered taste, difficulties with speech and swallowing, and dental caries.[4] Despite advances in radiation technology, around 40% of patients have xerostomia 12 months after treatment.[5, 6] The epidemiology of head and neck cancer has changed with

the rise in the incidence of human papilloma virus (HPV)-driven oropharyngeal cancer. Patients are often younger, [7] and the significant improvements in response to treatment and overall survival, [8, 9] mean that they will live for longer with the consequences of treatment. [10, 11] There is no effective treatment for RIX, and a Cochrane review concluded that "randomized controlled trials of topical interventions for dry mouth are required to provide evidence to guide clinical care". [12]

Lamellar bodies have surface active properties and are an essential lubricant of the body's tissues. They prevent mucosal surfaces from sticking to each other, and sticky secretions (such as thick saliva) from congesting the hollow organs. Visco-ease™ (Lamellar Biomedical Ltd), formerly known as LMS-611, is a multi-lipid mimetic of a naturally occurring lamellar body. Preclinical work has suggested that it may help to make the thick, sticky saliva seen after radiotherapy to the head and neck more fluid.[13]

The purpose of this study was to evaluate the safety and effectiveness of Visco-ease™ mouth spray for the treatment of RIX in patients with cancer of the head and neck. To our knowledge, this is the first study in humans. In line with published recommendations [14] we thought that reported outcomes were the most critical measures of its effectiveness, and we used the validated Groningen radiotherapy-induced xerostomia (GRIX) questionnaire [15] to evaluate patients' reports of RIX. The primary endpoint was the change in GRIX score from baseline to the end of treatment. Secondary objectives were assessments of safety and frequency of use.

7.3 MATERIALS AND METHODS

7.3.1 Participants

Patients with cancer of the head and neck who were planned to start radical primary radiotherapy or chemoradiotherapy, were recruited to this randomised, double-blind, placebo-controlled study.

Eligible patients were aged 18 years or older and were thought to be at high risk of RIX. Those with pre-existing xerostomia, or who had used any investigational drug or product within 30 days, and those who had had primary surgery for cancer of the head and neck or who had known allergies to egg, soya, or lanolin-based products, were excluded.

7.3.2 Randomisation and blinding

Patients were randomly assigned to be given Visco-ease™ or placebo (0.9% physiological saline) oral spray in a ratio of 2:1. Independent randomisation was done through an interactive web-response system at the Robertson Centre for Biostatistics. Neither the patient nor investigators were informed of the treatment allocated. All treatment and placebo kits were presented in an identical manner.

7.3.3 Procedures

In all cases, radical radiotherapy or chemoradiotherapy was delivered by volumetric modulated arc therapy (VMAT). Gross tumour and the all involved nodes were treated with doses of 65Gy/30# over six weeks. The prophylactic dose to areas considered at high risk of occult disease was 54Gy/30# over six weeks. Target volumes were selected and delineated according to international guidelines. [16] Cisplatin was delivered at 100mg/m2 on days 1 and 22 of treatment in those who had concurrent chemotherapy. Patients were asked to use the oral spray (Visco-ease™ or placebo) as required, but to use at least one spray twice a day during the course of their treatment, beginning on day one. They were instructed to spray under the tongue then to move the fluid around the mouth. To enable us to assess the tolerability of the product independent of the subsequent symptoms, they began to use the spray before they developed RIX. They were assessed weekly during treatment, and adverse events were recorded. Patients' scores of RIX using the GRIX questionnaire were collected weekly, and patients were also asked to keep a daily diary to record when they used the spray.

7.3.4 Statistical analysis (acknowledgment CM Messow)

Previous work has shown that changes in GRIX scores from baseline to week six of radiotherapy were normally distributed in untreated patients with a mean (SD) of 65.2 (22.3). [13]

To calculate the sample size we assumed a mean change of 65 in the placebo group and 35 in the treated group (SD 23 in both). Patients were allocated to the groups in a ratio of 2:1 (for each patient given placebo, two were given Visco-ease™). The sample size calculation was based on a comparison of the change in GRIX scores from baseline to week six of radiotherapy between the two groups

using a two-sided two-sample t test, with a significance level of 0.05. The number required to achieve a power of 90% was 30 (20 in the Visco-ease™ group and 10 in the placebo group). The sample size calculation was done with the help of Proc Power in SAS™ software 9.3 (SAS Institute Inc). To allow for a dropout rate of 25%, 41 patients were required (27 randomised to the Visco-ease™ group and 14 to the placebo group).

Baseline characteristics were summarised overall and for each treatment group using mean (SD), range for continuous variables, and number (%) for categorical variables.

Statistical analysis of the effect of treatment on the primary was done using linear regression, with adjustments for baseline GRIX scores. Analysis of variance (ANOVA) was used to analyse the difference in mean clinic GRIX scores at each time point. Fisher's exact test was used to compare categorical variables at baseline between the placebo and Viscoease ™ groups, and also for the comparison of patients with adverse events during the study. Independent two-sample t tests were used to compare the mean of continuous variables at baseline.

All analyses were done with the help of the statistical software platform R.17

7.3.5 Ethics approval and consent to participate

The protocol was approved by the Research Ethics Committee 4, (15/WS/0281) and MHRA (CI/2015/0053). Written informed consent was obtained from all participants. The study was sponsored by Lamellar Biomedical Limited and conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki.

7.4 RESULTS

7.4.1 Participants

A total of 43 patients (15 in the placebo group and 28 in the Visco-ease™ group) were recruited between March and December 2016 from 62 patients who were screened (Fig. 7-1). The intention-to-treat population reflected the number of patients actually recruited, which was slightly higher than planned, as the safety population (patients actually treated) was 25 in the Visco-ease™ arm and 14 in

the placebo arm. The per protocol population (19 Viscoease ™, 12 placebo) reflected those who completed the study.

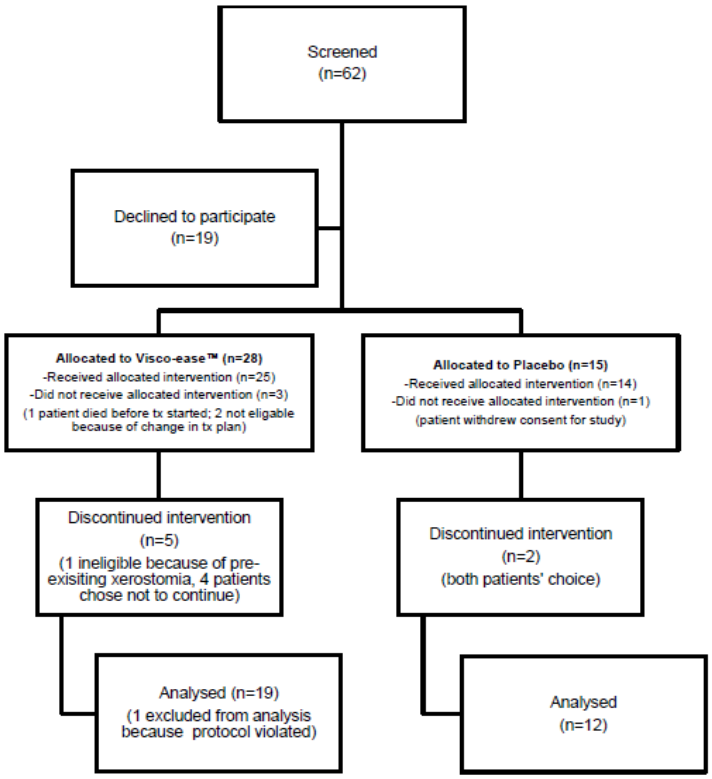

Figure 7-1. Trial profile, CONSORT flow diagram

7.4.2 Baseline demographics

Table 7-1 shows the patients' details (mean (range) age 59 (41-78) years). The demographics seemed well balanced between the two groups. A total of 39 participants were male and all had squamous cell carcinoma (SCC). The oropharynx was the most common subsite, (71%) of those tumours were HPV-positive across both groups. The most notable imbalance between the groups was in tumour staging. In the Visco-ease™ group a higher proportion of patients had stage III or IV disease or higher T stages than in the placebo group. The greater use of concurrent chemoradiotherapy in the Visco-ease™ group probably reflects this more advanced disease. The withdrawal of patients from both groups during the course of the study further increased this imbalance in disease stage.

	Placebo (n=14)	Visco-ease (n=25)	p-value
Age, mean (range), years	62 (51-78)	58 (41-70)	0.1513*
Sex			1**
Male	13	22	
Female	1	3	
Subsite			0.6735**
Oropharynx	9	15	
Larynx	3	5	
Hypopharynx	1	2	
Nasopharynx	1	0	
Unknown primary	0	3	
Disease – SCC	14	25	1**
T stage			0.2251**
T0-2	12	20	
T3-4	2	5	
Overall AJCC stage			
I-II	4	3	
III-IV	10	22	
Concurrent chemotherapy			
Yes	7	17	
No	7	8	

Table 7-1. Baseline demographics for APT. Data are number unless otherwise stated

* 2-sample t test ** Fisher's exact test.

7.4.3 Patient-reported xerostomia scores

Weekly GRIX scores are shown in Figure 7-2 and Table 7-2. Patients reported an increase in xerostomia throughout radiotherapy. There was no significant difference (calculated using ANOVA) between each group for mean clinic GRIX score at any time point. Changes in scores from baseline to the end of treatment were compared between groups using linear regression adjusted for the baseline GRIX score. No relation was found (effect = -1.26, CI - 21.77 to 19.24, p value = 0.90)

Variable	Placebo (n=12)	Visco-ease (n=19)	p-value *
Baseline	13.5 (12.1)	11.5 (11.6)	0.6592
Week 2	17.5 (16.4)	15.3 (9.8)	0.6840
Week 3	23.2 (17.6)	31.6 (22.9)	0.2621
Week 4	34.7 (23.1)	44.4 (25.0)	0.2837
Week 5	38.3 (22.5)	50.8 (25.3)	0.1652
Week 6	49.8 (29.8)	53.6 (24.2)	0.7122
End of treatment	55.0 (30.8)	53.1 (24.0)	0.8631
* independent 2-sample t tests between the groups			

Table 7-2. Weekly Groningen radiotherapy-induced xerostomia (GRIX) scores. Data are mean (SD)

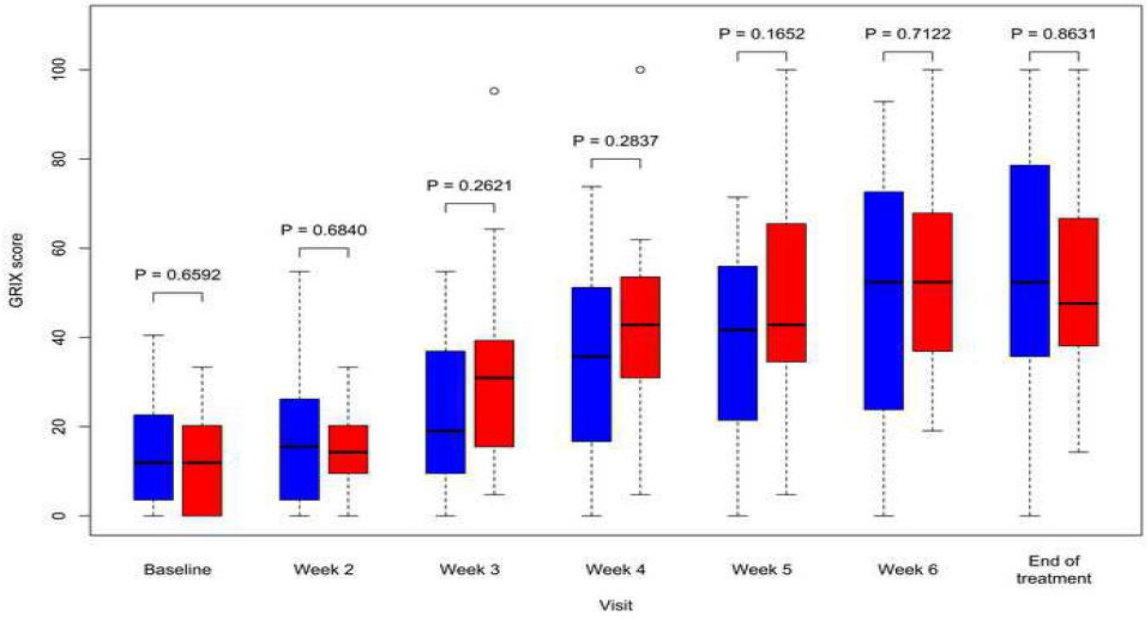

Figure 7-2. Weekly GRIX clinic scores (blue = placebo, red = Visco-easeTM). Acknowledgement CM Messow

7.4.4 Frequency of use of oral spray

The number of sprays used each day is shown in Figure 7-3 for each group. The number increased initially during radiotherapy but decreased again towards the end of treatment.

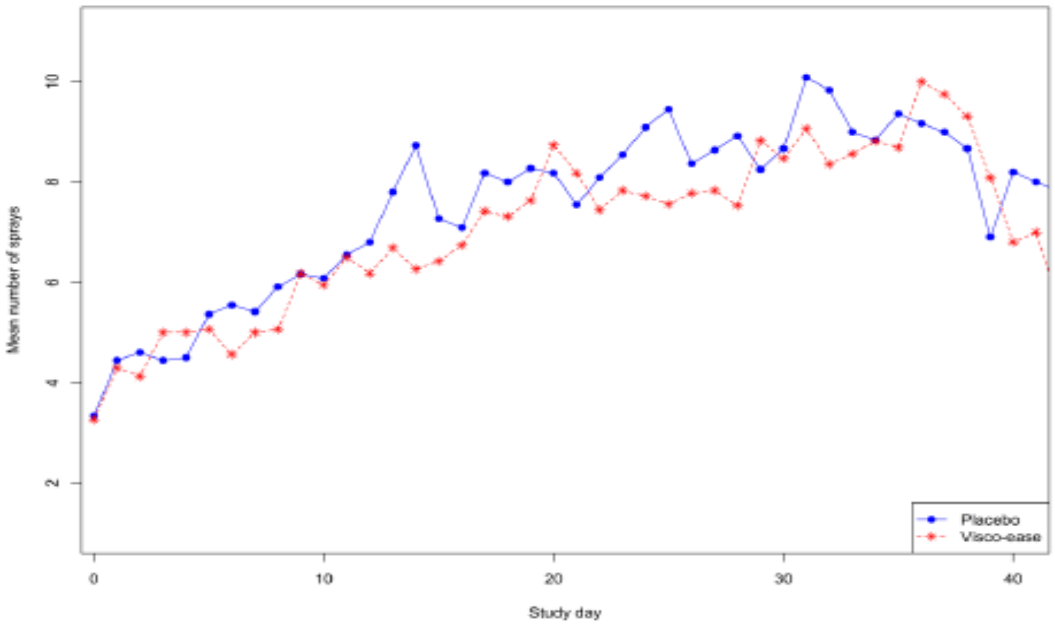

Figure 7-3. Number of sprays used per day.

Acknowledgement CM Messow

7.4.5 Safety endpoints

Serious adverse events that were "at least possibly device-related" were monitored throughout the study. There were none in either group. The number of participants with "at least one adverse event or serious adverse event" was compared between the groups as shown in Table 7-3. There was no significant difference between the groups in the percentage of participants affected. The relative high incidence of both reflects the serious acute toxicity experienced by these patients, and was anticipated.

Event	Placebo (n=14)	Visco-ease (n=25)	p-value
Non-serious AE	4	6	1.000
SAE	6	0	0.5145

Table 7-3. Comparison between patients who had at least one adverse event (AE) or serious adverse event (SAE) (APT).

7.5 DISCUSSION

The patients' characteristics were as expected for a group undergoing primary radiotherapy for cancer of the head and neck, and their xerostomia scores increased during treatment.

Previous work has shown a mean increase in GRIX score of 65.2 during six weeks of radiotherapy, [13] but the mean increase was 41.6 in the Visco-ease™ group in our study. What was unexpected, however, was a similar increase in the scores in the placebo arm of only 41.5. Laboratory work has already shown no efficacy of the placebo (physiological saline) on the visco-adhesive properties of RIX saliva. [13] The smaller-than-expected increase in GRIX scores in the placebo group may be the result of the placebo effect, and the subjective symptom of RIX could have been genuinely improved with the use of oral saline spray compared with no intervention.

Our findings confirm the importance of including a placebo for comparison when investigating new treatments for RIX. Some previous studies that have assessed interventions for RIX have not included a placebo arm.[18,19] Had the placebo group not been included we could have assumed that the smaller increase in scores compared with the historical controls was clinically significant, as they were around 36% less than those recorded previously in the same setting (41.6 compared with 65.2).

Comparison of the GRIX scores in both groups failed to show a significant difference at any time point. In particular the primary efficacy endpoint of a 30-point reduction in the score with Visco-ease™ compared with placebo was not met. This was partly because scores in the placebo group were lower than anticipated and, as four patients (one in the Visco-ease™ and three in the placebo group) did not develop RIX (as defined by not reaching a GRIX score of 30 or more at any point during the study), it was impossible for their scores to meet the primary efficacy endpoint.

The number of sprays used increased during the course of radiotherapy and decreased over the final one to two weeks of treatment. However, as we did not evaluate compliance formally, it is unclear if the reported numbers showed true compliance or a failure to record use as time went on. Good compliance in the early weeks suggests good tolerability.

There was no difference in the frequency of adverse events or serious adverse events between the groups, and no adverse event was related to the device. This first-in-man study of Visco-ease™ therefore has shown a safe toxicity profile.

It is disappointing that the study did not meet the primary efficacy endpoint of a significant reduction in RIX, and our results show some of the difficulties

involved in a study of a new intervention to treat it. It is widely accepted that patient-reported outcomes are the most important measure by which to judge the success of such an intervention [14] as physician reported scores or measures such as salivary flow do not necessarily correlate with the symptom experienced by the patient. [1, 20] Reports of RIX are therefore subjective,[14] making it a difficult metric to account for when designing a study such as this.

Our patients were currently being given radiotherapy for cancer of the head and neck. RIX is often considered to be a late or chronic side effect of the treatment but can also occur as an acute side effect during treatment. [13, 21] The acute group of patients were chosen from a safety perspective, as the device had not been tested in humans before and it was important to monitor its effects closely in those who were already attending hospital frequently. Patients who have completed radiotherapy generally attend monthly or less often as outpatients, and we thought that additional visits for safety monitoring would be an unjustified burden. Patients in the acute group are likely to have a considerably higher burden of symptoms (such as mucositis, dysphagia, skin reaction, pain, anorexia, weight loss, nausea, and vomiting) and a poorer QoL than those in the chronic group. [22] The modification of one acute symptom will probably make little difference to their QoL, as overall it will remain much poorer than it was at baseline. Now that we have shown the safety of Visco-ease™, further studies will be done in patients with established RIX after radiotherapy, as their symptoms are likely to be more stable. Its efficacy in those with chronic RIX will be the focus of future work.

During randomisation, the lack of stratification for any baseline characteristic (patients' characteristics, tumour, or treatment) resulted in a tendency for patients in the Visco-ease™ group to have stage III and IV cancer and for more to be treated by chemoradiotherapy. Unfortunately, given the nature of the withdrawals during the study, these imbalances were more pronounced at the end of treatment. Higher-stage disease will inevitably result in the treatment of larger volumes at higher doses and, compared with radiotherapy alone, concurrent chemoradiotherapy is well known to increase toxicity. [23, 24] It seems likely therefore that the symptoms in the Visco-ease™ group will have been worse than those in the placebo group, which may have skewed the results. Stratification for all potentially confounding variables in this study (age, concomitant medication, smoking/alcohol history, tumour stage, treatment; and

radiotherapy compared with chemoradiotherapy) would have meant that a larger sample size was required. We thought that this was inappropriate given that the study was the first to be done in humans.

Exploratory, post-hoc analyses were done after the initial results were examined and the difficulties described above were considered. All patients who failed to reach a GRIX clinic score of 30 or more were excluded. Multivariate regression showed that tumour staging, concurrent chemoradiotherapy, use of MST, and study treatment had the greatest influence on the scores. When using the restricted population and after adjusting for the potentially confounding covariates, compared to placebo, Visco-ease™ seemed to have a positive effect in reducing GRIX scores. This supplementary data will be used only to inform the design of future clinical studies and not to make any claims about efficacy. It does, however, suggest that a signal may be detected with an appropriately designed study, and this is worth investigating. An alternative formulation of Visco-ease™ - for example, an oral rinse rather than a spray, is also being considered for future studies.

7.6 POST VIVA ADDITIONS TO CHAPTER

The following section was added as correction to the thesis post viva, taking into account examiners' comments.

7.4.3 Patient-reported xerostomia scores

Weekly GRIX scores, shown in Figure 7-2 and Table 7-2, should be included for the intention to treat population rather than the per protocol population as per CONSORT guidelines. Reporting efficacy using the per protocol population, as was done here, may introduce bias and lead to spurious conclusions and should be regarded as a limitation of this work.

7.7 REFERENCES

1. Dirix P, Nuyts S, Van den Bogaert W. Radiation-induced xerostomia in patients with head and neck cancer: a literature review. Cancer 2006; 107:2525-34.

2. Bjordal K, Kaasa S. Psychological distress in head and neck cancer patients 7-11 years after curative treatment. Br J Cancer 1995; 71:592-7.

3. Dirix P, Nuyts S, Vander Poorten V, et al. The influence of xerostomia after radiotherapy on quality of life: results of a questionnaire in head and neck cancer.
Support Care Cancer 2008; 16:171-9.
4. Wijers OB, Levendag PC, Braaksma MM, et al. Patients with head and neck cancer cured by radiation therapy: a survey of the dry mouth syndrome in long-term survivors. Head Neck 2002; 24:737-47.
5. 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; 83:1007-14.
6. Vissink A, Mitchell JB, Baum BJ, et al. Clinical management of salivary gland hypofunction and xerostomia in head-and neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys 2010; 78: 983–91.
7. Mehanna H, Jones TM, Gregoire V, et al. Oropharyngeal carcinoma related to human papillomavirus. BMJ 2010; 340:c1439.
8. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus–positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008; 100:261-9.
9. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010; 363:24-35.
10. Goldstein DP, Hynds Karnell L, Christensen AJ, et al. Health-related quality of life profiles based on survivorship status for head and neck cancer patients. Head Neck 2007; 29:221-9.
11. Semple C. The role of the CNS in head and neck oncology. Nurs Stand 2001; 15:39-42.
12. Furness S, Worthington HV, Bryan G, et al. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev
2011;(12):CD008934.
13. Paterson C, Caldwell B, Porteous S, et al. Radiotherapy-induced xerostomia, preclinical promise of LMS-611. Support Care Cancer 2016;24:629-36.
14. Lovelace TL, Fox NF, Sood AJ, et al. Management of radiotherapy-induced salivary hypofunction and consequent xerostomia in patients with oral or head and neck cancer: meta-analysis and literature review. Oral Surg Oral Med Oral Pathol Oral Radiol 2014; 117:595-607.

15. Beetz I, Burlage FR, Bijl HP, et al. The Groningen Radiotherapy-Induced Xerostomia questionnaire: development and validation of a new questionnaire. Radiother Oncol 2010; 97:127-31.
16. Grégoire V, Coche E, Cosnard G, et al. 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 2000; 56:135-50.
17. R Core Team (2014). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from URL:
https://www.R-project.org/ (last accessed 26 March 2019).
18. Jacobs CD, van der Pas M. A multicenter maintenance study of oral pilocarpine tablets for radiation-induced xerostomia. Oncology (Williston Park) 1996; 10: 16-20.
19. Horiot JC, Lipinski F, Schraub S, et al. Post-radiation severe xerostomia relieved by pilocarpine: a prospective French cooperative study. Radiother Oncol 2000; 55:233-9.
20. 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; 12:127-36.
21. Tiwana MS, Mahajan MK, Uppal B, et al. Whole saliva physico-biochemical changes and quality of life in head and neck cancer patients following conventional radiation therapy: a prospective longitudinal study. Indian J Cancer 2011; 48:289-95.
22. List MA, Siston A, Haraf D, et al. Quality of life and performance in advanced head and neck cancer patients on concomitant chemoradiotherapy: a prospective
examination. J Clin Oncol 1999; 17:1020-8.
23. Cooper JS, Pajak TF, Forastiere AA, et al Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Eng J Med 2004; 350:1937-44.
24. Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med 2004; 350:1945-52.

8. Summary: Optimising radiotherapy outcomes for patients with head and neck cancer

8.1 Introduction

RT is well established as a radical, potentially curative treatment for patients with HNSCC. Advances in radiation technology have resulted in better survival outcomes, a decrease in the long-term toxicities of RT and improved quality of life. (Langendijk 2008, O'Sullivan 2012) However, further progress, both to improve disease control/survival, and minimise and manage toxicity remains an unmet need.

Strategies to achieve these aims have been evaluated in this work with approaches falling into two broad categories according to prognosis and a third category of managing toxicity which is cross cutting:

i. For patients with good prognosis HNSCC, strategies to safely de-intensify treatment have been evaluated

- Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection.
- Comparative cohort study of volumetric modulated arc therapy for squamous cell cancer of unknown primary in the head and neck-Involved neck only versus mucosal irradiation.

ii. For patients with poor prognosis HNSCC, individualised treatment escalation has been explored

- Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study

iii. Applicable to both prognostic groups are studies investigating the most common RT side effect, xerostomia (dry mouth).

- Feasibility of DW-MRI analysis of salivary glands during head and neck radiotherapy
- Radiotherapy-induced xerostomia, pre-clinical promise of LMS-611

- Radiotherapy-induced xerostomia: a randomised, double-blind, controlled trial of Visco-ease™ oral spray compared with placebo in patients with cancer of the head and neck

8.2 De-intensification of treatment

8.2.1 Omission of surgery

Zhou S, Chan C, Rulach R, Dyab H, Hendry F, Maxfield C, Dempsey MF, James A, Grose D, Lamb C, Schipani S, Wilson C, Cheng Lau Y, Paterson C. Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection. Oral Oncol. 2022 May; 128:105870. doi:0.1016/j.oraloncology.2022.105870. Epub 2022 Apr 18. PMID: 35447564.

Historically a neck dissection (ND) was planned before or after CRT for patients with HNSCC and advanced nodal disease (N2 or N3). The PET Neck study (Mehanna 2012) showed that PET-CT surveillance post CRT resulted in similar survival to those who underwent planned ND. However, the avoidance of planned surgery was only possible for patients who demonstrated a complete response (CR) on 12 week FDG PET-CT. All other patients (those achieving an equivocal (EQR) or incomplete response (ICR)) were still subject to multi-modality treatment with CRT then ND as per study protocol.

Since the inception of the PET Neck study, data has emerged that HPV-related tumours can take more than 12 weeks to involute completely and that 12-week FDG PET-CT has a low positive predictive value (PPV) for residual disease in those who achieve a less than CR (Huang 2013, Mirghani 2015, Bird 2016, Rulach 2019, Liu 2019, Urban 2020), especially in those with an EQR. Mehanna et al. acknowledged that the PET-Neck study population was not stratified for tumour HPV status, and therefore, FDG PET-CT at 12 weeks may overestimate the risk of residual disease (and therefore the need for ND) for the group with HPV-related disease. As ND post CRT has been shown to be associated with severe late toxicity (Machtay 2008) its omission is preferable, as long as this does not compromise disease control or survival.

With all these factors in mind, practice had already evolved after the publication of the PET Neck study, with some centres adopting a period of

further surveillance, rather than immediate ND (IND), in patients with HPV-related HNSCC and a less than CR on 12 week PET-CT. Other groups, however, continued to advocate IND for this group, regardless of tumour HPV status due to the lack of robust evidence to support the contrary strategy.

For the first time, my study demonstrated the long-term safety of the approach already adopted by several cancer centres internationally: the omission of an IND in patients with HPV-positive HNSCC achieving an EQR on 12-week FDG PET-CT. The survival outcomes reported are stratified by PET response. The EQR sub-group have no different survival to the group with a CR. This is despite IND being carried out in few cases, suggesting that IND is not needed to contribute to the good survival outcomes seen in the EQR group. A prospective randomised clinical trial to confirm this hypothesis is desirable but unlikely to be possible given that practice already takes account of this and factors described earlier.

This work was retrospective and subject to the limitations of this setting with possible imbalance between baseline patient/disease characteristics. Furthermore, the sample size was based on clinical experience and availability of data rather than a formal power calculation to ensure robustness of findings. However, selection bias is eliminated as all patients with node-positive HNSCC undergo post-treatment FDG PET-CT in my cancer network regardless of suitability for salvage treatment or any other imaging carried out. While sample sizes are modest when categorised into complete, equivocal, and incomplete nodal responses, the 2-year overall survival of 94.1% demonstrated in our cohort is comparable to that of the De-ESCALate study population (Mehanna 2019), suggesting our data is reflective of contemporary practice and outcomes. A prospective randomised clinical trial to assess long term outcomes with extended surveillance versus immediate neck dissections for those achieving an equivocal nodal response is desirable but unlikely to be feasible as extended surveillance has already been implemented into clinical practice in many centres. Without the prospect of being able to generate level one evidence, multi-centre validation of this strategy may be useful.
Further work is needed to standardise imaging protocols beyond the 12 week PET-CT for patients with a less than CR; currently modality and scheduling of subsequent imaging remains at the discretion of the local MDT. Soon to be

published UK guidelines, which update the 2016 edition (Simon 2016) and which I have co-written, will recommend that 'changes to the timing of post treatment imaging from three to four months for HPV+ve tumours has been proposed to reduce the rates of equivocal scans from involuting disease - known to be slower in HPV associated disease'. The combination of circulating biomarkers such as HPV DNA with post CRT PET-CT may allow a more refined strategy to identify patients with residual disease and work is ongoing in this area, with the multi-centre UK INOVATE study led by Dr Shreerang Bhide of the Royal Marsden Hospital due to report soon (Lee 2017).

I continue to investigate the use of post RT FDG PET-CT. I presented an oral abstract at the 2023 British Association of Head and Neck Oncologists (BAHNO) annual scientific meeting evaluating the positive predictive value of 6-month FDG PET-CT post radiotherapy in HPV- positive HNSCC. This work showed the PPV of post RT FDG PET-CT remains limited at 6 months, 7% for an EQR and 42% for ICR, suggesting that there is continued involution of HPV-positive HNSCC beyond 6 months.

A further manuscript examining the effects of HPV-status and treatment on the PPV of post-RT FDG PET-CT in HNSCC has recently been accepted for publication in Clinical Oncology (Zhou 2023). It demonstrated that the PPV of 12-week PET-CT is significantly lower for HPV-positive compared to HPV-unrelated HNSCC. It is poorer in patients with HPV-positive disease treated with CRT compared to RT alone.

8.2.2 Reduction in RT target volumes

Poon WY, Thomson M, McLoone P, Wilson C, Crosbie R, Schipani S, Grose D, James A, Lamb C, Rizwanullah M, Campbell F, Easton F, Paterson C. Comparative cohort study of volumetric modulated arc therapy for squamous cell cancer of unknown primary in the head and neck-Involved neck only versus mucosal irradiation. Clin Otolaryngol. 2020 Nov;45(6):847-852. doi: 10.1111/coa.13593. Epub 2020 Sep 17. PMID: 32501648.

In HNSCC with a confirmed, well lateralised, small primary tumour, unilateral RT has been shown to be safe and less morbid than bilateral RT with a reduction in both acute and late toxicity burden (Razavian 2023).

Conventionally, in HNSCCUP, potential mucosal sites and contralateral neck are treated with an elective dose of RT, and the ipsilateral neck is treated to a therapeutic dose. However, acute and late toxicity is of concern with this strategy. With the advent of IMRT, which allows some sparing of OARs and 'dose-painting' (treating different target volumes to different doses) there has been renewed interest in this approach with the hope that morbidity may be reduced compared to delivering comprehensive RT with conformal RT (Richards 2016). Nonetheless, a significant length of pharyngeal mucosa is treated to a dose of at least 50Gy meaning toxicity can be significant (Strojan 2013).
FDG PET-CT identifies the primary in approximately 40% of patients presenting with a head and neck cancer of unknown primary despite comprehensive evaluation with cross-sectional imaging and endoscopy (MacKenzie 2016). A true HNSCCUP in the PET-CT era is therefore a different entity to what was previously described, and the need to irradiate potential mucosal sites and contralateral neck must be questioned again. Furthermore, the biology of HNSCCUP has changed, such that an increasing proportion are associated with HPV infection. This group in particular carry an excellent prognosis and the avoidance of unnecessary toxicity is therefore highly relevant (Nieder 2001, Galloway 2015). Involved neck only (INO) RT avoids elective irradiation of potential primary sites and the contralateral neck. It is acknowledged however that this strategy is a 'departure from common practice' as numbers treated with this approach in the literature are generally outnumbered by those receiving RT to contralateral neck and/or mucosa (Galloway 2015).

For the first time, my paper has reported disease-related outcomes, dosimetry to OARS, and toxicities in patients investigated, diagnosed and treated for SCCUP with modern diagnostic and radiotherapy techniques. When compared with mucosal RT, INO RT resulted in statistically and clinically significant dose reduction to OARs. Significantly reduced rates of acute mucositis and dysphagia were experienced by this group as well as lower rates of late xerostomia during the first 12 months. Importantly, with a median follow up of 35 months, disease control was not compromised: the rate of mucosal emergence of the primary was not different between the two treatments. PFS and OS at 2 years were comparable between the 2 groups. The strength of the data is limited by its retrospective nature and any imbalance in patient/disease characteristics

between the two treatment groups which may confound results. The sample size was not decided via a formal power calculation, more on data availability which also limits the validity of findings. Clinical follow up with regards to toxicity was limited to a small number of patients with the remaining being lost to follow up which may also bias results.

While randomised evidence to guide practice remains optimal, it seems unlikely that this will ever be generated to guide future radiotherapy practice in HNSCCUP. EORTC 22205 was the only prospective randomised study of HNSCCUP. It was a phase III trial which compared comprehensive bilateral neck and mucosal radiotherapy to 50Gy, followed a 10Gy boost to the ipsilateral neck with ipsilateral neck radiotherapy to 60Gy alone. Planned accrual was 600 patients but the study closed after 2 years due to poor recruitment and no results have been reported. In the absence of high level evidence, confirmation of these findings in a larger, multi-centre cohort is desirable. To that end the author has developed a proposal which has been adopted by the National Oncology Trainees Collaborative for Healthcare Research (NOTCH). A national multi-centre project has been initiated, with members of the trainee collaborative collecting data on extent of radical radiotherapy volume and patient outcomes in patients with a diagnosis of HNSCCUP between 1/1/15 and 1/1/20 in the UK. Data from several hundred patients is anticipated and will generate significant evidence to guide future treatment strategies.

8.3 Individualised escalation of treatment

Paterson C, Allwood-Spiers S, McCrea I, et al. Study of diffusion weighted MRI as a predictive biomarker of response during radiotherapy for high and intermediate risk squamous cell cancer of the oropharynx: The MeRInO study. Clin Transl Radiat Oncol. 2017; 2:13-18. Published 2017 Jan 10. doi:10.1016/j.ctro.2016.12.003

Differences in survival between good and poor prognosis OPSCC are due to differences in loco-regional control (LRC) indicating treatment intensification to improve LRC may improve outcomes (Ang 2010).

While clinical variables such as tumour, node and metastases (TNM) staging and smoking history are prognostically robust they do not reliably predict response to

treatment. 6-7 weeks of radical (C) RT is delivered as standard for all locally advanced HNSCC. Disease response assessment is only reliable several weeks after treatment completion. Early prediction of outcome during RT is highly desirable and would allow adaptation of treatment based on response. There is currently no biomarker to predict treatment response during treatment and identification of the subset of patients that will benefit from more intensive treatment remains a clinical challenge.

Functional imaging parameters during treatment reflect changes in physiological activity and tumour radiobiology, potentially predicting response to treatment and long-term control. DW-MRI detects molecular diffusion i.e. the Brownian motion of water molecules. The apparent diffusion co-efficient (ADC) is the parameter that is used to quantify DW-MRI and it estimates the amount and speed of proton movement within the tissue (Koh 2007).

It is now established that solid tumour masses hinder diffusion and have low ADC values. Changes in ADC during RT have shown promise in predicting tumour control. A number of studies in HNSCC have shown that increases in ADC during treatment were significantly larger in those patients who achieved a complete response (Kim 2009, Vandecaveye 2010, King 2010, King 2013). The MeRInO study was the first to evaluate this in a single HNSCC sub-site, including only patients with poor prognosis OPSCC and is the largest study of this type.

The MeRInO study received ethical approval on 1st September 2015 and recruited 97 patients from June 2016 – September 2019. 77 patients were able to complete scanning requirements as per protocol, demonstrating the feasibility of obtaining DW MRIs during RT. The manuscript detailing the full results of my study is in preparation with submission to a peer reviewed journal planned for early 2024.

To implement adaptive dose-escalated RT, the feasibility of dose-escalation to disease but sparing of OARs must also be evaluated. This was previously difficult to achieve in HNSCC with conventional radiotherapy techniques because of the proximity of OARs to target volumes and increased risk of radiation-induced toxicity. However, with advances in radiotherapy technologies, such as intensity modulated radiotherapy (IMRT), highly conformal dose distributions and improved sparing of OARs can be achieved.

Pilot dosimetric work by the author and medical physics expert colleagues demonstrated a dose-escalation of 12.3% to gross tumour (GTV) was possible (79.8Gy EGQ2) with no clinically significant increase in dose to OARs (Grocutt 2022). Twenty representative patients who participated in the MeRInO study with high risk, locally advanced OPSCC, were re-planned retrospectively. The original clinical plans (65Gy/30#) were re-optimised then used to escalate the dose to the GTV in the 2nd half of treatment to a total of 73Gy. Comparisons were made between the 1) re-optimised 65Gy standard plans, 2) a plan sum of 32.5Gy in 15# and 40.5Gy in 15#. Despite the GTV being escalated by 12.3% to 73Gy, doses to all other target volumes remained within 1.3% of the standard dose plans. Furthermore, dose to OARs did not significantly increase when compared to the 65Gy plans, see table 8-1.

OAR	Dose constraint cGy	Dose with 65cGy/30# plan cGy (SD)	Dose with 73cGy/30# plan cGy (SD)
Ipsilateral parotid	Not applicable	3016 (823)	3170 (822)
Contralateral parotid	2400 mean	2148 (609)	2263 (628)
Upper midline mucosa	4000 mean	4327 (811)	4455 (816)
PRV spinal cord	4800 max	3638 (253)	3859 (257)
PRV brainstem	4800 max	3663 (554)	3712 (563)

Table 8-1.Dosimetry for OARs with standard and dose-escalated RT plans

The next step in this series of studies is to investigate DW MR guided, response adapted RT clinically. The author has developed a single centre study, **B**iologically **A**daptive **R**adi**o**therapy for **O**ropharyngeal Ca**n**c**e**r (BARitOne): An R-IDEAL stage 2a-2b technical optimisation, feasibility and safety study. Biologically adaptive RT is at an early stage of development in H&N cancer but is now a feasible clinical reality thanks to technological innovation and predicate studies. While the technologies and interventions proposed in BARitOne are not individually novel, the combined workflow is significantly different to the current paradigm. Prior to large scale implementation to allow randomised comparison to the current standard, technical optimisation and early proof of clinical effectiveness and safety must be robustly demonstrated. An R-IDEAL stage 2a (Development) and 2b (Exploration) study is therefore proposed. The

BAritOne study (REC Reference: 23/WS/0056, IRAS Project ID: 318567) received favourable ethical opinion from West of Scotland Research Ethics Committee 5 in May 2023 and the study is anticipated to open imminently at the time of writing (July 2023)

8.4 Predicting and managing toxicity – xerostomia

Radiotherapy-induced xerostomia is the most commonly reported late and permanent side effect of RT to the head and neck (H&N) (Dirix 2006). While technological advances such as parotid-sparing IMRT have resulted in modest improvements in observer-rated and patient-reported xerostomia (Nutting 2011), clinically significant xerostomia remains a problem for many patients (Little 2012, Vissink 2010).

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 (Makkonen 1986). This can cause discomfort, taste alteration, speech and swallowing difficulties, and accelerate dental caries (Wijers 2002).

8.4.1 Predicting xerostomia

Duffton A, Kemp O, Devlin L, Hay L, McLoone P, Paterson C. Feasibility of DW-MRI analysis of salivary glands during head and neck radiotherapy. Tech Innov Patient Support Radiat Oncol. 2021;19:46-51. Published 2021 Sep 4. doi:10.1016/j.tipsro.2021.07.002

There is a well-established relationship between radiation dose and adverse effects on normal tissues. Increased radiation dose results in increased permanent toxicity. This relationship has been well documented for xerostomia and salivary gland dose yet it is also acknowledged that xerostomia is multi-factorial (Beetz 2012, Eisbruch 1999). The possibility to identify intrinsic salivary gland factors that are associated with individual radiosensitivity may allow RT to be tailored, limiting the severity of normal tissue reactions, and improving patient's QOL

Functional imaging with serial quantification of tumour characteristics to predict treatment response was investigated in the MERINO study described above with a view to ultimately delivering biologically adaptive RT based on tumour response. Likewise, there may be an opportunity to measure physiological changes during RT in salivary glands or other organs at risk (OARs) and use these parameters to predict which patients are most at risk of severe toxicity. Again, biologically adaptive strategies may allow modification of RT for those at highest risk of long-term morbidity.

The first step in this potential new paradigm is to evaluate the feasibility of measuring any physiological changes in the salivary glands. This work demonstrated the feasibility of outlining the paired major salivary glands at baseline and during RT and measuring serial ADC on DW-MRI. On a small number of scans, it was not possible to delineate full parotid gland (PG) structures. This was due to the field of view being prioritised to include primary tumour and lymph nodes, meaning PG contours were incomplete. Going forward, image acquisition may need optimisation to ensure the field of view adequately allows both tumour and OAR evaluation. Whilst limited by the small number of patients included in the study, possible confounding patient/treatment factors and multiple testing of paired observations, this early data suggests that a lower rise in ADC during treatment is associated with more severe late xerostomia. This finding should be confirmed in a larger cohort but may represent an opportunity to identify patients at highest risk of severe toxicity and optimise RT to reduce dose delivered to salivary glands where possible.

8.4.2 Managing xerostomia

Paterson C, Caldwell B, Porteous S, McLean A, Messow CM, Thomson M. Radiotherapy-induced xerostomia, pre-clinical promise of LMS-611. Support Care Cancer. 2016 Feb; 24(2):629-636. doi: 10.1007/s00520-015-2823-5. Epub 2015 Jul 5. PMID: 26143037

Paterson C, Thomson MC, Caldwell B, Young R, McLean A, Porteous S, Clark S, Messow CM, Kean S, Grose D, Lamb C, Rizwannullah M, James A, Schipani S, Wilson C, Rulach R, Jones R. Radiotherapy-induced xerostomia: a randomised, double-blind, controlled trial of Visco-ease™ oral spray compared with placebo in patients with cancer of the head and neck. Br J Oral Maxillofac Surg. 2019

Dec;57(10):1119-1125. doi: 10.1016/j.bjoms.2019.10.300. Epub 2019 Oct 29. PMID: 31672256.

There is currently no effective topical treatment for radiotherapy induced xerostomia, 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' (Furness 2011). 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. These two studies investigated LMS-611, later known as Visco-ease™, in the ex-vivo and clinical settings and gathered data on patient reported xerostomia using the validated GRIX questionnaire.

The pre-clinical study demonstrated that saliva became more adhesive and viscous as RT progressed. However, the addition of LMS-611 (ex-vivo) at concentrations of 10 and 20 mg/ml reversed this change in visco-adhesive properties and restored its fluidity. It was therefore disappointing to find that in the clinical study comparision of GRIX scores between the 14 patients using placebo and 25 patients using Visco-ease™ sprays were no different. In particular the primary efficacy endpoint of a 30-point reduction in the score with Visco-ease™ compared with placebo was not met.
Looking more closely at outcomes in the observational, pre-clinical study may give some insights to this. 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 the reporting of xerostomia has also been previously documented in this setting (Franzen 1992, Lovelace 2014).The clinical study used a placebo for comparison. This was physiological saline, and had already been demonstrated to have no efficacy on the visco-adhesive properties of RIX saliva in the pre-clinical work. Yet there was a smaller-than-expected increase in GRIX scores in the placebo group of the clinical study. Patients' perception of RIX could have

been genuinely improved with the use of oral saline spray compared with no intervention. As a result comparison of the GRIX scores in both groups failed to show a significant difference at any time point. While it is widely accepted that patient-reported outcomes are the most important measure by which to judge the success of an intervention like this, reports of RIX are subjective (Lovelace 2014), making it a difficult metric to account for when designing a study such as this.

We also demonstrated in the pre-clinical study that there was no relevant correlation between patient-reported xerostomia and laboratory measurements of saliva properties. It is perhaps therefore unsurprising that an intervention that affects visco-adhesive saliva properties would not necessarily affect patient reported xerostomia. Lastly, the patient group evaluated in both studies were undergoing H&N RT and were experiencing significant acute toxicities associated with this (mucositis, dysphagia, skin reaction, pain, anorexia, weight loss, nausea, and vomiting) (List 1999). The modification of one acute symptom will probably make little difference to their QoL, as overall it will remain much poorer than it was at baseline.

The need for an effective topical intervention for xerostomia remains, further work is likely to be best carried out in the post-radiotherapy setting, where other toxicities may be less prominent and QOL scores more stable.

8.5 Conclusions

It is now evident that locally advanced HNSCC represents a spectrum of disease rather than a single entity, with variable response to standard (C) RT.

This series of papers has evaluated approaches which successfully de-escalate treatment for good prognosis HNSCC and lays the foundations for intensification of treatment for poor prognosis HNSCC.

Managing radiotherapy induced toxicity remains relevant for both prognostic groups and has also been explored.

I continue to work on studies which have led on from those described in the ‘De-intensification of treatment’ and ‘Individualised escalation of treatment’ sections.

The oral abstract I presented at the 2023 BAHNO annual scientific meeting investigating the positive predictive value of 6 month FDG PET-CT post

radiotherapy in HPV- positive HNSCC will be further developed with the intention of writing a full manuscript.

The data generated by the NOTCH study of RT practice in the UK for HNSCCUP is a major project for me to lead and may inform future RT practice and guidelines with significant interest already declared internationally.

The BaritOne study is now open and will be the focus of my prospective research efforts over the coming two years. It is important that this charity funded study recruits to time and target. As described above, the workflow proposed in the BARitOne study is significantly different to current protocols in all tumour types, where patients undergoing radical RT undergo several weeks of treatment, generally without modification. The principals learned in this study and optimised workflow will be applicable across a broad range of tumour sites and may ultimately be useful for toxicity reduction strategies as well as those targeting improved disease control. MR guided response adaptive RT is likely to be a prominent cross cutting theme in radiotherapy research in coming years and holds much promise in improving patient outcomes in clinical oncology. Early experience of this new paradigm will ensure that my institution is well placed to develop similar strategies in tumour types beyond head and neck cancer.

References

(For Introduction and Summary chapters only, see individual chapters for references as per publications)

Adelstein DJ, Ismaila N, Ku JA, et al. Role of Treatment Deintensification in the Management of p16+ Oropharyngeal Cancer: ASCO Provisional Clinical Opinion. J Clin Oncol. 2019;37(18):1578-1589. doi:10.1200/JCO.19.00441

Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363(1):24-35. doi:10.1056/NEJMoa0912217

Beetz I, Schilstra C, van der Schaaf A, 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(1):101-106. doi:10.1016/j.radonc.2012.03.004

Bell K, Licht N, Rübe C, Dzierma Y. Image guidance and positioning accuracy in clinical practice: influence of positioning errors and imaging dose on the real dose distribution for head and neck cancer treatment. Radiat Oncol. 2018;13(1):190. Published 2018 Oct 1. doi:10.1186/s13014-018-1141-8

Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med. 2004;350(19):1945-1952. doi:10.1056/NEJMoa032641

Bernier J, Cooper JS, Pajak TF, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck. 2005;27(10):843-850. doi:10.1002/hed.20279

Bird T, Barrington S, Thavaraj S, et al. (18)F-FDG PET/CT to assess response and guide risk-stratified follow-up after chemoradiotherapy for oropharyngeal

squamous cell carcinoma. Eur J Nucl Med Mol Imaging. 2016;43(7):1239-1247. doi:10.1007/s00259-015-3290-4

Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006;354(6):567-578. doi:10.1056/NEJMoa053422

Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival [published correction appears in Lancet Oncol. 2010 Jan;11(1):14]. Lancet Oncol. 2010;11(1):21-28. doi:10.1016/S1470-2045(09)70311-0

Brierly JD, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours, 8th edition. ISBN: 978-1-119-26357-9

Brotherston DC, Poon I, Le T, et al. Patient preferences for oropharyngeal cancer treatment de-escalation. Head Neck. 2013;35(2):151-159. doi:10.1002/hed.22930

Cooper JS, Pajak TF, Forastiere AA, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med. 2004;350(19):1937-1944. doi:10.1056/NEJMoa032646

Cooper JS, Zhang Q, Pajak TF, et al. Long-term follow-up of the RTOG 9501/intergroup phase III trial: postoperative concurrent radiation therapy and chemotherapy in high-risk squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys. 2012;84(5):1198-1205. doi:10.1016/j.ijrobp.2012.05.008

Demiroz C, Vainshtein JM, Koukourakis GV, et al. Head and neck squamous cell carcinoma of unknown primary: neck dissection and radiotherapy or definitive radiotherapy. Head Neck. 2014;36(11):1589-1595. doi:10.1002/hed.23479

Dirix P, Nuyts S, Van den Bogaert W. Radiation-induced xerostomia in patients with head and neck cancer: a literature review. Cancer. 2006;107(11):2525-2534. doi:10.1002/cncr.22302

Eisbruch A, Ten Haken RK, Kim HM, Marsh LH, Ship JA. Dose, volume, and function relationships in parotid salivary glands following conformal and intensity-modulated irradiation of head and neck cancer. Int J Radiat Oncol Biol Phys. 1999;45(3):577-587. doi:10.1016/s0360-3016(99)00247-3

Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100(4):261-269. doi:10.1093/jnci/djn011

Franzén L, Funegård U, Ericson T, Henriksson R. 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. 1992;28(2-3):457-462. doi:10.1016/s0959-8049(05)80076-0

Furness S, Worthington HV, Bryan G, Birchenough S, McMillan R. Interventions for the management of dry mouth: topical therapies. Cochrane Database Syst Rev. 2011;(12):CD008934. Published 2011 Dec 7. doi:10.1002/14651858.CD008934.pub2

Galloway TJ, Ridge JA. Management of Squamous Cancer Metastatic to Cervical Nodes With an Unknown Primary Site. J Clin Oncol. 2015;33(29):3328-3337. doi:10.1200/JCO.2015.61.0063

Garnaes E, Kiss K, Andersen L, et al. A high and increasing HPV prevalence in tonsillar cancers in Eastern Denmark, 2000-2010: the largest registry-based study to date. Int J Cancer. 2015;136(9):2196-2203. doi:10.1002/ijc.29254

Garnaes E, Kiss K, Andersen L, et al. Increasing incidence of base of tongue cancers from 2000 to 2010 due to HPV: the largest demographic study of 210 Danish patients. Br J Cancer. 2015;113(1):131-134. doi:10.1038/bjc.2015.198

Gebre-Medhin M, Brun E, Engström P, et al. ARTSCAN III: A Randomized Phase III Study Comparing Chemoradiotherapy With Cisplatin Versus Cetuximab in Patients With Locoregionally Advanced Head and Neck Squamous Cell Cancer. J Clin Oncol. 2021;39(1):38-47. doi:10.1200/JCO.20.02072

Gillison ML, Trotti AM, Harris J, et al. Radiotherapy plus cetuximab or cisplatin in human papillomavirus-positive oropharyngeal cancer (NRG Oncology RTOG 1016): a randomised, multicentre, non-inferiority trial [published correction appears in Lancet. 2020 Mar 7;395(10226):784]. Lancet. 2019;393(10166):40-50. doi:10.1016/S0140-6736(18)32779-X

Goldstein DP, Hynds Karnell L, Christensen AJ, Funk GF. Health-related quality of life profiles based on survivorship status for head and neck cancer patients. Head Neck. 2007;29(3):221-229. doi:10.1002/hed.20507

Grégoire V, Coche E, Cosnard G, Hamoir M, Reychler H. 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. 2000;56(2):135-150. doi:10.1016/s0167-8140(00)00202-4

Grégoire V, Levendag P, Ang KK, et al. CT-based delineation of lymph node levels and related CTVs in the node-negative neck: DAHANCA, EORTC, GORTEC, NCIC,RTOG consensus guidelines. Radiother Oncol. 2003;69(3):227-236. doi:10.1016/j.radonc.2003.09.011

Grégoire V, Eisbruch A, Hamoir M, Levendag P. Proposal for the delineation of the nodal CTV in the node-positive and the post-operative neck. Radiother Oncol. 2006;79(1):15-20. doi:10.1016/j.radonc.2006.03.009

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(1):172-181. doi:10.1016/j.radonc.2013.10.010

Grégoire V, Evans M, Le QT, et al. Delineation of the primary tumour Clinical Target Volumes (CTV-P) in laryngeal, hypopharyngeal, oropharyngeal and oral cavity squamous cell carcinoma: AIRO, CACA, DAHANCA, EORTC, GEORCC, GORTEC, HKNPCSG, HNCIG, IAG-KHT, LPRHHT, NCIC CTG, NCRI, NRG Oncology,

PHNS, SBRT, SOMERA, SRO, SSHNO, TROG consensus guidelines. Radiother Oncol. 2018;126(1):3-24. doi:10.1016/j.radonc.2017.10.016

Grocutt L, Paterson C, Valentine RM. Adaptive dose escalated radiotherapy in oropharyngeal cancers: a treatment planning feasibility study. Radiat Oncol. 2022;17(1):24. Published 2022 Feb 5. doi:10.1186/s13014-022-01991-x

Guerrero Urbano T, Clark CH, Hansen VN, et al. A phase I study of dose-escalated chemoradiation with accelerated intensity modulated radiotherapy in locally advanced head and neck cancer. Radiother Oncol. 2007;85(1):36-41. doi:10.1016/j.radonc.2007.07.011

Habbous S, Harland LT, La Delfa A, et al. Comorbidity and prognosis in head and neck cancers: Differences by subsite, stage, and human papillomavirus status. Head Neck. 2014;36(6):802-810. doi:10.1002/hed.23360

Herrero R, Castellsagué X, Pawlita M, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst. 2003;95(23):1772-1783. doi:10.1093/jnci/djg107

Huang SH, O'Sullivan B, Xu W, et al. Temporal nodal regression and regional control after primary radiation therapy for N2-N3 head-and-neck cancer stratified by HPV status. Int J Radiat Oncol Biol Phys. 2013;87(5):1078-1085. doi:10.1016/j.ijrobp.2013.08.049

Huang SH, Xu W, Waldron J, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. J Clin Oncol. 2015;33(8):836-845. doi:10.1200/JCO.2014.58.6412

Jordan RC, Lingen MW, Perez-Ordonez B, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. Am J Surg Pathol. 2012;36(7):945-954. doi:10.1097/PAS.0b013e318253a2d1

Junor EJ, Kerr GR, Brewster DH. Oropharyngeal cancer. Fastest increasing cancer in Scotland, especially in men. BMJ. 2010;340:c2512. Published 2010 May 11. doi:10.1136/bmj.c2512

Keller LM, Galloway TJ, Holdbrook T, et al. p16 status, pathologic and clinical characteristics, biomolecular signature, and long-term outcomes in head and neck squamous cell carcinomas of unknown primary. Head Neck. 2014;36(12):1677-1684. doi:10.1002/hed.23514

Kim S, Loevner L, Quon H, et al. Diffusion-weighted magnetic resonance imaging for predicting and detecting early response to chemoradiation therapy of squamous cell carcinomas of the head and neck. Clin Cancer Res. 2009;15(3):986-994. doi:10.1158/1078-0432.CCR-08-1287

King AD, Mo FK, Yu KH, et al. Squamous cell carcinoma of the head and neck: diffusion-weighted MR imaging for prediction and monitoring of treatment response. Eur Radiol. 2010;20(9):2213-2220. doi:10.1007/s00330-010-1769-8

King AD, Chow KK, Yu KH, et al. Head and neck squamous cell carcinoma: diagnostic performance of diffusion-weighted MR imaging for the prediction of treatment response. Radiology. 2013;266(2):531-538. doi:10.1148/radiol.12120167

Koh DM, Collins DJ. Diffusion-weighted MRI in the body: applications and challenges in oncology. AJR Am J Roentgenol. 2007;188(6):1622-1635. doi:10.2214/AJR.06.1403

Lacas B, Carmel A, Landais C, et al. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC Group. Radiother Oncol. 2021;156:281-293. doi:10.1016/j.radonc.2021.01.013

Lambert R, Sauvaget C, de Camargo Cancela M, Sankaranarayanan R. Epidemiology of cancer from the oral cavity and oropharynx. Eur J Gastroenterol Hepatol. 2011;23(8):633-641. doi:10.1097/MEG.0b013e3283484795

Langendijk JA, Doornaert P, Verdonck-de Leeuw IM, 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(22):3770-3776. doi:10.1200/JCO.2007.14.6647

Leclerc M, Maingon P, Hamoir M, et al. A dose escalation study with intensity modulated radiation therapy (IMRT) in T2N0, T2N1, T3N0 squamous cell carcinomas (SCC) of the oropharynx, larynx and hypopharynx using a simultaneous integrated boost (SIB) approach. Radiother Oncol. 2013;106(3):333-340. doi:10.1016/j.radonc.2013.03.002

Lee JY, Garcia-Murillas I, Cutts RJ, De Castro DG, Grove L, Hurley T, et al. Predicting response to radical (chemo) radiotherapy with circulating HPV DNA in locally advanced head and neck squamous carcinoma. Br J Cancer. 2017; 117: 876-883.

Leech M, Coffey M, Mast M, et al. ESTRO ACROP guidelines for positioning, immobilisation and position verification of head and neck patients for radiation therapists. Tech Innov Patient Support Radiat Oncol. 2017;1:1-7. Published 2017 Jan 5. doi:10.1016/j.tipsro.2016.12.001

Leong CH, Mohd Slim MA, Sabri H, Douglas CM, Montgomery J. Sociodemographics and survival characteristics of 253 human papilloma virus-related oropropharyngeal cancer cases in Glasgow, Scotland - A retrospective analysis. Clin Otolaryngol. 2021;46(4):854-860. doi:10.1111/coa.13732

Lewis-Jones H, Colley S, Gibson D. Imaging in head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;130(S2):S28-S31. doi:10.1017/S0022215116000396

Lingen MW, Xiao W, Schmitt A, et al. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncol. 2013;49(1):1-8. doi:10.1016/j.oraloncology.2012.07.002

List MA, Siston A, Haraf D, et al. Quality of life and performance in advanced head and neck cancer patients on concomitant chemoradiotherapy: a prospective examination. J Clin Oncol. 1999;17(3):1020-1028. doi:10.1200/JCO.1999.17.3.1020

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(3):1007-1014. doi:10.1016/j.ijrobp.2011.09.004

Liu HY, Milne R, Lock G, et al. Utility of a repeat PET/CT scan in HPV-associated Oropharyngeal Cancer following incomplete nodal response from chemo)radiotherapy. Oral Oncol. 2019;88:153-159. doi:10.1016/j.oraloncology.2018.11.033

Lovelace TL, Fox NF, Sood AJ, Nguyen SA, Day TA. Management of radiotherapy-induced salivary hypofunction and consequent xerostomia in patients with oral or head and neck cancer: meta-analysis and literature review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117(5):595-607. doi:10.1016/j.oooo.2014.01.229

Machtay M, Moughan J, Trotti A, et al. Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. J Clin Oncol. 2008;26(21):3582-3589. doi:10.1200/JCO.2007.14.8841

Mackenzie K, Watson M, Jankowska P, Bhide S, Simo R. Investigation and management of the unknown primary with metastatic neck disease: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;130(S2):S170-S175. doi:10.1017/S0022215116000591

Madani I, Duthoy W, Derie C, et al. Positron emission tomography-guided, focal-dose escalation using intensity-modulated radiotherapy for head and neck cancer. Int J Radiat Oncol Biol Phys. 2007;68(1):126-135. doi:10.1016/j.ijrobp.2006.12.070

Makkonen TA, Tenovuo J, Vilja P, Heimdahl A. Changes in the protein composition of whole saliva during radiotherapy in patients with oral or pharyngeal cancer. Oral Surg Oral Med Oral Pathol. 1986;62(3):270-275. doi:10.1016/0030-4220(86)90007-1

Mehanna H, Jones TM, Gregoire V, Ang KK. Oropharyngeal carcinoma related to human papillomavirus. BMJ. 2010;340:c1439. Published 2010 Mar 25. doi:10.1136/bmj.c1439

Mehanna H, Wong WL, McConkey CC, et al. PET-CT Surveillance versus Neck Dissection in Advanced Head and Neck Cancer. N Engl J Med. 2016;374(15):1444-1454. doi:10.1056/NEJMoa1514493

Mehanna H, Robinson M, Hartley A, et al. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial. Lancet. 2019;393(10166):51-60. doi:10.1016/S0140-6736(18)32752-1

Mehanna H. Update on De-intensification and Intensification Studies in HPV. Recent Results Cancer Res. 2017;206:251-256. doi:10.1007/978-3-319-43580-0_20

Miah AB, Bhide SA, Guerrero-Urbano MT, et al. Dose-escalated intensity-modulated radiotherapy is feasible and may improve locoregional control and laryngeal preservation in laryngo-hypopharyngeal cancers. Int J Radiat Oncol Biol Phys. 2012;82(2):539-547. doi:10.1016/j.ijrobp.2010.09.055

Mirghani H, Amen F, Tao Y, Deutsch E, Levy A. Increased radiosensitivity of HPV-positive head and neck cancers: Molecular basis and therapeutic perspectives. Cancer Treat Rev. 2015;41(10):844-852. doi:10.1016/j.ctrv.2015.10.001

Mentel A, Douglas CM, Montgomery J, McMahon J. External validation of OroGrams as a predictive model for overall and progression-free survival in Scottish patients with oropharyngeal squamous cell carcinoma: a retrospective cohort study. Br J Oral Maxillofac Surg. 2021;59(3):368-374. doi:10.1016/j.bjoms.2020.08.115

Mirghani H, Amen F, Blanchard P, et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: ongoing trials, critical issues and perspectives. Int J Cancer. 2015;136(7):1494-1503. doi:10.1002/ijc.28847

Mirghani H, Blanchard P. Treatment de-escalation for HPV-driven oropharyngeal cancer: Where do we stand?. Clin Transl Radiat Oncol. 2017;8:4-11. Published 2017 Nov 4. doi:10.1016/j.ctro.2017.10.005

Motz K, Qualliotine JR, Rettig E, Richmon JD, Eisele DW, Fakhry C. Changes in Unknown Primary Squamous Cell Carcinoma of the Head and Neck at Initial Presentation in the Era of Human Papillomavirus. JAMA Otolaryngol Head Neck Surg. 2016;142(3):223-228. doi:10.1001/jamaoto.2015.3228

Nieder C, Gregoire V, Ang KK. Cervical lymph node metastases from occult squamous cell carcinoma: cut down a tree to get an apple?. Int J Radiat Oncol Biol Phys. 2001;50(3):727-733. doi:10.1016/s0360-3016(01)01462-6

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(2):127-136. doi:10.1016/S1470-2045(10)70290-4

Nutting CM, Griffin CL, Sanghera P, et al. Dose-escalated intensity-modulated radiotherapy in patients with locally advanced laryngeal and hypopharyngeal cancers: ART DECO, a phase III randomised controlled trial. Eur J Cancer. 2021;153:242-256. doi:10.1016/j.ejca.2021.05.021

Nutting C, Finneran L, Roe J, et al. Dysphagia-optimised intensity-modulated radiotherapy versus standard intensity-modulated radiotherapy in patients with head and neck cancer (DARS): a phase 3, multicentre, randomised, controlled trial [published correction appears in Lancet Oncol. 2023 Aug;24(8):e328]. Lancet Oncol. 2023;24(8):868-880. doi:10.1016/S1470-2045(23)00265-6

Osazuwa-Peters N, Simpson MC, Zhao L, et al. Suicide risk among cancer survivors: Head and neck versus other cancers. Cancer. 2018;124(20):4072-4079. doi:10.1002/cncr.31675

O'Sullivan B, Rumble RB, Warde P; Members of the IMRT Indications Expert Panel. Intensity-modulated radiotherapy in the treatment of head and neck cancer. Clin Oncol (R Coll Radiol). 2012;24(7):474-487. doi:10.1016/j.clon.2012.05.006

O'Sullivan B, Huang SH, Su J, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncol. 2016;17(4):440-451. doi:10.1016/S1470-2045(15)00560-4

Perkins SM, Spencer CR, Chernock RD, et al. Radiotherapeutic management of cervical lymph node metastases from an unknown primary site. Arch Otolaryngol Head Neck Surg. 2012;138(7):656-661. doi:10.1001/archoto.2012.1110

Pignon JP, le Maître A, Maillard E, Bourhis J; MACH-NC Collaborative Group. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol. 2009;92(1):4-14. doi:10.1016/j.radonc.2009.04.014

Razavian NB, Shenker RF, D'Agostino RB Jr, Hughes RT. Association of ipsilateral radiation therapy with contralateral lymph node failure in patients with squamous cell carcinoma of the oral cavity: A systematic review and meta-analysis. Head Neck. 2023;45(8):1967-1974. doi:10.1002/hed.27421

Richards TM, Bhide SA, Miah AB, et al. Total Mucosal Irradiation with Intensity-modulated Radiotherapy in Patients with Head and Neck Carcinoma of Unknown Primary: A Pooled Analysis of Two Prospective Studies. Clin Oncol (R Coll Radiol). 2016;28(9):e77-e84. doi:10.1016/j.clon.2016.04.035

Rietbergen MM, Brakenhoff RH, Bloemena E, et al. Human papillomavirus detection and comorbidity: critical issues in selection of patients with oropharyngeal cancer for treatment De-escalation trials. Ann Oncol. 2013;24(11):2740-2745. doi:10.1093/annonc/mdt319

Rischin D, Young RJ, Fisher R, et al. Prognostic significance of p16INK4A and human papillomavirus in patients with oropharyngeal cancer treated on TROG 02.02 phase III trial. J Clin Oncol. 2010;28(27):4142-4148. doi:10.1200/JCO.2010.29.2904

Rulach R, Zhou S, Hendry F, et al. 12 week PET-CT has low positive predictive value for nodal residual disease in human papillomavirus-positive oropharyngeal cancers. Oral Oncol. 2019;97:76-81. doi:10.1016/j.oraloncology.2019.08.011

Semple C. The role of the CNS in head and neck oncology. Nurs Stand. 2001;15(23):39-42. doi:10.7748/ns2001.02.15.23.39.c2987

Shi W, Kato H, Perez-Ordonez B, et al. Comparative prognostic value of HPV16 E6 mRNA compared with in situ hybridization for human oropharyngeal squamous carcinoma. J Clin Oncol. 2009;27(36):6213-6221. doi:10.1200/JCO.2009.23.1670

Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17-48. doi:10.3322/caac.21763

Simon R, Homer J, Clarke P, et al. Follow-up after treatment for head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;130(S2):S208-S211. doi:10.1017/S0022215116000645

Sivars L, Näsman A, Tertipis N, et al. Human papillomavirus and p53 expression in cancer of unknown primary in the head and neck region in relation to clinical outcome. Cancer Med. 2014;3(2):376-384. doi:10.1002/cam4.199

Straetmans J, Vent J, Lacko M, et al. Management of neck metastases of unknown primary origin united in two European centers. Eur Arch Otorhinolaryngol. 2015;272(1):195-205. doi:10.1007/s00405-014-2934-5
Strojan P, Ferlito A, Langendijk JA, et al. Contemporary management of lymph node metastases from an unknown primary to the neck: II. a review of therapeutic options. Head Neck. 2013;35(2):286-293. doi:10.1002/hed.21899

Tiong A, Rischin D, Young RJ, et al. Unilateral radiotherapy treatment for p16/human papillomavirus-positive squamous cell carcinoma of unknown primary in the head and neck. Laryngoscope. 2018;128(9):2076-2083. doi:10.1002/lary.27131

Tribius S, Hoffmann AS, Bastrop S, et al. HPV status in patients with head and neck of carcinoma of unknown primary site: HPV, tobacco smoking, and outcome. Oral Oncol. 2012;48(11):1178-1184. doi:10.1016/j.oraloncology.2012.05.022

Urban R, Godoy T, Olson R, et al. FDG-PET/CT scan assessment of response 12 weeks post radical radiotherapy in oropharynx head and neck cancer: The impact of p16 status. Radiother Oncol. 2020;148:14-20. doi:10.1016/j.radonc.2020.03.032

Vandecaveye V, Dirix P, De Keyzer F, et al. Predictive value of diffusion-weighted magnetic resonance imaging during chemoradiotherapy for head and neck squamous cell carcinoma. Eur Radiol. 2010;20(7):1703-1714. doi:10.1007/s00330-010-1734-6

Van Dyne EA, Henley SJ, Saraiya M, Thomas CC, Markowitz LE, Benard VB. Trends in Human Papillomavirus-Associated Cancers - United States, 1999-2015. MMWR Morb Mortal Wkly Rep. 2018;67(33):918-924. Published 2018 Aug 24. doi:10.15585/mmwr.mm6733a2

Vissink A, Mitchell JB, Baum BJ, et al. Clinical management of salivary gland hypofunction and xerostomia in head-and-neck cancer patients: successes and barriers. Int J Radiat Oncol Biol Phys. 2010;78(4):983-991. doi:10.1016/j.ijrobp.2010.06.052

Wijers OB, Levendag PC, Braaksma MM, Boonzaaijer M, Visch LL, Schmitz PI. Patients with head and neck cancer cured by radiation therapy: a survey of the dry mouth syndrome in long-term survivors. Head Neck. 2002;24(8):737-747. doi:10.1002/hed.10129

Wilson JA, Carding PN, Patterson JM. Dysphagia after nonsurgical head and neck cancer treatment: patients' perspectives. Otolaryngol Head Neck Surg. 2011;145(5):767-771. doi:10.1177/0194599811414506
Yu Y, Lee NY. JAVELIN Head and Neck 100: a Phase III trial of avelumab and chemoradiation for locally advanced head and neck cancer. Future Oncol. 2019;15(7):687-694. doi:10.2217/fon-2018-0405

Zamani M, Grønhøj C, Jensen DH, et al. The current epidemic of HPV-associated oropharyngeal cancer: An 18-year Danish population-based study with 2,169 patients. Eur J Cancer. 2020;134:52-59. doi:10.1016/j.ejca.2020.04.027

Zhou S, Chan C, Lau YC et al. The Effects of Human Papillomavirus Status and Treatment on the Positive Predictive Value of Post Radiotherapy 18F-Fluorodeoxyglucose Positron Emission Tomography- Computed Tomography in Advanced Head and Neck Squamous Cell Carcinoma. Clinical Oncology, https://doi.org/10.1016/j.clon.2023.09.006