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Contemporary Assessment and Management of Head and Neck Cancer Surgical Margins

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1. Introduction

Head and neck cancer (HNC) is the sixth most common cancer worldwide,[1] which includes cancers of the aerodigestive tract, including lip, oral cavity, nasal cavity, paranasal sinuses, pharynx, larynx, oropharynx, hypopharynx, salivary glands, and local lymph nodes.[2] More than 90% of these are head and neck squamous cell carcinomas (HNSCC), arising from the mucosal lining in these regions.[3, 4]

Although oral squamous cell carcinomas (OSCC) can arise de novo from clinically normal appearing mucosa,[5, 6] they are typically preceded by clinically apparent changes in the tissue, termed oral potentially malignant lesions (OPML) and include leukoplakia, erythroplakia, oral submucous fibrosis, oral lichen planus and actinic keratosis.[7-9] Oral epithelial dysplasia (OED) is a histopathologic diagnosis that describes this tissue transformation and is characterised by cellular and morphological changes similar to those in OSCC but are limited to epithelial cells and remain non-invasive, hence termed premalignant or potentially malignant.[10] The histological grading system developed by the World Health Organisation is used widely to describe the degree of OED in oral mucosa – mild, moderate and severe dysplasia, and carcinoma in situ.[10] The histopathological diagnosis of OED, and its severity as interpreted by pathologists, is used as a predictor of a lesion's risk of malignant transformation, and also the type of intervention required – surgical treatment or watchful waiting.[11] However, a recent study found that this system was not useful for predicting patient outcomes or determining management strategies and recommended definitive treatment of all OED until a more reliable progression/transformation system is developed.[10] In addition, the presence of a non-homogeneous mucosal lesion has been shown to be a significant independent clinical indicator of underlying OED.[8]

Despite the reported transformation rate of 31.4% of OPMLs to OSCC,[12] clinical and histological characteristics have limited potential as predictors of transformation and do not aid in early diagnosis of HNSCC.[5, 13] It has been shown that as many as 50% of HNSCCs may arise from apparently clinically normal mucosa, thus posing an inherent diagnostic challenge.[5, 6] Although it is established that OPML and OED are statistically more likely to progress to cancer, the actual underlying mechanisms are poorly understood, and it is not inevitable that a dysplastic lesion will progress to cancer.[5, 6] Thus upon clinical diagnosis of HNSCC, the disease staging is often advanced with worsened prognosis.[5, 11]

The diagnostic process for OPML, OED or suspected HNSCC involves visual and tactile inspection using white light and other adjunctive visual aids, histopathological assessment of a biopsy sample, and one or more diagnostic imaging methods by radiography or molecular methods (positron emission tomography (PET), computerised tomography (CT), magnetic resonance imaging (MRI)).[14] All these approaches are necessary to aid in accurate tumour staging which directs therapeutic planning, and have to overcome significant challenges including delineation of tumour volume and accurate location, cervical lymph node involvement, distant metastasis, and presence of second primary tumours.[15] The main treatment modality for HNSCC, determined at the stage of diagnosis, continues to be surgical resection in combination with chemoradiotherapy depending on anatomical location.[16] Stratified treatment approaches exist based on HPV status. Newer treatment modalities involve drug/molecular targets used in conjunction with radionuclide tracers leading to personalised medicine.[14, 17] However, despite advanced techniques for early detection and management of HNSCC, the 5-year survival rate of smoking associated HNSCC is still 30-50%, with survivors experiencing poor quality of life.[4, 18] Overall, patients with advanced disease continue to have a poor prognosis and high locoregional and distant recurrences,[19] supporting the need for hybrid technologies both pre-, post-, and during surgery to attain maximum information in minimum time.

2. Surgical margin assessment

The aim of cancer surgery is to remove as much diseased tissue and retain as much healthy tissue as possible.[20] The key issue with surgical management of OSCC is predicting the risk of locoregional relapse, reported to occur in up to 20% of cases, accounting for ongoing modest survival rates.[21-24] One key predictor of locoregional relapse is the presence of carcinoma in or close to the surgical margins of the primary tumour, which is currently not reliably possible despite surgeon's conventional gross assessment (limited to white light tissue reflectance assessing colour and texture) and thorough histopathological examination, as relapse can occur in cases with clear margins.[19, 20, 24] Histopathological examination reports margins as clean/clear (>5 mm between carcinoma and margin), involved (carcinoma exists within 1 mm of the margin), or close (carcinoma exists between 1 to 5 mm from the margin). [24-26] Even though this method has a reported accuracy ratio of >95%, around 30% of patients with histologically negative margins undergo treatment failure raising concerns about its sensitivity.[19, 27-30] In addition, intraoperative histopathological assessment relies on the

quality of samples and degree of sampling, extends the time of operation and yields incomplete results.[20]

While in some cases histopathological examination shows tumour cells in the surgical margins thus implying that residual tumour cells could still exist in patients, most patients with local relapse have histologically clear surgical margins.[24] In these cases, relapse may be due to minimal residual disease (MRD) or field cancerisation.[24] In the case of MRD, small clusters of histopathologically undetectable tumour cells proliferate leading to local recurrence.[24] The field cancerisation model describes a field of premalignant epithelium, which may be rather expansive due to the process of lateral cancerisation, containing preneoplastic cells from which the primary carcinoma may have developed, and second primary tumours (SPT) can develop, following additional genetic hits.[24, 31] Differentiation between SPT and local recurrence due to MRD must be made where possible in order to determine appropriate therapeutic measures – while the latter may be treated with post-surgical radiotherapy or resection, treatment of the former is more complicated.[24] Surgery is not feasible due to the large extent of disease and radiotherapy may even be contraindicated as it could aid in the progression of preneoplastic cells into neoplasia, and thus more intensive surveillance during follow-up may be the best option.[24]

Most studies demonstrate an association between involved or close margins and a worse prognosis,[21, 32] with involved margins resulting in shorter disease-free survival,[21, 23, 32] and shorter overall survival.[33] The presence of close margins has prognostic significance, with a recent study finding margins at a cut-off of ≤1.6mm from the tumour to be prognostic of shorter disease-free survival and shorter overall survival.[32] However, molecular changes indicating early tumour development have been demonstrated in surgical margins of tumours from the larynx, pharynx, and oral cavity considered histologically 'normal'.[23, 34, 35] The rate of local recurrence (and thus failure of treatment) even in margins diagnosed as tumour-free is quoted in studies to be anywhere from 6.9-22%.[36]

It has been hypothesized that the majority of genetic alterations may occur during the early cancer progression process and can precede the observation of certain cytological changes.[37] It is thus believed that if given a reliable set of molecular or genetic biomarkers of epithelial transition/progression to malignancy, the subsequent removal of the altered tissue may prevent the future development of malignancy at that site.[38, 39] Such molecular biomarkers may also be used to assess the margins of tumours subsequent to surgical resection,[24, 40] allowing a means of objective assessment which may detect MRD, and predict potential for local recurrence in the surrounding tissues. Furthermore, molecular studies on genetic markers have shown there is a clonal relationship between the primary tumour and premalignant epithelium adjacent to the tumour,[24] suggesting that molecular analysis of histologically negative surgical margins may be a more sensitive method for detecting malignant trans-formed cells.[19] Ultimately we would like to propose a shift from conventional histopatho-logical assessment of surgical margins to molecular analysis, either through laboratory testing or imaging techniques.

3. Biomarkers in HNSCC

Since there is a need to reconsider methods of surgical margin assessment during SCC resection, margins should not only be examined macroscopically and microscopically, but also at a molecular level for dysregulated gene expression, which is also applicable in the diagnosis of OED and OPML. It is currently accepted that genetic and epigenetic changes within a clonal population of cells drives carcinogenesis by influencing oncogenes and tumour suppressor genes (TSG).[41-43]

Current modelling postulates that the development of cancer is driven by the accumulation of genetic and epigenetic changes within a clonal population of cells.[44] These genotypic alterations can affect hundreds of genes, leading to phenotypic changes in critical cellular functions such as resistance to cell death, increased proliferation, induction of angiogenesis, and the ability to invade and metastasize.[45] The mechanisms underlying these genetic and epigenetic aberrations can include genomic instability through chromosomal rearrangement, amplification, deletion, methylation, or mutation.[45]

These genetic alterations have been shown to contribute directly to cancer development and progression, and have a direct effect upon oncogenes and TSGs as well as the phenotypes they regulate.[41-43] There has been considerable investigation into the genotypic and phenotypic alterations observed in HNC,[42] and many studies have attempted to identify the genetic and molecular aberrations occurring in HNC surgical margins as a means of predicting local recurrence and relapse.

De Carvalho *et al.* examined 55 HNSCC patients undergoing operative therapy with histologically negative surgical margins and found 36.4% of these patients (20/55) showed overexpression of one of three genes they reported to being overexpressed in tumour samples – *MMP9*, *EPCAM* and *PTHLH*, with *MMP9* overexpression correlating with the risk of developing SPT.[19] Santhi *et al.* showed that both cytoplasmic and nuclear NF-κB proteins had a significant negative correlation from tumour to surgical margin to extra margin (2 cm away from the actual surgical margin), with COX-2 paralleling its expression, suggesting that these molecules are involved in tumour progression and may be used as markers in assessing MRD.[46] In a later study, they compared the expression microRNAs (miRs) in oral tumour progression and oral surgical margins.[47] They reported a decreased expression of I-miR-125a, I-miR-184, and I-miR-16 and an increased expression of I-miR-96 in the progression from normal mucosa to OED to OSCC, supported by the same pattern of expression retrogressing from extra margin samples to margin samples to tumour samples.[47]

Potential molecular markers for OSCC or OED include: protein markers (e.g. *TP53*,[35, 48-53] MMP9,[54] CDKN2A (p16),[49, 55-57] *EIF4E[58], epigenetic markers (promoter hypermethylation), {Sinha, 2009 #1258, 59-61]* microRNA expression (e.g. miR-16, miR-125a, miR-184),[47] DNA copy number changes, [50, 62-65] and loss of heterozygosity (e.g. 3p, 9p, 13q, 11q, 17p).[63, 66-68] Table 1 summarises studies that have investigated molecular markers with the potential to predict local relapse. Table 2 details the list of studies investigating the performance of molecular markers in HNSCC surgical margin analysis using a case (with relapse) and control (without local relapse) approach, with the development of local relapse being the end-point.

Study	Marker (analysis method)	Patients and Tumours	Samples studied	Results (number of positive/total)	Notes
Jin et al.[292]	TP53 mutation (PCR-SSCP)	Laryngeal carcinoma (n = 20)	Tumour-adjacent tissue histologically normal	5/20	
Cruz et al.[48]	TP53 mutation (IHC)	OSCC (n = 42)	Tumour-adjacent tissue non-malignant mucosa	7/42	
Tunca et al.[51]	TP53 mutation (PCR-SSCP and sequencing)	HNSCC (n = 15)	Surgical margin tissue	5/15	
Van der Toorn et al.[293]	TP53 mutation (IHC)	OSCC (n = 20)	Tumour-free surgical margin	11/20	
Blide et al.[49]	TP53 mutation, CDKN2A (p16), CHEK2, LAMA5 (via IHC)	OSCC (n = 16)	Tumour-free surgical margin	12/16 (TP53) 11/16 (p16) 1/16 (CHEK2) 0/16 (LAMA5)	
Shin et al.[294]	TP53 mutation (IHC)	HNSCC (n = 31)	Tumour-adjacent normal epithelium	6/31	
Van Houten et al. [94]	TP53 mutation (IHC)	HNSCC (n = 30)	Tumour-free surgical margin	19/30	Only margin samples with TP53 mutation in tumour were investigated
Nathan et al.[54]	TP53 mutation, 4E, MMP-9 (via IHC)	HNSCC (n = 52)	Tumour-free surgical margin	- 23/52 (TP53) - 27/52 (4E) - 28/52 (MMP-9)	
Tabor et al.[82]	Microsatellite (LOH)	HNSCC (n = 28)	- Tumour-adjacent non-malignant mucosa samples (n = 140) - Tumour-free surgical margins (n = 42)	- 10/28 - 7/28	
Szukala et al.[295]	Microsatellite (LOH at 13q)	Laryngeal carcinoma (n = 65)	Cancer-free surgical margin	8-20/65	
Poh et al.[68]	Microsatellite (LOH at 3p and 9p)	OSCC (n = 20)	Tumour-adjacent tissue, cancer free (n = 32)	15/32	
Breiger et al.[67]	Microsatellite at 3q26	HNSCC (n = 20)	- Biopsy 1cm from tumour (n = 20)	- 4/20 - 3/20	

Study	Marker (analysis method)	Patients and Tumours	Samples studied	Results (number of positive/total)	Notes
			- Biopsy 2cm from tumour (n = 20)		
Bremmer et al.[63]	Microsatellite (LOH at 3p, 9p, 11q and 17p), DNA ploidy, MLPA	HNSCC (n = 10)	Cancer-free surgical margin	- 10/10 - 4/10 - 10/10	Only margins with TP53 mutations were analysed
Martone et al.[55]	Promoter hypermethylation of MGMT, CDKN2A (p16), DAPK1	HNSCC (n = 11)	Cancer-free surgical margin	5/11 (MGMT) 3/11 (CDKN2A (p16)) 8/11 (DAPK1)	
Goldenberg et al. [34]	Promoter hypermethylation MGMT and CDKN2A (p16)	HNSCC (n = 6)	Surgical margins	3/6	Intraoperative margin analysis
Wong et al.[57]	Promoter-methylation CDKN2A (p16) and p15	HNSCC (n = 73)	Tumour-adjacent mucosa histologically normal (n = 29)	5/29 (CDKN2A (p16)) 18/29 (p15)	
Supic et al.[61]	Promoter hypermethylation of p16, DAPK, RASSF1A, APC, WIF1, RUNX3, E-cad, MGMT, hMLH1	OSCC (n = 47)	Tumour-adjacent mucosa histologically normal	44/47 (any marker) 27/47 (p16) 14/47 (DAPK) 17/47 (RASSF1A) 6/47 (APC) 19/47 (WIF1) 11/47 (RUNX3) 6/47 (E-cad) 7/47 (MGMT) 6/47 (hMLH1)	
Shaw et al.[296]	Promoter hypermethylation of p16, CYGB (via PMA)	OSCC (n = 20)	Deep margins histologically tumour-free	11/20 (p16) 17/20 (CYGB)	Possible contamination from adjacent tumour

Study	Marker (analysis method)	Patients and Tumours	Samples studied	Results (number of positive/total)	Notes
Roh et al.[60]	Promoter hypermethylation of p16, DCC, KIF1A, EDNRB (via qMSP)	HNSCC (n = 12)	Deep margins grossly tumour-free	8/12 (any marker)	
Kato et al.[72]	Promoter hypermethylation of p16, MGMT (via MSP)	OSCC (n = 51)	Tumour-adjacent mucosa histologically normal (n = 22)	6/22 (p16) 9/22 (MGMT)	
Barrera et al.[297]	Chromosome imbalance (Interphase-FISH)	HNSCC (n = 10)	Cell brushings of clinically normal tumour-adjacent margins	10/10	Possible contamination from adjacent tumour
Voravud et al. [298]	Chromosome imbalance (Interphase-FISH)	HNSCC (n = 20)	Epithelium adjacent to tumour, histologically normal	8/20	
Ott et al.[62]	Chromosome imbalance (Interphase-FISH)	HNSCC (n = 20)	Tumour-adjacent margins	Most cases/20 (any genomic change)	Various chromosomes targeted
Stafford et al.[64]	Chromosome imbalance (CGH)	HNSCC (n = 19)	Clinically normal tumour-adjacent mucosa	0/19	
Fabricius et al. [299]	Telomerase (DNA-PCR)	HNSCC (n = 40)	Tumour margin biopsy	13/40	
Preuss et al.[65]	DNA Ploidy	HNSCC (n = 20)	- Biopsy 1cm from tumour (n = 20) - Biopsy 2cm from tumour (n = 20)	Greater DNA irregularity at 1cm than 2cm	
De Carvalho et al. [19]	Expression of PTHLH, EPCAM, MMP-9,	HNSCC (n = 55)	Tumour-adjacent mucosa histologically normal	20/55 (any marker) 13/55 (MMP-9) 6/55 (EPCAM) 5/55 (PTHLH)	

Study	Marker (analysis method)	Patients and Tumours	Samples studied	Results (number of positive/total)	Notes
Graveland et al. [300]	LY6D (qRT-PCR)	HNSCC (n=55)	'Clean' or 'close' deep margins histologically tumour-free	12/55	
Dasgupta et al. [301]	Mitochondrial DNA (mtDNA) mutation	HNSCC (n = 50)	Histologically normal margins (n = 24)	17/24	Only margins with mtDNA mutation in tumour were assessed
Santhi et al.[47]	microRNA expression	OSCC (n = 84)	- Surgical margin tissues (histologically mild to moderate dysplastic) - Extra margin tissue (histologically normal) (n = 56)	- Increased expression (miR-125a, miR-184, miR-16) in margin vs. tumour - Decreased expression (miR-96) in margin vs. tumour	

Table 1. Molecular markers at HNSCC surgical margins with potential to predict local relapse

Study	Marker (analysis method)	Patients and margins	(Number of patients with positive molecular margins/total patients)	Sensitivity (positive margins/ number of cases)	Specificity (negative margins/ number of controls)	Significance	Notes
Brennan et al. [93]	TP53 mutation (Sanger sequencing)	HNSCC (n = 30) Evaluated (n = 25) Margins (n = 72)	13/25 (48%)	5/5 (100%)	12/20 (60%)	Yes (KM-logrank)	
Van Houten et al.[97]	TP53 mutation (Sanger sequencing)	HNSCC (n = 179) Evaluated (n = 76) Margins (4 to 5 per tumour, 3-4 superficial	50/76 (66%)	9/9 (100%)	25/62 (40%)	Yes (KM-logrank)	

Study	Marker (analysis method)	Patients and margins	(Number of patients with positive molecular margins/total patients)	Sensitivity (positive margins/ number of cases)	Specificity (negative margins/ number of controls)	Significance	Notes
		and 1 deep mucosal margin)					
Bergshoeff et al.[50]	TP53 mutation (IHC), Chromosome instability (CIN) (via Interphase-FISH)	OSCC (n = 20) Evaluated (n = 19)	8/19 (42%)	3/4 (75%) – TP53 4/4 (100%) - CIN	10/15 (67%) - TP53 11/15 (73%) - CIN	No – TP53 (Fisher-exact test) Yes - CIN (Fisher-exact test)	
Huang et al. [302]	TP53 mutation (Sanger sequencing)	OSCC (n = 58) Evaluated (n = 25)	16/25 (64%)	11/13 (85%)	7/12 (58%)	Yes (KM-logrank)	
Partridge et al. [35]	TP53 mutation (p53 phage plaque assay, immunocytochemistry, FASAY)	OSCC (n = 18) Evaluated (n = 11)	6/11 (55%)	4/5 (80%)	4/6 (67%)	Not performed	
Nathan et al. [110]	TP53 mutation (IHC), eIF4E (IHC)	Laryngeal carcinomas (n = 54)	6/54 (11%) - TP53 32/54 (59%) – EIF4	6/23 (26%) – TP53 21/25 (84%) – EIF4	31/31 (100%) – TP53 18/29 (82%) – EIF4	Yes (KM-logrank)	
Graveland et al.[52]	TP53 mutation (IHC), LOH (PCR), Ki-67 (IHC)	HNSCC (n = 35)	17/35 (49%) - LOH	11/16 (69%) – LOH (75%) – positive TP53 staining (62%) – positive TP53 with >5% total epithelium positive (62%) - LOH 9p present (88%) – LOH 9p and/or >5% TP53 staining positive	6/19 (31%) – LOH (47%) – positive TP53 staining (84%) – positive TP53 with >5% total epithelium positive (74%) - LOH 9p present (63%) – LOH 9p and/or >5% TP53 staining positive	Yes - LOH 9p and/or >5% TP53 staining positive (KM-logrank) No – Ki-67 (KM-logrank)	

Study	Marker (analysis method)	Patients and margins	(Number of patients with positive molecular margins/total patients)	Sensitivity (positive margins/ number of cases)	Specificity (negative margins/ number of controls)	Significance	Notes
Pena Murillo et al.[95]	TP53 mutation, Ly-6D (qRT-PCR)	OSCC (n = 142) Evaluable (n = 102) Carcinoma-free resection margins	51/102 (50%) – TP53 14/51 (27%) – Ly-6D	(92%) – TP53 (42%) – Ly-6D (70%) – Combined TP53 and Ly-6D	(56%) – TP53 (81%) – Ly-6D (70%) – Combined TP53 and Ly-6D	Yes – TP53 (KM-logrank) No – Ly-6D (KM-logrank)	46 cases received post-op radiotherapy Only wild-type TP53 positive margins were analysed for Ly-6D
Yi et al.[96]	TP53 mutation, Cyclin D1, eIF4E (ISH)	Laryngeal carcinoma (n = 115)	47/115 (41%) – TP53 34/115 (30%) - D1 35/115 (30%) - eIF4E	21/33 (64%) – TP53 17/33 (51%) - D1 28/33 (85%) - eIF4E	56/82 (68%) – TP53 65/82 (79%) - D1 75/82 (91%) - eIF4E	Yes (Chi-square)	
Nathan et al. [58]	eIF4E (IHC)	HNSCC (n = 65)	36/65 (55%)	20/22 (91%)	27/43 (63%)	Yes (KM-logrank)	
Ogbureke et al. [303]	Bone sialoprotein (BSP), Dentin sialophosphoprotein (DSSP), Osteopontin (OPN), MMP-9 (via IHC)	OSCC (n = 20) Surgical margins (histologically negative) (n = 200)	10/20 (50%) – BSP 14/20 (70%) – DSPP 14/20 (70%) – OPN 16/20 (80%) MMP-9	6/9 (67%) – BSP 8/9 (89%) – DSPP 7/9 (78%) – OPN 6/9 (67%) MMP-9	4/11 (36%) – BSP 6/11 (55%) – DSPP 7/11 (64%) – OPN 10/10 (100%) MMP-9	Yes (KM-logrank)	
Reis et al. [304]	4-gene signature of MMP-1, COL4A1, P4HA2, THBS2 (via qRT-PCR)	OSCC (n = 30) Margins (n = 136)	Not reported	Not reported	Not reported	Yes (KM-logrank)	All four genes were up-regulated in margins of patients with disease recurrence compared to those without recurrence.
Monteobugnol i et al. [305]	Ki-67 expression	OSCC (n = 42)	13/42 (30%) - High Ki-67 values	2/4 (50%) - High Ki-67 values	25/38 (65%) - High Ki-67 values	Yes (KM-logrank)	

Study	Marker (analysis method)	Patients and margins	(Number of patients with positive molecular margins/total patients)	Sensitivity (positive margins/ number of cases)	Specificity (negative margins/ number of controls)	Significance	Notes
			29/42 (69%) – Low Ki-67 values	1/4 (25%) - Low Ki-67 values	9/38 (24%) - Low Ki-67 values		
Sardi et al.[86]	Microsatellite analysis (MSI and LOH)	HNSCC (n = 41)	11/25 (44%)	7/8 (88%)	13/17 (76%)	Yes (KM-logrank)	
Temam et al. [306]	Microsatellite analysis (MSI)	HNSCC (n = 76) Evaluated (n = 26) Margins (n = 113)	7/26 (27%)	5/5 (100%)	19/21 (90%)	Yes (KM-logrank)	
Handschel et al.[307]	DNA ploidy	HNSCC (n = 40)	16/40 (40%)	14/20 (70%)	18/20 (90%)	Not reported	
Zhao et al. [308]	CD44v6, BIRC5 (survivin) (via IHC)	Laryngeal carcinoma (n = 146) Evaluated (n = 112)	35/112 (31%) CD44v6 44/112 (39%) BIRC5	20/41 (49%) CD44v6 26/41 (63%) BIRC5	56/71 (79%) CD44v6 53/71 (75%) BIRC5	Yes (univariate cox-proportional hazard)	
Schaaij-Visser et al. [309]	KRT4 (cytokeratin 4), CRNN (cornulin) (via IHC)	HNSCC (n = 46)	23/46 (50%) 23/46 (50%)	17/23 (74%) 16/23 (70%)	17/23 (74%) 16/23 (70%)	Yes (KM-logrank)	
Sinha et al.[56]	Methylation of CDKN2A (p16)	OSCC (n = 30)	13/30 (43%)	5/6 (67%)	16/24 (67%)	Yes (KM-logrank)	
Tan et al.[59]	Methylation of CDKN2A (p16, CCNA1, DCC	HNSCC (n = 42) Evaluated (n = 27)	11/27 (41%)	5/5 (100%)	16/22 (73%)	Yes (KM-logrank)	

Table 2. Performance of molecular markers in HNSCC surgical margin analysis of patients with (cases) and without (controls) local relapse [adapted and modified from Braakhuis *et al.*[24]

3.1. Epigenetic events

Unlike genetic alterations, epigenetic changes are heritable and potentially reversible.[69] Epigenetic changes refer to any heritable modifications in gene expression without alterations of the DNA sequence; they occur more frequently than gene mutations and may persist for the entire cell life and even for multiple generations.[43] The transcription of each gene may change from high-level expression to complete silencing, depending on the influence of the "epimutations" which interfere with the action of activators and suppressors on specific promoters in the chromatin context.[41] Epigenetic inheritance includes DNA methylation, histone modifications and RNA-mediated silencing.

Promoter hypermethylation is a well-documented mechanism for tumour-specific alteration of suppressor gene activity in human malignancy, including head and neck cancer.[70] In normal tissues, unmethylated cytosine is found in high densities in CpG islands; areas with high concentration of cytosine and guanine that map close to a promoter region in 40% of mammalian genes.[41] This unmethylated state is associated with a high rate of transcriptional activity; vital for maintaining TSG levels. Where hypermethylation of TSG occurs (via the enzyme DNA methyltransferase), stable transcriptional silencing of tumour suppressor activity occurs.[42, 69]

Studies have shown that methylation of the p16INK4a gene is a frequent event in primary HNC, with hypermethylation occurring in 50-73% of cases.[34, 71] In an analysis of 22 OSCC cases where paired cancerous tissues and the surrounding normal mucosa were simultaneously analysed, methylation of p16 and O6-methylguanine-DNA-methyltransferase (MGMT; a gene which produces a DNA repair enzyme essential for removing adducts caused by alkylating agents) were shown in 27-40% of specimen margins considered 'normal'.[72] In a recent study on the prognostic significance of tumour-related gene hypermethylation in cancer-free surgical margins of OSCC, Supic *et al.* selected a number of genes involved in a wide range of cellular processes for analysis.[61] These included cell cycle control (*p16*), apoptosis (*DAPK* and *RASSF1A*), *Wnt* signalling (*APC*, *WIF1* and *RUNX3*), cell–cell adhesion (*E-cad*), and DNA repair (*MGMT* and *hMLH1*).[61] Results showed that whilst DNA hypermethylation in histologically negative surgical margins is a frequent event, hypermethylation of p16 did not have prognostic significance.[61] Sinha *et al.* found that patients with positive molecular margins for p16 hypermethylation had a 6.3-fold increased risk of having local recurrence compared to patients with negative margins.[56] However, positivity of margins did not show any significant correlation with T classification, nodal status, histological presence or status of marginal dysplasia, or any other patient or tumour parameter.[56] However, in a recent systematic review and meta-analysis of literature, Rainsbury *et al.* concluded that based on 6 studies analysed, significantly better overall survival rates was seen in p16-positive oropharyngeal squamous cell carcinoma (OPSCC) tumours.[73] Differences in methodologies and cut-off points for analysis between the studies remain a limitation for analysis, and there remains inadequate evidence at this time to determine whether or not hypermethylation of p16 can be used as a biomarker for use in determination of clear surgical resection margins or to predict the risk of local relapse.

3.2. Loss of heterozygosity

Loss of heterozygosity (LOH) may occur when one copy of a polymorphic marker with two slightly different alleles is lost or amplified (allelic gain).[45] LOH in key chromosomal loci represents one of the more promising markers; consistently being identified as a potentially independent risk predictor, supported by data from several laboratories, including studies by Sidransky, Califano, Mao, Hong, Lippman, and Lee.[12, 74-78]

Califano and Sidransky developed genetic progression models based on their studies of gene alterations in squamous cell carcinoma of the head and neck.[79, 80] They reported LOH at 9p21, 3p and 17p13 in squamous hyperplasia, as well as LOH at 13q11, 13q21 and 14q31 in dysplasia, with loss of chromosomal region 9p21 being the most common genetic alteration in HNSCC (occurring in 70-80% of dysplastic lesions of the oral mucosa).[75, 79] Consensus has emerged that LOH at 3p and 9p provides evidence of the accumulation of genetic damage in potentially malignant lesions.[40, 81, 82] This has led to a number of investigations into the predictive value of LOH at these specific chromosomal loci in malignant risk of low-grade OED.[83-85] There is a general trend for lesions with greater disturbance in cellular architecture and organization to harbor more genetic alterations at 3p and 9p, however this is not noted in all studies. [40, 74, 76, 80]

Bremmer *et al.* implemented a range of genetic assays to screen for oral pre-malignant fields in histologically 'normal' mucosa, and concluded that LOH may be a valuable screening tool to detect oral pre-malignant fields in high risk patients.[63] The utility of LOH to evaluate risk of local recurrence in surgical margins was also noted by Sardi *et al.*[86] However, a study by Szukala *et al.* found no predictive value of LOH to determine risk of local relapse in laryngeal cancer patients, with a low frequency of LOH detected in collected 'clean' margin samples.[66]

The predictive and prognostic capacity of LOH at 3p and 9p to predict risk of transition from OED to malignancy has also been recently explored. A study by Zheng *et al.* in 2012 aimed to prospectively validate their retrospective "2000 LOH progression model" proposed by Rosin *et al.*[84, 87] Using a prospective cohort of 296 subjects with a histologic diagnosis of primary mild/moderate dysplasia, the authors first validated their original model for predicting progression to severe dysplasia, carcinoma in situ, or invasive cancer.[84, 88] High-risk (3p and/or 9p LOH) lesions were found to have a 22.6-fold increased risk of progression when compared with the low-risk (3p and 9p retention) lesions; findings consistent with the previous study.[84, 87] By further refining their model with the inclusion of two further markers (4q and 17p), prospective validation of the new model was performed. It demonstrated that low-grade lesions showing retention of 9p had approximately a 5% risk of progression over 5 years to severe dysplasia or more advanced disease.[87] This finding has important implications, as it could suggest that individuals falling into this category might not require aggressive treatment or monitoring despite having a histologic diagnosis of dysplasia. Conversely, a high-progression rate (approximately 65%) for high-risk lesions was found, and would suggest that this group should be aggressively monitored for clinical progression.[87]

From this study, a number of areas for future investigation arise. It is important to evaluate the capacity of LOH to predict risk of progression within the immediate surrounding field and

of secondary oral malignancy, given that a portion of HNSCC may not arise from the exact site of the visually distinguished pre-malignancy.[85, 88]

Ultimately, comparison amongst existing studies is hindered by methodological differences, adjustment for confounders, and controls. Whilst early evidence appears promising, the clinical utility of LOH in 3p and 9p as a predictive tool to screen for progression of OED at surgical resection margins still requires further long-term prospective validation and/or investigation.[45, 84]

3.3. p53 family

p53 is a TSG located on chromosome 17p13, and plays a major role in cell-cycle progression, cellular differentiation and DNA repair and apoptosis.[89, 90] Loss of p53 function impairs the regulation of cell cycle arrest and apoptosis, thus altering the ability of cells to respond to stress or damage (such as DNA damage, hypoxia, and oncogene activation).[89, 90] This can then lead to genomic instability, and the accumulation of additional genetic alterations.[91] Loss of p53 has long been implicated in early carcinogenesis, including HNSCC.[92]

Several studies have investigated the expression of p53 in HNSCC tumour resection margins. [24, 49] Three groups have used p53 mutation-specific probes to detect aberrant cells in the resection margins,[93, 94] with 100% sensitivity achieved in identifying the tumours that had a local relapse in two independent studies.[93, 94] However, the assays used had a relatively low specificity (40%),[94] and contamination of margin samples by mutated DNA derived from cells leaking from the tumour could not be excluded.[93, 94] In a recent study by Bilde *et al.*, immunohistochemistry was used to analyse surgical specimens from 16 consecutive OSCC patients with surgical margins deemed negative.[49] Histologically normal epithelium adjacent to oral carcinomas showed upregulation of both p53 and p16, but with very little overlap.[49] It was not possible to conclude whether the observed changes represented early malignant changes or simply a reaction to cellular stress.[49] In case-control comparisons using immunohistochemistry, p53 appears to have problems with respect to marker sensitivity, which may be due to the presence of mutations not resulting in protein overexpression.[83, 93, 95-97] Ultimately, there remains insufficient evidence to determine whether p53 alterations can be used as predictive markers to identify surgical margins at risk of local recurrence.

A number of studies have shown a correlation between p53 expression and early recurrence, risk for secondary recurrence, metastatic spread and more aggressive disease progression.[49, 98] Studies involving immunohistochemical staining for the p53 tumour suppressor protein, image cytometry of abnormal DNA content, and promoter methylation of the p16 tumour suppressor gene have all attempted to establish potential markers for malignant progression. [99-101] Inactivation of p53 has been associated with a reduction in post-surgical patient survival in OSCC.[102, 103] Suprabasal p53 staining was found to be correlated with increasing grades of dysplasia in a recent study by Vered *et al.*,[104] consistent with an earlier study by Bortoluzzi *et al.*[104, 105] However, Cruz *et al.*[99] did not find a correlation between grade of dysplasia and p53 expression, with Murti *et al.*[106] finding a similar level of p53 expression in biopsies of patients who did or did not progress to OSCC; thus concluding that p53 expression was not predictive of the risk of malignant transformation. p53, in combination

with p16INK4a and Ki-67 alteration, has been proposed as potential markers to define high risk leukoplakia, with further validation in larger sample sizes required.[107]

Due to the many differences in study design, methodology and laboratory techniques, there are currently conflicting reports regarding the value of p53 as a biomarker for the prediction of relapse in HNSCC surgical margins. There is yet to be sufficient validated evidence on its utility in adoption for predictive assessment of dysplastic progression.

The proto-oncogene eIF4E (eukaryotic initiation factor 4E) is a eukaryotic translation initiation factor.[108] eIF4E regulates the translation of cap-dependent mRNAs, and an aberrant increase in eIF4E shifts the balance in favour of translation of transcripts that promote cell proliferation and malignancy.[96, 108] eIF4E protein is commonly elevated in HNSCCs,[109] and its overexpression in surgical margins has been found in a number of studies associated with increased risk of local recurrence.[96] In an investigation into the prognostic value of p53 and eIF4E expression in laryngeal carcinoma surgical margins, Nathan *et al.* concluded that eIF4E overexpression appeared to be a more sensitive indicator of recurrence, and suggested that it may occur as an earlier event in the tumourigenesis process.[110] A recent study by Yi *et al.* investigating the prognostic value of p53, eIF4E and cyclin D1 in laryngeal carcinoma surgical margins found similar results, finding that eIF4E overexpression positivity of margins displayed a greater sensitivity than the other two studied factors.[96] Ultimately, further studies are still required to validate and assess the clinical utility of eIF4E in the surgical margin assessment of HNSCC.

Other genes in the p53 family have also been analysed, such as p63, p73 (both structurally and functionally related to p53) and CDK inhibitor (CDKI) p21. There is again insufficient data to determine the predictive value of p63 and p73 in the progression of dysplastic HNSCC lesions, and whilst general trends have been elucidated in the literature, there is no published data that correlates p63 or p73 expression with the prediction of progression to HNSCC.[104, 111, 112] There are conflicting reports on the expression of p21 in the progression of dysplasia in HNSCC, with Choi *et al.* concluding that whilst an increasing trend in p21 expression was detected in histological progression, there was no significant correlation or progression to OSCC.[113] Future studies aimed at assessing the p53 pathway as a whole may be beneficial to further explore the mechanisms of its deregulation in dysplastic progression to HNSCC.

3.4. microRNA

There has been increasing evidence of the role of non-coding microRNAs (miRs) in the regulation of fundamental processes such as cell cycle, differentiation and apoptosis; and by extension, the impact of their dysregulation on the process of carcinogenesis.[114-117] MiRs are single-stranded endogenous, non-coding RNA transcribed from DNA, ranging between 18 and 24 nucleotides in length. They have the ability to regulate expression of other genes on a post-transcriptional level through various processes by degradation or repression of target mRNA; influencing organ development, cell differentiation, proliferation, apoptosis and stress responses.[118, 119] Recent studies have suggested that miRs may also regulate mRNA targets through less stringent mechanisms, such as binding to non-complementary regions and binding to sites located within the coding regions of transcripts.[120] Given their pivotal

function as post-transcriptional regulators of gene expression, miRs affect almost every cellular process; and have been implicated in numerous disease types, including cancer.[116, 119, 121]

The role of miRs in cancer development was first established by Calin *et al.* in a study that reported a specific miR cluster (miR-15/16) was deleted and/or down-regulated in the majority of chronic lymphocytic leukemia (CLL) cases.[122] The link to cancer was further strengthened by the discovery that miR genomic positioning appeared to be non-random,[121, 122] and that a significant number of miR genes were located at fragile sites (unstable regions that have been shown to promote DNA instability in cancer cells) or genomic regions that have been linked to cancers.[123] *RAS*, *HMGA2*, and *MYC* oncogenes have been identified as let-7 targets, indicating significant tumour-suppressive importance for this family of miRs.[118, 123]

There have been many molecular studies investigating the expression and dysregulation of miRs in HNSCC.[13, 115, 116, 124-126] Using a candidate-gene approach, most have attempted to examine the role of expression and proposed targets of specific miRs in HNSCC cell lines compared to normal samples.[13, 125-128] The underlying process by which miR deregulation affects the process of transition from dysplasia to HNSCC has not yet been fully elucidated, with a main impediment being the multifactorial aetiology of HNSCC and wide heterogeneity of lesions. However, Zhang *et al.* reported that Dicer and Drosha, enzymes involved in processing miRNA, were upregulated in salivary gland pleomorphic adenomas.[129]

In HNSCC, Li *et al.* found an increased level of miR-21 expression that was negatively associated with low levels of tropomyosin 1 (TPM1) and phosphate tensin homologue (PTEN); TSGs that mediate apoptotic and cell-cycle events.[126] In the same study, miR-21 was found to facilitate anchorage-independent growth of HNSCC cells, partly through the down-regulation of TPM1.[126] This finding has been validated in further studies observing elevated miR-21 expression contribution to neoplastic phenotypes.[130, 131] It is pertinent to note that most studies to date investigating miR expression profiles in HNSCC have used cancer cell lines, and only a few have been in solid tumour samples.[13, 132] Cell lines may not reflect the miR profiles of solid tumours, as particular culture conditions and clonal selection may radically change miR expression.[128] Of the few studies which have selected specific miRs for further investigation using patient samples, Childs *et al.* showed that miR-21 was also found in neoplastic head and neck cells, and may have potential as a prognostic marker in HNC.[124]

To date, there are limited studies investigating the role of miRs in surgical margins.[47] Santhi *et al.* analysed 72 miRs reported to be differentially expressed in OSCC and detected decreased expression of miR-125a, miR-184 and miR-16 and an increased expression of miR-96 in both progressive oral mucosal samples and dysplastic surgical margin samples.[47] Langevin *et al.* found miR-137 promoter hypermethylation to be associated with poor overall survival in patients with HNSCC, but found no significant associations with surgical resection margin positivity[133]. Further studies are required to define a broader set of miR profiles within a wider range of surgical samples, and to correlate results with patient outcomes. Ultimately, there remains insufficient evidence to determine whether these alterations could be used as predictive markers to identify dysplastic progression to HNSCC.

Despite the increasing number of studies into miR expression in HNSCC, there remain few publications that have investigated the deregulation of miRs in the transition process from dysplasia to malignancy. In an investigation of miR pre-cursors in oral leukoplakia (OL), Xiao et al. found up-regulation of both miR-31 and its passenger strand (termed miR-31*).[134] miR-31* was negatively associated with recurrent/newly formed OL, and they hypothesized that miR-31* may play an important role during OL progression via the regulation of fibroblast growth factor 3 (FGF3).[134] This was consistent with miR expression profile findings in a prospective translational study by Lajer et al., who examined global miR expression in a series of consecutive tumours and biopsies obtained from patients with OSCC and OPSCC.[128] Of the one hundred and fourteen miRs differentially expressed between OSCC and normal epithelium, the upregulation of miR-31 and downregulation of miR-375 were found to be the most significant aberrations.[128] Thus there is evidence to suggest that the upregulation of miR-31 may be an early event in the transition process from dysplasia to OSCC; however, further elucidation of its role in the progression process and its predictive value still requires further investigation.

Ultimately, whilst early results of molecular prognostic indicators such as LOH and eIF4E appear promising, the routine use of these markers for HNSCC surgical resection margins assessment is yet to be validated. Further research is required which can ideally integrate the convenience of histopathology with the objectivity of molecular panel analysis, supported by a distinct outline of clinical parameters, baseline data, and sufficiently sizable homogeneous patient populations amenable to long-term follow-up.

4. Imaging techniques

It has now been established that molecular profiling of tissue changes enable clinicians to "visualise" more of the disease, indeed diagnose altered tissue early. While macroscopic changes may be detected under white light examination and tissue/cell level changes through histopathology, molecular dysregulation may be identified using special imaging techniques. While most current methods assess tissue in the plane parallel to the lesion, methods aiding assessment in the vertical cross-section (plane perpendicular to the mucosal surface) are required to detect lesions below the mucosal surface and evaluate submucosal tumour invasion.[135]

All optical imaging techniques detect and analyse backscattered photons from mucosa.[135] Visible light (400-700 nm) is used for conventional white light inspection, however shorter wavelengths in ultraviolet (UV) and longer wavelengths in the near-infrared (NIR) regions of the light spectrum can also be used for imaging. UV and blue light are absorbed by biomole-cules to produce fluorescence.[135] In order to detect targeted tumour cells, the tumour-specific signal must be significantly discriminated from the non-specific background signals, thus optimising the signal-to-background ration (SBR).[136] The visible light spectrum has relatively short penetration depths useful for imaging (<100 μm) as it is mostly absorbed by haemoglobin, and is significantly associated with a high level of nonspecific surrounding

signals, resulting in a low SBR.[135, 136] NIR is less susceptible to tissue scattering and haemoglobin absorption, yielding penetration depths >1000 µm through the mucosa and a high SBR, with an optical imaging window of about 650-900 nm in which the absorption coefficient is at a minimum.[135, 136]

Optical imaging techniques using Optical Fluorescence Imaging (OFI) and Narrow Band Imaging (NBI) reflect tissue changes at the microscopic and molecular levels. Optical Coherence Tomography (OCT) and Angle-Resolved Low-Coherence Interferometry (a/LCI) non-invasively provide information in the vertical and axial planes. Raman spectroscopy is a point detection technique based on the inelastic scattering of light, also enabling molecular histo-pathological examination. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are methods traditionally used to detect carcinoma and metastasis (hence staging), and assess treatment response, providing anatomical and physiological information. Positron emission tomography (PET) is a true form of molecular imaging, opening the door for drug delivery and molecular surgical guidance. Hybrid imaging methods, PET/CT and PET/MRI, offer the best of both these imaging approaches. All these methods, collectively termed "optical biopsy", are non-destructive in situ assays of mucosal histopathologic states using the spectral and spatial properties of scattered light to measure cellular and/or tissue morphology, providing an instantaneous diagnosis.[135, 137]

4.1. Optical imaging

4.1.1. Optical coherence tomography

Optical Coherence Tomography (OCT) is based on the principle of low-coherence interfer-ometry.[135] It provides high resolution (~1-20 µm) cross-sectional images of tissue *in situ*, higher than conventional ultrasound, MRI, or CT, and comparable to conventional histology but being non-destructive, it aids real-time surgical diagnostics and an "optical biopsy" of the tissue.[138] Initial success with this modality was with retinal pathology[139] and broncho-pulmonary diseases.[140] More recently, it has been deemed useful in diagnosing diseases of the oropharynx/larynx and other oral tissues.[138, 141, 142]

OCT is similar to ultrasound B-mode imaging except that OCT uses light instead of acoustic waves, measuring the echo time delay and intensity of backscattered light.[143] The system uses NIR light, split into reference and sample beams, and plots the back-reflected light from structures within the tissue against depth (up to 2-3 mm).[139, 143, 144] Since the velocity of light is extremely high, optical echoes cannot be measured directly by electronic detection, but instead uses low-coherence interferometry – the back scattered light waves interfere with the reference beam and this interference pattern is used to measure the light echoes versus the depth profile of the tissue *in vivo*.[144] OCT also uses fibre optic technology, allowing for low-profile imaging to be performed through small optical fibres attached directly to a scalpel, tissue probe, endoscope, or microscope.[144] The device is compact and portable.[144]

In healthy mucosa, the basement membrane can be easily identified at the junction of the bright lamina propria and the darker epithelium, which is lost in the presence of invasive cancer.[145] However, one study had inconsistent results, showing a deceptive change in the histological

layers when compared to conventional biopsy of oral lesions (various anatomical sites).[138] The authors also noted that OCT image analysis is unique, requiring special training, and associated with a wide range of variability when interpreting its parameters (mainly epithelium thickness and status of basement membrane).[138] The authors previously aimed to generate a bank of normative and pathological OCT data from oral tissues to identify cellular structures of normal and pathological processes, thus creating a diagnostic algorithm.[146]

While OCT is useful for clinical detection of OSCC and OPML,[147] it also has potential in evaluating surgical margins for MRD in HNSCC just as it has been proven useful in cancers of other tissues such as breast,[148, 149] skin,[150, 151] vulva,[152] and prostate.[153]

4.1.2. Angle-resolved low-coherence interferometry

Angle-resolved low-coherence interferometry (a/LCI) is a light scattering technique which isolates the angle scattering distribution from cellular nuclei at various tissue depths.[137] In doing so, it is able to provide biomarkers based on morphology that are highly correlated with the presence of dysplasia.[137] It measures the angular intensity distribution of light scattered by a tissue sample, quantifying subcellular morphology as a function of depth in the tissue. [137] For each depth layer, signatures from cell nuclei are extracted by collecting and processing the angular scattering signal using a Mie theory-based light-scattering model to produce measurements of average nuclear diameter with submicron-level accuracy.[137] Studies that have investigated the use of a/LCI have confirmed that neoplastic tissue transformation is accompanied by an increase in the average cell nuclei size,[137, 154-156] thus detecting potentially malignant lesions as well as malignant lesions. The diameter of a non-dysplastic epithelial cell nucleus is typically 5-10 μm, while dysplastic nuclei can be as large as 20 μm across.[157] When this is optimized to 11.84 μm for the classification of tissue health, a/LCI yields a sensitivity of 100%, specificity of 84%, overall accuracy of 86%, positive predictive value of 34% and negative predictive value of 100% in oesophageal epithelium *in vivo*.[137, 155] This technique has been studied in animal models, *ex vivo* human studies, and more recently in *in vivo* studies, predominantly associated with cases of Barrett's Oesophagus (which is associated with an increased risk of oesophageal adenocarcinoma) and oesophageal epithelium.[137] The system is portable and the probe can be used through the accessory channel of a standard endoscope, thus providing surgical guidance.[137]

a/LCI could have a role in assessing surgical margins in HNSCC by assessing size of nuclei in the margins although currently there are no studies that have investigated this.

4.1.3. Optical fluorescence imaging

The basis of optical imaging techniques is the ability of photons to travel through tissue and interact with tissue components.[158] Fluorescence is the property of certain molecules to absorb light at a particular wavelength and to emit light of a longer wavelength after a brief interval called fluorescence lifetime.[158] Fluorescence spectroscopy, a major form of optical imaging, is a non-invasive diagnostic tool that evaluates the biochemical composition and structure of tissue autofluorescence (AF).[159] It is relatively simple, fast, accurate, and can aid

in real-time cancer detection.[159] While microscopic imaging systems for intraoperative surgical margin assessment based on endogenous contrast or AF are useful, high resolution of the diseased tissue is limited to a small field of view, making it difficult to survey the entire surgical excision margin intraoperatively.[20] Extrinsic approaches are more effective, which use fluorescent dyes detected by probes.[20] The signals can also be integrated into the white light image, which enables real-time intraoperative visualisation.[20] OFI is advantageous and convenient as it can be used intraoperatively for surgical guidance in resecting malignant tissue and for pathological sampling.[160] Various devices implementing OFI, both commercially available as well as those developed by researchers, using visible light or NIR, with or without excitable dyes, have been investigated mostly in breast cancer,[160, 161] but is now being tested in HNSCC as well.[162-166]

Francisco *et al.*[159] recently showed that fluorescence spectroscopy could discriminate between oral mucosa, injury, margins, and areas of recurrence, using a homemade fluorescence spectroscopy system, at 406 nm wavelength [159] without using injectable dyes, and providing macroscopic visualisation of affected and unaffected tissue. The VELscope™ and Identafi™ are commercially available tools that use the principles of AF and tissue reflectance to discriminate between normal and abnormal tissue. These tools are described below in order to illustrate loss of AF (LAF) and diascopic fluorescence as indicators of tissue change, which provide the clinician with additional information aiding diagnosis. Miyamoto *et al.* investigated intraoperative molecular imaging (multispectral fluorescence images) to identify tumour extensions in a murine HNC model.[167] They reported 86% sensitivity and 100% specificity in the diagnostic accuracy analysis compared to histology, the gold standard. They also reported a 60-day improvement in survival rate when using molecular imaging during surgery, compared to standard surgery (37% versus 5% respectively). Thus fluorescence can be used both for diagnosis and for surgical guidance to improve patient outcomes.

The Visually Enhanced Lesion Scope (VELscope™; LED Medical Diagnostics Inc., Barnaby Canada) uses direct tissue AF to enhance oral mucosal abnormalities.[7] An external light source, in this case blue light excitation between 400-460 nm, is used to excite endogenous fluorophores (typically nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD)) in the oral epithelium and collagen cross-links in the underlying stroma, which absorb the extrinsic photons and emit lower energy photons which appear clinically as fluorescence.[168-173] Since each fluorophore is associated with specific excitation and emission wavelengths, changes in tissue architecture and concentrations of fluorophores (as in the case of mucosal abnormalities and neoplastic development) results in altered absorption and scattering properties of the tissue,[7] with decreased tissue AF being reported in OED and mucosal inflammation.[68, 169, 174] Under the VELscope™, normal oral mucosa appears pale green when viewed under a filter while abnormal tissue exhibits LAF and appears dark.

While the VELscope™ has assisted in the detection of OED and OSCC not visible by conventional oral examination (COE) warranting tissue biopsy and aiding in demarcating margins, [68, 175] clinicians have been advised to use the VELscope™ in conjunction with COE as LAF may also be displayed in tissues with mucosal inflammation.[176] Complete diascopic fluorescence, wherein tissues display normal fluorescence pattern with the application of

pressure, can differentiate inflammatory lesions from neoplastic lesions.[176] However, the challenge of completely blanching tissues and inter-operator variation in the interpretation of partial blanching (i.e. low specificity and variable sensitivity) grades the VELscope™ as a useful clinical tool for clinically visualising abnormalities but not an accurate discriminator of the condition of the mucosa under inspection.[176] Nevertheless, a recent clinical study suggested the use of a decision making protocol incorporating the VELscope™ in routine general dental practice allows for the detection of additional oral mucosal lesions requiring specialist referral.[177]

The Identafi™ (DentalEZ, PA, USA) is a multispectral screening device which uses direct fluorescence as well as tissue reflectance to visualise intraoral tissues by incorporating three different lights which are to be used sequentially.{#1120;Bhatia, 2013 #729} The light emitting diode (LED) white light enables superior visualisation of oral tissues but cannot differentiate between OPML and other more benign abnormalities of the oral mucosa, in a manner similar to that displayed by Microlux/DL™.[7, 178] Visualisation of oral mucosa under violet light (405 nm wavelength) through the accompanying photosensitive filter glasses, assesses the AF properties of tissue, with normal mucosa exhibiting natural fluorescence and abnormal tissues displaying LAF in a similar fashion to VELscope™.[7] Despite the dubious sensitivity and specificity of this wavelength of light,[173] areas of LAF visualised were often larger than what was clinically visible which might be due to the visualisation of deeper neovascularisation and stromal changes that accompany lesion progression, thus having a potential application in the determination of surgical margins for lesion excision.[163, 164] The green-amber light (545 nm wavelength) uses the concept of reflectance spectroscopy to characterise the connective tissue vasculature.[7] The process of carcinogenesis involves angiogenesis resulting in altered vascular morphology and it has been suggested that these tissue changes can be used to determine the prognosis of oral lesions, enabling the differentiation between benign lesions and OPML.[7, 179-181] Reflectance spectroscopy uses light within the absorption spectrum of haemoglobin (400-600 nm) which would reflect the degree of angiogenesis in the tissue. A significantly reduced reflectance spectra is observed in OSCC and OPML due to greater light absorption from increased microvasculature density and oxygenated haemoglobin content in neoplastic tissue.[7]

The underlying principles have enormous potential for application. Ongoing clinical trials by our group have shown excellent lesion visibility compared to COE under incandescent light. Violet light examination provided improved lesion visibility compared to COE, and improved visualisation of lesion borders and slight increase in lesion size compared to incandescent and white light. It also has a high level of clinical utility for evaluating inflammatory pathology. However, a high level of clinical experience is required to interpret the results of AF examination as the violet light displays low sensitivity for detection of OED. The green light helps uncover subtle vascular and inflammatory patterns providing additional clinical information.

Both these technologies highlight the usefulness of detecting LAF and diascopic fluorescence, however, additional information is required to diagnose the tissue change. Molecular studies in this area aid in our understanding of the phenomena of AF, LAF and diascopic fluorescence in oral tissues, enabling more informed use of such devices and superior interpretation of

changes in AF patterns. One study in oral cancer patients showed that all their tumour samples (confirmed by histopathology) had displayed LOH intraorally when a simple hand-held device, similar to the VELscope™, was used.[68] Molecular analysis in this study on margins with low-grade or no dysplasia showed a significant association between LAF samples and LOH at 3p and/or 9p, which is strongly associated with tumour recurrence after tumour removal.[68] Furthermore, this study found that LAF extended beyond the clinical visible lesion, and these areas displayed dysplasia/cancer on histology and/or genetic alterations associated with molecular risk, thus showing that the VELscope™ can distinguish between dysplasia and normal oral mucosa.[68]

4.1.4. Narrow band imaging

Narrow Band Imaging (NBI; Olympus Medical Systems Corporation, Tokyo, Japan) utilises the concept that the depth of light penetration is dependent on its wavelength to enhance mucosal surface texture and underlying vasculature.[182, 183] The spectral bandwidth of the filtered light is narrowed.[183] The system has two modes, white light and NBI.[7] In NBI mode, only blue light (400 – 430 nm) and green light (525 – 555 nm) are emitted in parallel which make blood vessels in the superficial mucosa appear brown, and the deeper larger vessels in the submucosa appear cyan.[183] Blue light (centred at 415 nm) penetrates shallowly and corresponds to the peak absorption spectrum of haemoglobin, while green light (centred at 540 nm) penetrates deeper.[183] In NBI mode, inflammatory lesions have an ill-demarcated border and can be differentiated from neoplastic lesions which appear as areas with scattered dark spots and a well-demarcated border.[184, 185] These scattered dark brown spots represent superficial blood vessels; interpapillary capillary loops (IPCL).[7] Visualisation of the vasculature, as well as the degree of dilation, meandering, tortuosity, and calibre of IPCLs all indicate the true extent of lesions and severity of pathology, thus guiding the position of biopsy and resection margins.[184, 186-188] Takano *et al.*'s[184] IPCL classification for oral mucosa is tabulated in Table 3. It has been recommended that lesions with Types III and IV IPCL patterns must always be biopsied.[189] The presence of keratinised tissue can pose a hindrance to optimal visualisation of the lesion itself.[7]

Gono *et al.*[183] used NBI in colonoscopy and upper gastrointestinal endoscopy and concluded that magnified NBI enhanced the capillary pattern and the crypt pattern on the mucosa, which are useful features for diagnosing early cancer.[190] Later, Muto *et al.* reported that carcinoma in situ at oropharyngeal and hypopharyngeal mucosal sites can be clinically recognised using magnified NBI endoscopy, confirming the usefulness of evaluating in situ angiogenesis in solid tumours in the head and neck region.[191, 192] Yoshida *et al.* validated the use of NBI with magnifying endoscopy in oesophageal lesions. [193] A case report by Katada *et al*. showed the usefulness of NBI combined with gastrointestinal endoscopy (GIE) in detecting OSCC in the floor of the mouth.[187] Further case reports and investigations also supported the use of NBI in the oropharynx,[194] nasopharynx,[195, 196] hypopharynx,[197] larynx,[198] and oesophagus.[199] Its use in determining tumour size and margins in gastrointestinal cancers of the bile duct,[200, 201] duodenal papilla,[202] stomach[203, 204] was also investigated, all with encouraging results boast-

ing higher sensitivities and specificities than the current detection method used at the time. All these studies hailed NBI as a method with improved detection and diagnostic accuracy of cancers in areas that are difficult to examine, and critically allowing for early diagnosis, having an impact on treatment options, quality of life and patient survival.[205] It was considered that NBI would develop into a useful tool in the future pre-, intra-, and post-operative endoscopic assessment of neoplastic lesions in the upper aerodigestive tract.[206]

Type	Description/Features
I	· Normal mucosa · Regular brown dots – when loops are perpendicular to the surface of the mucosa · Waved lines – loops are parallel · Study by Yang *et al.*[189] – 17% frequency of dysplasia in these lesions, hence remain cautious and utilise clinical judgement as well
II	· Non neoplastic and inflammatory lesions, but dysplasia present most of the time · Dilated and crossing IPCL pattern
III	· Non neoplastic lesions, but dysplasia is almost definitely present · Elongated and meandering IPCL pattern
IV	· Neoplastic lesions · Large vessels IPCL pattern destruction · Presence of angiogenesis

Table 3. Summary of IPCL classification for oral mucosa by Takano et al.[184]

In a multicentre, prospective, randomised controlled trial (n = 320), Muto *et al.*[207] found that NBI detected superficial cancer more frequently than white light imaging in both the head and neck region (100% vs 8%) and the oesophagus (97% vs 55%). They reported a sensitivity and accuracy of 100% and 86.7% in the detection of superficial cancer in the head and neck region using NBI, and 97.2% and 88.9% in the oesophagus, respectively.[207] Piazza *et al.*[208] showed that 27% (26 of 96) of patients with OSCC and OPSCC had a diagnostic advantage by applying NBI and high definition television (HDTV) compared to white light and HDTV. In a later study, [209] they concluded that NBI and HDTV were of value in defining superficial tumour extension, in the detection of synchronous lesions in the pre-/intra-operative settings, and in post-treatment surveillance for early detection of persistence, recurrence, and metachronous tumours.

Fielding *et al.* combined white light and AF to the bronchoscopic and laryngoscopic assessments of head and neck cancer patients, and reported improved sensitivity but low specificity, increasing the number of unnecessary biopsies.[210] A later study combined AF and NBI for the detection of mucosal lesions during panendoscopy in head and neck cancer patients, and reported higher specificity than when using AF or white light alone, thus directly impacting on patient management.[211]

A recently published systematic review by Vu & Farah[212] on the efficacy of NBI for detection and surveillance of OPML analysed data from a prospective cohort study by Piazza *et al.*,[209] and a retrospective cohort study by Yang *et al.*;[213] both of which aimed to evaluate the efficacy of NBI endoscopy compared to WL in oral mucosal examination. Vu & Farah concluded that based on available evidence, there is a demonstrable improvement in the ability of NBI visualization to stage tumours, assess margins and detect synchronous, metachronous and recurrent lesions compared to visualization using broadband WL.[212]

A prospective study by Nguyen *et al.* utilized white light (WL), NBI and AFAF to inspect the oral cavity, larynx/hypopharynx and bronchus of 73 patients with known HNSCC, patients with SCC of unknown primary origin, and surgically treated HNSCC patients requiring panendoscopy for suspected recurrent disease.[211] The authors found a significant improvement in the detection of moderate dysplasia or worse by NBI compared to WL, and that the combined use of AF and NBI had significant implications upon mapping and guiding the surgical resection borders of three assessed oral cases.[211] This study demonstrated increased specificity with NBI for the detection of mucosal lesions.[211]

While OFI and NBI can detect tissue and molecular changes in a localised region, imaging modalities such as Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) provide anatomical information, including nodal involvement and metastasis which influence staging and treatment protocol employed. Ultimately, multimodal imaging can provide additional diagnostic information than white light illumination or a single imaging modality alone.[214, 215]

Both CT and MRI involve 3D sectional imaging and have extremely high diagnostic value.[216] CT scans require ionising radiation (with shorter scan times) while MRI does not but has a longer scan time.[216] CT is currently the most commonly used modality for head and neck imaging, and can improve delineation of soft tissue pathologies with intravenously administered contrast media,[216] however MRI provides the most detailed view of soft tissues and is routinely used to visualise such tumours.[216]

4.2. Molecular imaging

Molecular imaging modalities have the potential to be indispensable in every aspect of cancer care, from early detection to staging, drug delivery, molecular surgical guidance and treatment response.[20, 217, 218] Oncological molecular imaging is defined as the non-invasive imaging of distinctive cellular and sub-cellular events in malignant cells.[20, 219] Molecular imaging probes target the production of genetically determined biomolecules by cancer cells by displaying these directly in or on individual malignant cells, in the extracellular matrix, or cells in the vicinity such as T cells, macrophages, dendritic cells, fibroblasts or endothelial cells.[158, 220, 221] For example, probes paired with positron emitters and novel target-specific anti-cancer drugs could be quantitatively imaged by PET, providing information on tumour biology, guiding drug development, and furthering personalized medicine.[14, 17] Diseased tissue may also be detected through this imaging modality based on hypoxia [222, 223] or pH changes.[17, 224] It is clearly useful to detect changes at the cellular and molecular level rather than rely on anatomical characteristics alone which is commonly the case at present.[20]

Tumours may be able to be characterised without biopsies or surgery, and allow for accurate staging, re-staging and drug response monitoring, paving the way towards true personalised medicine.[20] Molecular imaging modalities may also be used for intraoperative surgical guidance and evaluation of surgical margins, thus improving outcomes.[20]

4.2.1. Raman spectroscopy

Raman spectroscopy is a non-invasive technique that can analyse the molecular composition of a tissue, enabling surgeons to identify, examine and determine the quality of the tumour's molecular margins.[145] It is based on the phenomenon that intramolecular bonds cause light to scatter in a manner that is both measurable and predictable, albeit for a very short time constituting <1 part per million of the total reflected light.[145] Point detection techniques can be used to collect molecular information during endoscopy with optical fibre probes, and they have the potential to be extended to imaging.[135] Raman spectroscopy produces inelastic light scattering (returning photons have longer wavelength than the incident photons) and diffuse NIR photons (photons that return after several scattering events and are useful for measuring fine pathological structures) which aid molecular histopathologic examination.[135] It is performed by illuminating tissue with NIR photons that are absorbed by the vibrational/rotational nodes of molecular bonds associated with chemical functional groups specific to mucosal proteins, lipids, and nucleic acids. [135, 225, 226] Some of these photons are then inelastically scattered, forming detailed spectral patterns that can be reduced to the principal components using multivariate statistics. However, the Raman effect is much weaker than fluorescence and can be easily obscured by fluorescence from the tissue or optical fibre itself.[136]

Shim et al. demonstrated the use of CCD detector in collecting Raman spectra in vivo in the gastrointestinal tract.[227] Molckovsky and colleagues showed that Raman spectroscopy could be used to distinguish between adenomatous and hyperplastic polyps in the colon, with 100% sensitivity, 89% specificity, and 95% accuracy when used in vivo.[228] Haka et al. used Raman spectroscopy to examine breast tissue in vivo, and reported perfect sensitivity and specificity when using their diagnostic algorithm.[229] They highlighted the feasibility of using it for real-time intraoperative margin assessment during partial mastectomy surgery, which could be similarly used for intraoperative margin assessment in HNSCC cases. Stone et al. examined biopsy specimens of laryngeal mucosa using Raman spectroscopy and conventional histopathological analysis, and reported 92% sensitivity and 90% specificity for Raman spectra generated over 30 seconds in the diagnosis of invasive cancer (compared to reference spectra generated from histopathologically normal mucosa).[230] In membranous vocal cord specimens, Lau et al. reported 69% sensitivity and 94% specificity for invasive carcinoma using Raman spectra recorded over 5 seconds.[231]

Spatially offset Raman spectroscopy (SORS) has been shown to be an effective tool in recovering Raman spectra from up to several millimetres beneath the surface of turbid media.[232] Keller et al. found that, using source-detector separations of up to 3.75 mm, SORS can detect sub-millimetre-thick tumours under a 1mm normal layer, and tumours at least 1 mm thick can be detected under a 2 mm normal layer using the Monte Carlo simulation model of breast

tumour margin analysis.[232] Other recent developments within Raman spectroscopy include surface enhanced Raman spectroscopy (SERS), coherent anti-Stokes Raman spectroscopy (CARS), and stimulated Raman scatters (SRS),[165, 233] which could all have applications in HNSCC margin analysis. Visualising molecular information using Raman spectroscopy has also been shown to aid in identifying patients with prostate cancer who are at risk of cancer progression from those with no evidence of disease.[234]

Raman spectroscopy provides an objective analysis of the tissue's molecular structure compared to the *ex vivo* histopathological analysis and grading based on tissue morphology. It may provide a more clinically relevant measure of the tumour margin on which to guide surgical excision. It has been possible to stage and grade malignancies from a spectral measurement on the surface of bladder tissue using Raman spectroscopy.[235] Representative reference spectra need to be developed by analysing a large cohort of histologically diagnosed mucosal lesions, against which spectra captured *in vivo* can be compared and leading to algorithms that can quickly produce a diagnosis.[145]

4.2.2. Positron emission tomography and hybrid technologies

PET provides a 3-D image of the functional processes in the body, wherein (18F)-fluorodeoxyglucose (18F-FDG), a glucose analogue, is commonly used as the radiopharmaceutical delivering the positron-emitting radionuclide (tracer), thus reflecting tissue metabolic activity by regional glucose uptake, with cancer cells exhibiting increased use of glucose.[20, 236] 18F-FDG PET highlights metabolic differences between malignant and healthy cells and is the first true molecular imaging modality.[20] A hand-held PET probe to detect high-energy gamma rays during breast cancer surgery has been developed for intra-operative evaluation of tumour localisation and margin status.[162] PET is limited in its use due to high cost, use of ionising radiation, and relatively low spatial resolution (it is difficult to detect small tumours (<1 cm) using this hand-held probe).[20, 136] Since PET on its own provides low anatomical information, it is commonly used in conjunction with CT, and more recently, with MRI which has the advantage of greater soft tissue contrast and fewer artefacts.[14] The PET/MRI hybrid imaging technology combines the functional sequences of MR with the molecular information of PET to provide information about tumour biology and microenvironment [237] – hence the best of both worlds.

A number of studies have evaluated the effectiveness of PET/CT versus PET/MRI in HNSCC. Many studies have found no significant difference between diagnostic capability and anatomic localisation of lesions as detected by both modalities,[238-243] however, there is agreement for tailored use of PET/MRI in the head and neck region since higher soft tissue contrast would aid in diagnosis.[238, 242, 244, 245] Kanda *et al.* found that PET/MRI and PET/CT had equal sensitivities and specificities, and both these were more sensitive compared to MRI alone but less specific, when investigating these modalities in HNSCC.[244] Kubiessa *et al.*[239] showed that PET alone had the highest sensitivity while MRI alone showed best specificity when comparing PET/CT to PET/MRI in patients with HNSCC. Evangelista *et al.* evaluated contrast-enhanced CT (ceCT) with PET in HNSCC cases and found this to be particularly adept at diagnosing small lymph node metastases in positions usually difficult to interpret in the head

and neck region.[246] Lee *et al.* found PET/MRI to have acceptable accuracy in T staging compared to endoscopic ultrasound and even higher accuracy (although not statistically significant) than PET/CT in predicting N staging, demonstrating its value as preoperative diagnostic imaging tool in HNSCC.[245] PET/CT has been deemed useful in staging, identification of second primaries, and monitoring in the head and neck region aiding treatment planning.[216, 236, 244, 245, 247]

Hybrid technologies can be used to assess treatment response.[246, 248, 249] Adkins *et al.* found that PET/CT is better than CT in evaluating tumour response to cetuximab in patients with incurable HNSCC.[248] PET/CT has also exhibited high sensitivity to detecting distant metastases, assessing the response to chemotherapy or chemoradiation treatment and in predicting outcome.[246] PET/CT can be used post-therapeutically to assess treatment response, detect residual/recurrent tumours, and exclude distant metastases.[15] "Activatable molecular probes" or "smart" probes may be used to elicit a change in signal upon enzymatic activity or in response to specific biomolecular interactions.[20, 220] These allow very high signal-to-background ratios compared to conventional targeted contrast agents, and lead to the possibility of imaging intracellular targets.[220] Nguyen and Tsien summarise that the use of these fluorescent-labelled molecularly targeted probes would provide real-time, intraoperative distinction of the molecular edge between cancer and adjacent normal tissue, and could aid in discerning and preservation of vital structures such as nerves during surgery.[218] Bhatnagar *et al.* reviewed the use of different PET tracers in hybrid imaging modalities such as PET/CT and PET/MRI to detect and monitor biomarker status to assess effectiveness of therapy in patients with HNSCC.[250] They suggest that treatment strategies could be modulated and adapted by comparing an initial baseline measurement of biomarker expression before treatment to measurements taken during therapy, thus strategically addressing treatment response.[250] Some examples of biomarkers that may be used in this instance are tissue hypoxia, cell proliferation and apoptosis, and epidermal growth factor receptor (EGFR) status.[250] Examples of PET tracers that may be used in relation to biomarkers of response to therapy are described below.

4.2.3. Tumour hypoxia

Hypoxia has been established as an indicator of poor prognosis in HNSCC patients, causing radiation resistance in tumour cells by preventing irreversible damage to DNA by free radicals induced by ionising radiation (oxygen is required for the production of free radicals), thus allowing DNA repair and tumour cell survival.[251, 252] The critical partial pressure of oxygen below which solid tumours resist radiation therapy is about 10-15mm Hg.[252] In comparison, three times the amount of radiation needed to kill tumour cells in normoxic conditions is required to achieve the same in hypoxia. Hypoxia mapping can be performed with the use of molecular imaging to identify tumours that would benefit from hypoxia-reducing treatments.[250]

Fluoromisonidazole (FMISO) has been investigated widely as a PET imaging agent in HNSCC.[253-256] It has been shown that FMISO and FDG uptake do not necessarily correlate, thus representing different tumour properties, with high uptake of FMISO before

radiation therapy indicative of locoregional treatment failure and associated poor prognosis. [250, 255, 256] Nevertheless, FMISO may be used in HNSCCs to delineate hypoxic tumour volumes as an indicator to escalate radiation doses.[257-260] The key challenge though is that tumour hypoxia is a dynamic process with a constant change in relative contribution of acute and chronic hypoxia to the total hypoxic volume.[250] One study showed just 46% correlation between two sequential FMISO scans, just 3 days apart, in 20 HNSCC patients. [261] A smaller study with 7 HNSCC patients found correlation between hypoxic volumes on sequential scans in only three patients.[262] Further research is required to investigate the normal variation in FMISO uptake and changes in tumour oxygenation kinetics prior and during therapy, before FMISO imaging can clinically guide hypoxia-mediate intensity modulated radiation therapy (IMRT).[250, 259, 263]

Fluorine 18 fluoroazomycin arabinoside (FAZA) is also a hypoxia-specific PET agent that clears the blood more rapidly than FMISO, thus producing a higher target-to-background signal ratio.[264] Fluorine 18 fluoroerythronitroimidazole (FETNIM) is in theory a stronger indicator of hypoxia than FMISO due to its greater hydrophilia and better pharmacokinetics.[265] Both agents show promise as hypoxia radiotracers, but further research is needed, especially in comparison to FMISO.

Radioactive copper–labelled diacetyl-bis-(N4- methylthiosemicarbazone), or Cu-ATSM, is a neutral lipophilic compound that can permeate cell membranes.[250] In hypoxic conditions, Cu-ATSM molecules are reduced and negatively charged, while they wash out rapidly from normoxic cells, thus selectively accumulating in hypoxic cells resulting in a high SBR.[266] It has been shown that Cu-ATSM showed a significant difference in its uptake in HNSCC patients with residual or recurrent tumour compared to those without, which was not reflected in FDG uptake.[267] Others have shown that Cu-ATSM may be used to identify hypoxic subvolumes for IMRT.[268]

4.2.4. Tumour cell proliferation

While radiation therapy and chemotherapy can lead to a rapid decrease in the rate of cellular proliferation in responding tumours, which precedes a decrease in tumour size, accelerated tumour cell repopulation is an indicator of underlying radiation resistance and hence, treatment failure.[251, 269] Early identification of tumour cell repopulation as part of response assessment through imaging can identify target areas for dose escalation.[250] 3'-Fluoro-3' deoxythymidine (FLT)-PET is used widely to assess cellular proliferation, and unlike FDG, is only taken up by actively dividing cells and not surrounding inflammatory cells, allowing for specific detection of cellular division and subsequent dose escalation in these areas.[270, 271] Changes in the intensity of FLT uptake can be used to reflect cellular response to treatment, even prior to changes in tumour volume.[251, 271] However, FLT does not distinguish between benign and malignant abnormal cervical lymph nodes because its uptake by the germinal centres of reactive lymph nodes leads to a low positive predictive value.[272]

4.2.5. Apoptosis

Chemotherapy and radiation therapy rely on apoptosis to induce tumour cell death. Radiation resistance and treatment failure can result from mutations that lead to uncontrolled cellular

proliferation and dysregulation of apoptotic mechanisms.[273] Technetium 99m (99mTc)–labelled annexin V is a protein that binds to a major phospholipid constituent of cell membranes and has been investigated for imaging apoptosis in various malignancies including HNSCC.[274] The difficulty of radiolabeling annexin V with fluorine 18 has led to the development of other such tracers such as 18F-ML- 10 (2-[5-fluoro-pentyl]-2-methyl-malonic acid) (Aposense; Petach Tikva, Israel). This is a novel small-molecule probe designed to allow visualisation of apoptosis related cellular alterations, useful for differentiating between apoptotic and necrotic cells.[275]

4.2.6. Amino acid transport and protein synthesis

Carbon 11 (11C) methionine is a PET tracer that has been investigated to assess amino acid transport and accelerated protein synthesis in malignant tissue.[276] 11C-methionine allows for effective visualisation of HNSCC, demonstrating a good correlation with FDG demonstrating similar sensitivities and specificities for tumour detection, but does not distinguish between histological grade.[277, 278] It has been shown that there is a decline in 11C-methionine uptake at tumour sites with histology-confirmed complete treatment response in HNSCC patients, in comparison with sites of residual tumour tissue after radiation therapy.[279] Early decrease of 11C-methionine uptake correlates to final tumour volume reduction seen at MRI at the conclusion of treatment in HNSCC patients, suggesting that 11C-methionine can be used for early treatment adaptation.[280] Conversely, Nuutinen *et al.* showed a substantial early decline in 11C-methionine uptake in HNSCC patients after radiation therapy, but the rate of decrease in tracer uptake was comparable between patients with disease recurrence and those with preserved local control.[281] At present, there is no clear role for 11C-methionine in the imaging of HNSCC.[250]

Fluorine 18 fluoroethyltyrosine (FET), an amino acid analogue that is taken up by tumour cells through amino acid transport systems, has shown high diagnostic accuracy in patients with brain tumours, but has lower sensitivity (64-75%) when compared to FDG (89-95%) in the evaluation of HNSCC, making it unsuitable to replace FDG in the initial assessment of HNSCC despite superior specificity (90-100%) than FDG (50-79%).[282-285] FET could still have a role in differentiating between residual tumour tissue and inflammatory tissue after therapy.[250]

4.2.7. Cell membrane synthesis

Choline is incorporated with phospholipids during cell membrane synthesis.[286] A preliminary study[287] using 11C-choline in HNSCC patients found it to be just as effective as FDG for detecting malignant head and neck tumours with PET, however, another study[288] did not find 11C-choline PET/CT to be superior to FDG PET/CT for the detection of recurrent HNSCC.

While this is useful, carcinogenesis and transformation of tissue in HNSCC involves elaborate modification of numerous biomarkers. It would be more useful to assess the overall imbalance in biomarker regulation rather than rely on one marker alone. This considerably complicates the process of molecular diagnosis and analysis through imaging and requires further research.

OFI techniques can be used intraoperatively, in conjunction with information from pre-operative MRI, CT or PET, providing a more holistic knowledge of macroscopic and molecular

level tissue alterations, enabling ideal surgical guidance.[289-291] This will ultimately improve patient outcome by decreasing MRD in surgical margins.

5. Conclusion

Methods for early detection, molecular assessment of margins and surgical guidance, and assessment of treatment response are instrumental to changing the rate of local recurrence and resultant reduced prognosis in patients with HNSCC by enabling personalised medicine. There are a number of biomarkers that alter expression as tissue transforms. These can be used to assess MRD in surgical margins. Furthermore, optical and molecular imaging techniques can be used to identify molecular changes in biomarker expression, enabling immediate intraoperative decisions on extent of lesion and margin status, reducing the need for repeat surgery and the risk of recurrence. Multimodal imaging will provide more information about diseased tissue, enabling the surgeon to visualise the tumour in terms of its molecular extent and not simply its visual extent (whether with white light or fluorescence). Further research into molecular biomarkers as potential targets for "smart" probes for assessment of MRD in surgical margins is required to enhance current molecular imaging modalities which have applications pre-operatively to delineate lesion location and volume, intra-operatively to assess surgical margins and for surgical guidance, and post-operatively to assess treatment response. Research is also required to assess projected improvements in overall recurrence rates following introduction of these technologies to reveal whether these technologies have improved outcomes in practice.

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References

[1] Warnakulasuriya S: Global epidemiology of oral and oropharyngeal cancer. *Oral Oncol* 2009, 45(4-5):309-316.

[2] Alibek K, Kakpenova A, Baiken Y: Role of infectious agents in the carcinogenesis of brain and head and neck cancers. *Infect Agent Cancer* 2013, 8(1):7.

[3] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010, 127(12):2893-2917.

[4] Farah CS, Simanovic B, Dost F: Oral cancer in Australia 1982-2008: a growing need for opportunistic screening and prevention. *Australian dental journal* 2014, 59(3): 349-359.

[5] John K, Wu J, Lee BW, Farah CS: MicroRNAs in Head and Neck Cancer. *International journal of dentistry* 2013, 2013:650218.

[6] Speight PM: Update on oral epithelial dysplasia and progression to cancer. *Head and neck pathology* 2007, 1(1):61-66.

[7] Bhatia N, Lalla Y, Vu AN, Farah CS: Advances in optical adjunctive aids for visualisation and detection of oral malignant and potentially malignant lesions. *International journal of dentistry* 2013, 2013:194029.

[8] Dost F, Le Cao KA, Ford PJ, Farah CS: A retrospective analysis of clinical features of oral malignant and potentially malignant disorders with and without oral epithelial dysplasia. *Oral surgery, oral medicine, oral pathology and oral radiology* 2013, 116(6): 725-733.

[9] Warnakulasuriya S, Johnson NW, van der Waal I: Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med* 2007, 36(10): 575-580.

[10] Dost F, Le Cao K, Ford PJ, Ades C, Farah CS: Malignant transformation of oral epithelial dysplasia: a real-world evaluation of histopathologic grading. *Oral surgery, oral medicine, oral pathology and oral radiology* 2014, 117(3):342-352.

[11] Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E: Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. *J Oral Pathol Med* 2008, 37(3):127-133.

[12] Lee JJ, Hong WK, Hittelman WN, Mao L, Lotan R, Shin DM, Benner SE, Xu XC, Lee JS, Papadimitrakopoulou VM *et al*: Predicting cancer development in oral leukoplakia: ten years of translational research. *Clin Cancer Res* 2000, 6(5):1702-1710.

[13] Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, Pintilie M, Jurisica I, Perez-Ordonez B, Gilbert R *et al*: Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. *Hum Mol Genet* 2009, 18(24):4818-4829.

[14] Farah C, Bhatia N, John K, Lee B: Minimum intervention dentistry in oral medicine. *Australian dental journal* 2013, 58 Suppl 1:85-94.

[15] Tantiwongkosi B, Yu F, Kanard A, Miller FR: Role of F-FDG PET/CT in pre and post treatment evaluation in head and neck carcinoma. *World journal of radiology* 2014, 6(5):177-191.

[16] Gillison ML, Castellsague X, Chaturvedi A, Goodman MT, Snijders P, Tommasino M, Arbyn M, Franceschi S: Eurogin 2012 roadmap: Comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix. *Int J Cancer* 2013, 134(3):497-507.

[17] Heuveling DA, de Bree R, van Dongen GA: The potential role of non-FDG-PET in the management of head and neck cancer. *Oral Oncol* 2011, 47(1):2-7.

[18] Nagadia R, Pandit P, Coman WB, Cooper-White J, Punyadeera C: miRNAs in head and neck cancer revisited. *Cell Oncol* 2013, 36(1):1-7.

[19] de Carvalho AC, Kowalski LP, Campos AH, Soares FA, Carvalho AL, Vettore AL: Clinical significance of molecular alterations in histologically negative surgical margins of head and neck cancer patients. *Oral Oncol* 2012, 48(3):240-248.

[20] Hussain T, Nguyen QT: Molecular imaging for cancer diagnosis and surgery. *Advanced drug delivery reviews* 2014, 66:90-100.

[21] Eckardt A, Barth EL, Kokemueller H, Wegener G: Recurrent carcinoma of the head and neck: treatment strategies and survival analysis in a 20-year period. *Oral Oncol* 2004, 40(4):427-432.

[22] Karim-Kos HE, de Vries E, Soerjomataram I, Lemmens V, Siesling S, Coebergh JWW: Recent trends of cancer in Europe: A combined approach of incidence, survival and mortality for 17 cancer sites since the 1990s. *Eur J Cancer* 2008, 44(10):1345-1389.

[23] Slootweg PJ, Hordijk GJ, Schade Y, van Es RJJ, Koole R: Treatment failure and margin status in head and neck cancer. A critical view on the potential value of molecular pathology. *Oral Oncol* 2002, 38(5):500-503.

[24] Braakhuis BJ, Bloemena E, Leemans CR, Brakenhoff RH: Molecular analysis of surgical margins in head and neck cancer: more than a marginal issue. *Oral Oncol* 2010, 46(7):485-491.

[25] Woolgar JA, Triantafyllou A: A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. *Oral Oncol* 2005, 41(10):1034-1043.

[26] Lyons A: Current concepts in the management of oral cancer. *Dental update* 2006, 33(9):538-539, 542-535.

[27] Gandour-Edwards RF, Donald PJ, Lie JT: Clinical utility of intraoperative frozen-section diagnosis in head and neck-surgery - a quality assurance perspective. *Head Neck* 1993, 15(5):373-376.

[28] Gandour-Edwards RF, Donald PJ, Wiese DA: Accuracy of intraoperative frozen section diagnosis in head and neck-surgery - experience at a university medical-center. *Head Neck* 1993, 15(1):33-38.

[29] Laccourreye O, Hans S, Menard M, Garcia D, Brasnu D, Holsinger C: Transoral lateral oropharyngectomy for squamous cell carcinoma of the tonsillar region - II. An analysis of the incidence, related variables, and consequences of local recurrence. *Arch Otolaryngol Head Neck Surg* 2005, 131(7):592-599.

[30] Spiro RH, Guillamondegui O, Paulino AF, Huvos AG: Pattern of invasion and margin assessment in patients with oral tongue cancer. *Head Neck* 1999, 21(5):408-413.

[31] Slaughter DP, Southwick HW, Smejkal W: Field cancerization in oral stratified squamous epithelium: Clinical implication of multicentric origin. *Cancer* 1953, 6(5): 963-968.

[32] Wong LS, McMahon J, Devine J, McLellan D, Thompson E, Farrow A, Moos K, Ayoub A: Influence of close resection margins on local recurrence and disease-specific survival in oral and oropharyngeal carcinoma. *Br J Oral Maxillofac Surg* 2012, 50(2): 102-108.

[33] Nason RW, Binahmed A, Pathak KA, Abdoh AA, Sandor GKB: What is the adequate margin of surgical resection in oral cancer? *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009, 107(5):625-629.

[34] Goldenberg D, Harden S, Masayesva BG, Ha P, Benoit N, Westra WH, Koch WM, Sidransky D, Califano JA: Intraoperative molecular margin analysis in head and neck cancer. *Arch Otolaryngol Head Neck Surg* 2004, 130(1):39-44.

[35] Partridge M, Li SR, Pateromichelakis S, Francis R, Phillips E, Huang XH, Tesfa-Selase F, Langdon JD: Detection of minimal residual cancer to investigate why oral tumors recur despite seemingly adequate treatment. *Clin Cancer Res* 2000, 6(7):2718-2725.

[36] Yanamoto S, Yamada S, Takahashi H, Yoshitomi I, Kawasaki G, Ikeda H, Minamizato T, Shiraishi T, Fujita S, Ikeda T *et al*: Clinicopathological risk factors for local recurrence in oral squamous cell carcinoma. *International journal of oral and maxillofacial surgery* 2012, 41(10):1195-1200.

[37] Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, Pevsner J, Westra WH, Califano J: A transcriptional progression model for head and neck cancer. *Clin Cancer Res* 2003, 9(8):3058-3064.

[38] Smith J, Rattay T, McConkey C, Helliwell T, Mehanna H: Biomarkers in dysplasia of the oral cavity: a systematic review. *Oral Oncol* 2009, 45(8):647-653.

[39] Pitiyage G, Tilakaratne WM, Tavassoli M, Warnakulasuriya S: Molecular markers in oral epithelial dysplasia: review. *J Oral Pathol Med* 2009, 38(10):737-752.

[40] Tabor MP, Braakhuis BJ, van der Wal JE, van Diest PJ, Leemans CR, Brakenhoff RH, Kummer JA: Comparative molecular and histological grading of epithelial dysplasia of the oral cavity and the oropharynx. *J Pathol* 2003, 199(3):354-360.

[41] Egger G, Liang G, Aparicio A, Jones PA: Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004, 429(6990):457-463.

[42] Gasche JA, Goel A: Epigenetic mechanisms in oral carcinogenesis. *Future oncology (London, England)* 2012, 8(11):1407-1425.

[43] Feinberg AP, Ohlsson R, Henikoff S: The epigenetic progenitor origin of human cancer. *Nature reviews Genetics* 2006, 7(1):21-33.

[44] Nagpal JK, Das BR: Oral cancer: reviewing the present understanding of its molecular mechanism and exploring the future directions for its effective management. *Oral Oncol* 2003, 39(3):213-221.

[45] Lingen MW, Pinto A, Mendes RA, Franchini R, Czerninski R, Tilakaratne WM, Partridge M, Peterson DE, Woo SB: Genetics/epigenetics of oral premalignancy: current status and future research. *Oral diseases* 2011, 17 Suppl 1:7-22.

[46] Santhi WS, Sebastian P, Varghese BT, Prakash O, Pillai MR: NF-kappaB and COX-2 during oral tumorigenesis and in assessment of minimal residual disease in surgical margins. *Experimental and molecular pathology* 2006, 81(2):123-130.

[47] Santhi WS, Prathibha R, Charles S, Anurup KG, Reshmi G, Ramachandran S, Jissa VT, Sebastian P, Radhakrishna Pillai M: Oncogenic microRNAs as biomarkers of oral tumorigenesis and minimal residual disease. *Oral Oncol* 2013, 49(6):567-575.

[48] Cruz IB, Meijer C, Snijders PJF, Snow GB, Walboomers JMM, van der Waal I: p53 immunoexpression in non-malignant oral mucosa adjacent to oral squamous cell carcinoma: potential consequences for clinical management. *J Pathol* 2000, 191(2):132-137.

[49] Bilde A, von Buchwald C, Dabelsteen E, Therkildsen MH, Dabelsteen S: Molecular markers in the surgical margin of oral carcinomas. *J Oral Pathol Med* 2009, 38(1):72-78.

[50] Bergshoeff VE, Hopman AHN, Zwijnenberg IR, Ramaekers FCS, Bot FJ, Kremer B, Manni JJ, Speel EJM: Chromosome instability in resection margins predicts recurrence of oral squamous cell carcinoma. *J Pathol* 2008, 215(3):347-348.

[51] Tunca B, Erisen L, Coskun H, Cecener G, Ozuysal S, Egeli U: p53 gene mutations in surgical margins and primary tumor tissues of patients with squamous cell carcinoma of the head and neck. *Tumori* 2007, 93(2):182-188.

[52] Graveland AP, Golusinski PJ, Buijze M, Douma R, Sons N, Kuik DJ, Bloemena E, Leemans CR, Brakenhoff RH, Braakhuis BJ: Loss of heterozygosity at 9p and p53 immunopositivity in surgical margins predict local relapse in head and neck squamous cell carcinoma. *Int J Cancer* 2011, 128(8):1852-1859.

[53] Graveland AP, Bremmer JF, de Maaker M, Brink A, Cobussen P, Zwart M, Braakhuis BJ, Bloemena E, van der Waal I, Leemans CR *et al*: Molecular screening of oral pre-cancer. *Oral Oncol* 2013, 49(12):1129-1135.

[54] Nathan CAO, Amirghahri N, Rice C, Abreo FW, Shi RH, Stucker FJ: Molecular analysis of surgical margins in head and neck squamous cell carcinoma patients. *Laryngoscope* 2002, 112(12):2129-2140.

[55] Martone T, Gillio-Tos A, De Marco L, Fiano V, Maule M, Cavalot A, Garzaro M, Merletti F, Cortesina G: Association between hypermethylated tumor and paired surgical margins in head and neck squamous cell carcinomas. *Clin Cancer Res* 2007, 13(17): 5089-5094.

[56] Sinha P, Bahadur S, Thakar A, Matta A, Macha M, Ralhan R, Gupta SD: Significance of promoter hypermethylation of p16 gene for margin assessment in carcinoma tongue. *Head Neck* 2009, 31(11):1423-1430.

[57] Wong TS, Man MW, Lam AK, Wei WI, Kwong YL, Yuen AP: The study of p16 and p15 gene methylation in head and neck squamous cell carcinoma and their quantitative evaluation in plasma by real-time PCR. *Eur J Cancer* 2003, 39(13):1881-1887.

[58] Nathan CAO, Franklin S, Abreo FW, Nassar R, De Benedetti A, Glass J: Analysis of surgical margins with the molecular marker eIF4E: A prognostic factor in patients with head and neck cancer. *J Clin Oncol* 1999, 17(9):2909-2914.

[59] Tan HK, Saulnier P, Auperin A, Lacroix L, Casiraghi O, Janot F, Fouret P, Temam S: Quantitative methylation analyses of resection margins predict local recurrences and disease-specific deaths in patients with head and neck squamous cell carcinomas. *BrJ Cancer* 2008, 99(2):357-363.

[60] Roh JL, Westra WH, Califano JA, Sidransky D, Koch WM: Tissue imprint for molecular mapping of deep surgical margins in patients with head and neck squamous cell carcinoma. *Head Neck* 2012, 34(11):1529-1536.

[61] Supic G, Kozomara R, Jovic N, Zeljic K, Magic Z: Prognostic significance of tumor-related genes hypermethylation detected in cancer-free surgical margins of oral squamous cell carcinomas. *Oral Oncol* 2011, 47(8):702-708.

[62] Ott CE, Skroch E, Steinhart H, Verdorfer I, Pahl S, Iro H, Gebhart E, Bohlender JE: Thin section arrays for I-FISH analysis of chromosome-specific imbalances in squamous cell carcinomas of the head and neck. *Int J Oncol* 2002, 20(3):623-630.

[63] Bremmer JF, Braakhuis BJM, Brink A, Broeckaert MAM, Belien JAM, Meijer GA, Kuik DJ, Leemans CR, Bloemena E, van der Waal I *et al*: Comparative evaluation of genetic assays to identify oral pre-cancerous fields. *J Oral Pathol Med* 2008, 37(10): 599-606.

[64] Stafford ND, Ashman JNE, MacDonald AW, Ell SR, Monson JRT, Greenman J: Genetic analysis of head and neck squamous cell carcinoma and surrounding mucosa. *Arch Otolaryngol Head Neck Surg* 1999, 125(12):1341-1348.

[65] Preuss SF, Brieger J, Essig EK, Stenzel MJ, Mann WJ: Quantitative DNA measurement in oropharyngeal squamous cell carcinoma and surrounding mucosa. *ORL J Otorhinolaryngol Relat Spec* 2004, 66(6):320-324.

[66] Szukala K, Sowinska A, Wierzbicka M, Biczysko W, Szyfter W, Szyfter K: Does loss of heterozygosity in critical genome regions predict a local relapse in patients after laryngectomy? *Mutat Res* 2006, 600(1-2):67-76.

[67] Brieger J, Kastner J, Gosepath J, Mann WJ: Evaluation of microsatellite amplifications at chromosomal locus 3q26 as surrogate marker for premalignant changes in mucosa surrounding head and neck squamous cell carcinoma. *Cancer Genet Cytogenet* 2006, 167(1):26-31.

[68] Poh CF, Zhang L, Anderson DW, Durham JS, Williams RM, Priddy RW, Berean KW, Ng S, Tseng OL, MacAulay C *et al*: Fluorescence visualization detection of field alterations in tumor margins of oral cancer patients. *Clin Cancer Res* 2006, 12(22): 6716-6722.

[69] Mascolo M, Siano M, Ilardi G, Russo D, Merolla F, De Rosa G, Staibano S: Epigenetic disregulation in oral cancer. *International journal of molecular sciences* 2012, 13(2): 2331-2353.

[70] Roh J-L, Wang XV, Manola J, Sidransky D, Forastiere AA, Koch WM: Clinical correlates of promoter hypermethylation of four target genes in head and neck cancer: A cooperative group correlative study. *Clin Cancer Res* 2013, 19(9):2528-2540.

[71] Perez-Sayans M, Suarez-Penaranda JM, Gayoso-Diz P, Barros-Angueira F, Gandara-Rey JM, Garcia-Garcia A: p16(INK4a)/CDKN2 expression and its relationship with oral squamous cell carcinoma is our current knowledge enough? *Cancer letters* 2011, 306(2):134-141.

[72] Kato K, Hara A, Kuno T, Mori H, Yamashita T, Toida M, Shibata T: Aberrant promoter hypermethylation of p16 and MGMT genes in oral squamous cell carcinomas and the surrounding normal mucosa. *Journal of cancer research and clinical oncology* 2006, 132(11):735-743.

[73] Rainsbury JW, Ahmed W, Williams HK, Roberts S, Paleri V, Mehanna H: Prognostic biomarkers of survival in oropharyngeal squamous cell carcinoma: systematic review and meta-analysis. *Head Neck* 2013, 35(7):1048-1055.

[74] Califano J, Westra WH, Meininger G, Corio R, Koch WM, Sidransky D: Genetic progression and clonal relationship of recurrent premalignant head and neck lesions. *Clin Cancer Res* 2000, 6(2):347-352.

[75] Sidransky D: Molecular markers in cancer diagnosis. *Journal of the National Cancer Institute Monographs* 1995(17):27-29.

[76] Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S, Hittelman W, Hong WK: Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. *Nature medicine* 1996, 2(6):682-685.

[77] Lippman SM, Sudbo J, Hong WK: Oral cancer prevention and the evolution of molecular-targeted drug development. *J Clin Oncol* 2005, 23(2):346-356.

[78] Hong WK, Spitz MR, Lippman SM: Cancer chemoprevention in the 21st century: genetics, risk modeling, and molecular targets. *J Clin Oncol* 2000, 18(21 Suppl):9S-18S.

[79] Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W *et al*: Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Res* 1996, 56(11):2488-2492.

[80] Sidransky D: Molecular genetics of head and neck cancer. *Current opinion in oncology* 1995, 7(3):229-233.

[81] Tsui IF, Rosin MP, Zhang L, Ng RT, Lam WL: Multiple aberrations of chromosome 3p detected in oral premalignant lesions. *Cancer Prev Res (Phila)* 2008, 1(6):424-429.

[82] Tabor MP, Brakenhoff RH, van Houten VM, Kummer JA, Snel MH, Snijders PJ, Snow GB, Leemans CR, Braakhuis BJ: Persistence of genetically altered fields in head and neck cancer patients: biological and clinical implications. *Clin Cancer Res* 2001, 7(6): 1523-1532.

[83] Partridge M, Pateromichelakis S, Phillips E, Emilion GG, A'Hern RP, Langdon JD: A case-control study confirms that microsatellite assay can identify patients at risk of developing oral squamous cell carcinoma within a field of cancerization. *Cancer Res* 2000, 60(14):3893-3898.

[84] Rosin MP, Cheng X, Poh C, Lam WL, Huang Y, Lovas J, Berean K, Epstein JB, Priddy R, Le ND *et al*: Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. *Clin Cancer Res* 2000, 6(2):357-362.

[85] Rosin MP, Lam WL, Poh C, Le ND, Li RJ, Zeng T, Priddy R, Zhang L: 3p14 and 9p21 loss is a simple tool for predicting second oral malignancy at previously treated oral cancer sites. *Cancer Res* 2002, 62(22):6447-6450.

[86] Sardi I, Franchi A, Ferriero G, Frittelli A, Bruschini L, Montali E, Gallo O: Prediction of recurrence by microsatellite analysis in head and neck cancer. *Genes Chromosomes Cancer* 2000, 29(3):201-206.

[87] Zhang L, Poh CF, Williams M, Laronde DM, Berean K, Gardner PJ, Jiang H, Wu L, Lee JJ, Rosin MP: Loss of heterozygosity (LOH) profiles--validated risk predictors for progression to oral cancer. *Cancer Prev Res (Phila)* 2012, 5(9):1081-1089.

[88] Lingen MW, Szabo E: Validation of LOH profiles for assessing oral cancer risk. *Cancer Prev Res (Phila)* 2012, 5(9):1075-1077.

[89] Chin D, Boyle GM, Theile DR, Parsons PG, Coman WB: Molecular introduction to head and neck cancer (HNSCC) carcinogenesis. *British journal of plastic surgery* 2004, 57(7):595-602.

[90] Choi S, Myers JN: Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. *Journal of dental research* 2008, 87(1):14-32.

[91] Kim MM, Califano JA: Molecular pathology of head-and-neck cancer. *Int J Cancer* 2004, 112(4):545-553.

[92] Mirzayans R, Andrais B, Scott A, Murray D: New insights into p53 signaling and cancer cell response to DNA damage: implications for cancer therapy. *Journal of biomedicine & biotechnology* 2012, 2012:170325.

[93] Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, Goodman SN, Sidransky D: Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1995, 332(7):429-435.

[94] van Houten VMM, Tabor MP, van den Brekel MWM, Kummer JA, Denkers F, Dijkstra J, Leemans R, van der Waal I, Snow GB, Brakenhoff RH: Mutated p53 as a molecular marker for the diagnosis of head and neck cancer. *J Pathol* 2002, 198(4):476-486.

[95] Pena Murillo C, Huang X, Hills A, McGurk M, Lyons A, Jeannon JP, Odell E, Brown A, Lavery K, Barrett W *et al*: The utility of molecular diagnostics to predict recurrence of head and neck carcinoma. *Br J Cancer* 2012, 107(7):1138-1143.

[96] Yi HJ, Zhang BQ, Guo W, Zhao LD, Yang SM: The role of molecular margins as prognostic factors in laryngeal carcinoma in Chinese patients. *Acta oto-laryngologica* 2012, 132(8):874-878.

[97] van Houten VMM, Leemans CR, Kummer JA, Dijkstra J, Kuik DJ, van den Brekel MWM, Snow GB, Brakenhoff RH: Molecular diagnosis of surgical margins and local recurrence in head and neck cancer patients: A prospective study. *Clin Cancer Res* 2004, 10(11):3614-3620.

[98] Warnakulasuriya S: Lack of molecular markers to predict malignant potential of oral precancer. *J Pathol* 2000, 190(4):407-409.

[99] Cruz IB, Snijders PJ, Meijer CJ, Braakhuis BJ, Snow GB, Walboomers JM, van der Waal I: p53 expression above the basal cell layer in oral mucosa is an early event of malignant transformation and has predictive value for developing oral squamous cell carcinoma. *J Pathol* 1998, 184(4):360-368.

[100] Hall GL, Shaw RJ, Field EA, Rogers SN, Sutton DN, Woolgar JA, Lowe D, Liloglou T, Field JK, Risk JM: p16 Promoter methylation is a potential predictor of malignant transformation in oral epithelial dysplasia. *Cancer epidemiology, biomarkers & preven-*

tion : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2008, 17(8):2174-2179.

[101] Bradley G, Odell EW, Raphael S, Ho J, Le LW, Benchimol S, Kamel-Reid S: Abnormal DNA content in oral epithelial dysplasia is associated with increased risk of progression to carcinoma. *Br J Cancer* 2010, 103(9):1432-1442.

[102] Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J *et al*: TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med* 2007, 357(25):2552-2561.

[103] Van der Vorst S, Dekairelle AF, Weynand B, Hamoir M, Gala JL: Assessment of p53 functional activity in tumor cells and histologically normal mucosa from patients with head and neck squamous cell carcinoma. *Head Neck* 2012, 34(11):1542-1550.

[104] Vered M, Allon I, Dayan D: Maspin, p53, p63, and Ki-67 in epithelial lesions of the tongue: from hyperplasia through dysplasia to carcinoma. *J Oral Pathol Med* 2009, 38(3):314-320.

[105] Bortoluzzi MC, Yurgel LS, Dekker NP, Jordan RC, Regezi JA: Assessment of p63 expression in oral squamous cell carcinomas and dysplasias. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004, 98(6):698-704.

[106] Murti PR, Warnakulasuriya KA, Johnson NW, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS: p53 expression in oral precancer as a marker for malignant potential. *J Oral Pathol Med* 1998, 27(5):191-196.

[107] Nasser W, Flechtenmacher C, Holzinger D, Hofele C, Bosch FX: Aberrant expression of p53, p16INK4a and Ki-67 as basic biomarker for malignant progression of oral leukoplakias. *J Oral Pathol Med* 2011, 40(8):629-635.

[108] Nathan CO, Liu L, Li BD, Abreo FW, Nandy I, De Benedetti A: Detection of the proto-oncogene eIF4E in surgical margins may predict recurrence in head and neck cancer. *Oncogene* 1997, 15(5):579-584.

[109] Sunavala-Dossabhoy G, Palaniyandi S, Clark C, Nathan CO, Abreo FW, Caldito G: Analysis of eIF4E and 4EBP1 mRNAs in head and neck cancer. *Laryngoscope* 2011, 121(10):2136-2141.

[110] Nathan CAO, Sanders K, Abreo FW, Nassar R, Glass J: Correlation of p53 and the proto-oncogene eIF4E in larynx cancers: Prognostic implications. *Cancer Res* 2000, 60(13):3599-3604.

[111] Chen YK, Hsue SS, Lin LM: Expression of p63 protein and mRNA in oral epithelial dysplasia. *J Oral Pathol Med* 2005, 34(4):232-239.

[112] Takeda T, Sugihara K, Hirayama Y, Hirano M, Tanuma JI, Semba I: Immunohistological evaluation of Ki-67, p63, CK19 and p53 expression in oral epithelial dysplasias. *J Oral Pathol Med* 2006, 35(6):369-375.

[113] Choi HR, Tucker SA, Huang Z, Gillenwater AM, Luna MA, Batsakis JG, El-Naggar AK: Differential expressions of cyclin-dependent kinase inhibitors (p27 and p21) and their relation to p53 and Ki-67 in oral squamous tumorigenesis. *Int J Oncol* 2003, 22(2):409-414.

[114] Calin GA, Croce CM: MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006, 6(11):857-866.

[115] Clague J, Lippman SM, Yang H, Hildebrandt MA, Ye Y, Lee JJ, Wu X: Genetic varia‐ tion in microRNA genes and risk of oral premalignant lesions. *Molecular carcinogene‐ sis* 2010, 49(2):183-189.

[116] Gorenchtein M, Poh CF, Saini R, Garnis C: MicroRNAs in an oral cancer context - from basic biology to clinical utility. *Journal of dental research* 2012, 91(5):440-446.

[117] Barker EV, Cervigne NK, Reis PP, Goswami RS, Xu W, Weinreb I, Irish JC, Kamel-Reid S: MicroRNA evaluation of unknown primary lesions in the head and neck. *Mol Cancer* 2009, 8.

[118] Malumbres M: miRNAs and cancer: An epigenetics view. *Molecular aspects of medicine* 2012, 34(4):863-874.

[119] Wu BH, Xiong XP, Jia J, Zhang WF: MicroRNAs: new actors in the oral cancer scene. *Oral Oncol* 2011, 47(5):314-319.

[120] Perez-Sayans M, Pilar GD, Barros-Angueira F, Suarez-Penaranda JM, Fernandez AC, Gandara-Rey JM, Garcia-Garcia A: Current trends in miRNAs and their relationship with oral squamous cell carcinoma. *J Oral Pathol Med* 2012, 41(6):433-443.

[121] Scapoli L, Palmieri A, Lo Muzio L, Pezzetti F, Rubini C, Girardi A, Farinella F, Maz‐ zotta M, Carinci F: MicroRNA expression profiling of oral carcinoma identifies new markers of tumor progression. *International journal of immunopathology and pharmacol‐ ogy* 2010, 23(4):1229-1234.

[122] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K *et al*: Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002, 99(24):15524-15529.

[123] Calin GA, Sevignani C, Dan Dumitru C, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M *et al*: Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci U S A* 2004, 101(9):2999-3004.

[124] Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M, Chen Q, Burk RD, Smith RV, Prystowsky MB *et al*: Low-level expression of micro-RNAs let-7d and mir-205 are prognostic markers of head and neck squamous cell carcinoma. *Am J Pathol* 2009, 174(3):736-745.

[125] Kozaki K-i, Imoto I, Mogi S, Omura K, Inazawa J: Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. *Cancer Res* 2008, 68(7): 2094-2105.

[126] Li J, Huang H, Sun L, Yang M, Pan C, Chen W, Wu D, Lin Z, Zeng C, Yao Y *et al*: miR-21 indicates poor prognosis in tongue squamous cell carcinomas as an apoptosis inhibitor. *Clin Cancer Res* 2009, 15(12):3998-4008.

[127] Lajer CB, Garnaes E, Friis-Hansen L, Norrild B, Therkildsen MH, Glud M, Rossing M, Lajer H, Svane D, Skotte L *et al*: The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer. *Br J Cancer* 2012, 106(9):1526-1534.

[128] Lajer CB, Nielsen FC, Friis-Hansen L, Norrild B, Borup R, Garnaes E, Rossing M, Specht L, Therkildsen MH, Nauntofte B *et al*: Different miRNA signatures of oral and pharyngeal squamous cell carcinomas: a prospective translational study. *Br J Cancer* 2011, 104(5):830-840.

[129] Zhang X, Cairns M, Rose B, O'Brien C, Shannon K, Clark J, Gamble J, Tran N: Alterations in miRNA processing and expression in pleomorphic adenomas of the salivary gland. *Int J Cancer* 2009, 124(12):2855-2863.

[130] Reis PP, Tomenson M, Cervigne NK, Machado J, Jurisica I, Pintilie M, Sukhai MA, Perez-Ordonez B, Grenman R, Gilbert RW *et al*: Programmed cell death 4 loss increases tumor cell invasion and is regulated by miR-21 in oral squamous cell carcinoma. *Mol Cancer* 2010, 9:238.

[131] Zheng ZM, Wang X: Regulation of cellular miRNA expression by human papillomaviruses. *Biochim Biophys Acta* 2011, 1809(11-12):668-677.

[132] Babu JM, Prathibha R, Jijith VS, Hariharan R, Pillai MR: A miR-centric view of head and neck cancers. *Biochim Biophys Acta* 2011, 1816(1):67-72.

[133] Langevin SM, Stone RA, Bunker CH, Grandis JR, Sobol RW, Taioli E: MicroRNA-137 promoter methylation in oral rinses from patients with squamous cell carcinoma of the head and neck is associated with gender and body mass index. *Carcinogenesis* 2010, 31(5):864-870.

[134] Xiao W, Bao ZX, Zhang CY, Zhang XY, Shi LJ, Zhou ZT, Jiang WW: Upregulation of miR-31* is negatively associated with recurrent/newly formed oral leukoplakia. *PLoS One* 2012, 7(6):1-10.

[135] Wang TD, Van Dam J: Optical biopsy: a new frontier in endoscopic detection and diagnosis. *Clin Gastroenterol Hepatol* 2004, 2(9):744-753.

[136] Keereweer S, Kerrebijn JD, van Driel PB, Xie B, Kaijzel EL, Snoeks TJ, Que I, Hutteman M, van der Vorst JR, Mieog JS *et al*: Optical image-guided surgery--where do we stand? *Mol Imaging Biol* 2011, 13(2):199-207.

[137] Zhu Y, Terry NG, Wax A: Angle-resolved low-coherence interferometry: An optical biopsy technique for clinical detection of dysplasia in Barretts esophagus. *Expert Review of Gastroenterology and Hepatology* 2012, 6(1):37-41.

[138] Hamdoon Z, Jerjes W, Upile T, McKenzie G, Jay A, Hopper C: Optical coherence tomography in the assessment of suspicious oral lesions: An immediate ex vivo study. *Photodiagnosis and Photodynamic Therapy* 2013, 10(1):17-27.

[139] Adhi M, Duker JS: Optical coherence tomography-current and future applications. *Curr Opin Ophthalmol* 2013, 24(3):213-221.

[140] Whiteman SC, Yang Y, Gey van Pittius D, Stephens M, Parmer J, Spiteri MA: Optical coherence tomography: real-time imaging of bronchial airways microstructure and detection of inflammatory/neoplastic morphologic changes. *Clin Cancer Res* 2006, 12(3 Pt 1):813-818.

[141] Lee CK, Tsai MT, Lee HC, Chen HM, Chiang CP, Wang YM, Yang CC: Diagnosis of oral submucous fibrosis with optical coherence tomography. *J Biomed Opt* 2009, 14(5): 054008.

[142] Ozawa N, Sumi Y, Shimozato K, Chong C, Kurabayashi T: In vivo imaging of human labial glands using advanced optical coherence tomography. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009, 108(3):425-429.

[143] Fujimoto JG: Optical coherence tomography for ultrahigh resolution in vivo imaging. *Nature Biotechnol* 2003, 21(11):1361-1367.

[144] Brezinski ME, Tearney GJ, Boppart SA, Swanson EA, Southern JF, Fujimoto JG: Optical biopsy with optical coherence tomography: Feasibility for surgical diagnostics. *J Surg Res* 1997, 71(1):32-40.

[145] Hughes OR, Stone N, Kraft M, Arens C, Birchall MA: Optical and molecular techniques to identify tumor margins within the larynx. *Head Neck* 2010, 32(11):1544-1553.

[146] Hamdoon Z, Jerjes W, Al-Delayme R, McKenzie G, Jay A, Hopper C: Structural validation of oral mucosal tissue using optical coherence tomography. *Head and Neck Oncology* 2012, 4(1).

[147] Jerjes W, Upile T, Conn B, Hamdoon Z, Betz CS, McKenzie G, Radhi H, Vourvachis M, El Maaytah M, Sandison A *et al*: In vitro examination of suspicious oral lesions using optical coherence tomography. *British Journal of Oral and Maxillofacial Surgery* 2010, 48(1):18-25.

[148] Savastru D, Chang EW, Miclos S, Pitman MB, Patel A, Iftimia N: Detection of breast surgical margins with optical coherence tomography imaging: a concept evaluation study. *J Biomed Opt* 2014, 19(5):056001.

[149] South FA, Chaney EJ, Marjanovic M, Adie SG, Boppart SA: Differentiation of ex vivo human breast tissue using polarization-sensitive optical coherence tomography. *Biomed Opt Express* 2014, 5(10):3417-3426.

[150] Alawi SA, Kuck M, Wahrlich C, Batz S, McKenzie G, Fluhr JW, Lademann J, Ulrich M: Optical coherence tomography for presurgical margin assessment of non-melanoma skin cancer - a practical approach. *Exp Dermatol* 2013, 22(8):547-551.

[151] Pelosini L, Smith HB, Schofield JB, Meeckings A, Dhital A, Khandwala M: In vivo optical coherence tomography (OCT) in periocular basal cell carcinoma: correlations between in vivo OCT images and postoperative histology. *Br J Ophthalmol* 2013, 97(7): 890-894.

[152] Wessels R, van Beurden M, de Bruin DM, Faber DJ, Vincent AD, Sanders J, van Leeuwen TG, Ruers TJ: The value of optical coherence tomography in determining surgical margins in squamous cell carcinoma of the vulva: a single-center prospective study. *Int J Gynecol Cancer* 2014.

[153] Skarecky DW, Brenner M, Rajan S, Rodriguez E, Jr., Narula N, Melgoza F, Ahlering TE: Zero positive surgical margins after radical prostatectomy: is the end in sight. *Expert Rev Med Devices* 2008, 5(6):709-717.

[154] Pyhtila JW, Chalut KJ, Boyer JD, Keener J, D'Amico T, Gottfried M, Gress F, Wax A: In situ detection of nuclear atypia in Barrett's esophagus by using angle-resolved low-coherence interferometry. *Gastrointest Endosc* 2007, 65(3):487-491.

[155] Terry NG, Zhu Y, Rinehart MT, Brown WJ, Gebhart SC, Bright S, Carretta E, Ziefle CG, Panjehpour M, Galanko J *et al*: Detection of dysplasia in barrett's esophagus with in vivo depth-resolved nuclear morphology measurements. *Gastroenterology* 2011, 140(1):42-50.

[156] Kelloff GJ, Sigman CC: Assessing intraepithelial neoplasia and drug safety in cancer-preventive drug development. *Nat Rev Cancer* 2007, 7(7):508-518.

[157] Backman V, Wallace MB, Perelman LT, Arendt JT, Gurjar R, Muller MG, Zhang Q, Zonios G, Kline E, McGilligan JA *et al*: Detection of preinvasive cancer cells. *Nature* 2000, 406(6791):35-36.

[158] Weissleder R, Pittet MJ: Imaging in the era of molecular oncology. *Nature* 2008, 452(7187):580-589.

[159] Francisco AL, Correr WR, Pinto CA, Filho JG, Chulam TC, Kurachi C, Kowalski LP: Analysis of surgical margins in oral cancer using in situ fluorescence spectroscopy. *Oral Oncol* 2014.

[160] Bydlon TM, Barry WT, Kennedy SA, Brown JQ, Gallagher JE, Wilke LG, Geradts J, Ramanujam N: Advancing optical imaging for breast margin assessment: an analysis of excisional time, cautery, and patent blue dye on underlying sources of contrast. *PLoS One* 2012, 7(12):e51418.

[161] Pleijhuis R, Timmermans A, De Jong J, De Boer E, Ntziachristos V, Van Dam G: Tissue-simulating Phantoms for Assessing Potential Near-infrared Fluorescence Imaging Applications in Breast Cancer Surgery. *J Vis Exp* 2014(91).

[162] Keereweer S, Sterenborg HJ, Kerrebijn JD, Van Driel PB, Baatenburg de Jong RJ, Lowik CW: Image-guided surgery in head and neck cancer: current practice and future directions of optical imaging. *Head Neck* 2012, 34(1):120-126.

[163] Lane P, Follen M, MacAulay C: Has fluorescence spectroscopy come of age? a case series of oral precancers and cancers using white light, fluorescent light at 405 nm, and reflected light at 545 nm using the Trimira identafi 3000. *Gend Med* 2012, 9(1 SUPPL.):S25-S35.

[164] Lane P, Lam S, Follen M, MacAulay C: Oral fluorescence imaging using 405-nm excitation, aiding the discrimination of cancers and precancers by identifying changes in collagen and elastic breakdown and neovascularization in the underlying stroma. *Gend Med* 2012, 9(1 SUPPL.):S78-S82.e78.

[165] Olivo M, Bhuvaneswari R, Keogh I: Advances in bio-optical imaging for the diagnosis of early oral cancer. *Pharmaceutics* 2011, 3(3):354-378.

[166] Ragazzi M, Piana S, Longo C, Castagnetti F, Foroni M, Ferrari G, Gardini G, Pellacani G: Fluorescence confocal microscopy for pathologists. *Mod Pathol* 2014, 27(3):460-471.

[167] Miyamoto S, Sperry S, Yamashita T, Reddy NP, O'Malley BW, Jr., Li D: Molecular imaging assisted surgery improves survival in a murine head and neck cancer model. *Int J Cancer* 2012, 131(5):1235-1242.

[168] Richards-Kortum R, Sevick-Muraca E: Quantitative optical spectroscopy for tissue diagnosis. *Annu Rev Phys Chem* 1996, 47:555-606.

[169] Pavlova I, Williams M, El-Naggar A, Richards-Kortum R, Gillenwater A: Understanding the biological basis of autofluorescence imaging for oral cancer detection: High-resolution fluorescence microscopy in viable tissue. *Clin Cancer Res* 2008, 14(8): 2396-2404.

[170] Rethman MP, Carpenter W, Cohen EEW, Epstein J, Evans CA, Flalfz CM, Graham FJ, Hujoel PP, Kalmar JR, Koch WM *et al*: Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. *J Am Dent Assoc* 2010, 141(5): 509-520.

[171] Poh CF, MacAulay CE, Zhang L, Rosin MP: Tracing the "at-risk" oral mucosa field with autofluorescence: Steps toward clinical impact. *Cancer Prev Res (Phila)* 2009, 2(5): 401-404.

[172] De Veld DCG, Witjes MJH, Sterenborg HJCM, Roodenburg JLN: The status of in vivo autofluorescence spectroscopy and imaging for oral oncology. *Oral Oncol* 2005, 41(2): 117-131.

[173] Roblyer D, Kurachi C, Stepanek V, Williams MD, El-Naggar AK, Lee JJ, Gillenwater AM, Richards-Kortum R: Objective detection and delineation of oral neoplasia using autofluorescence imaging. *Cancer Prev Res (Phila)* 2009, 2(5):423-431.

[174] Pavlova I, Weber CR, Schwarz RA, Williams MD, Gillenwater AM, Richards-Kortum R: Fluorescence spectroscopy of oral tissue: Monte Carlo modeling with site-specific tissue properties. *J Biomed Opt* 2009, 14(1):014009.

[175] Kois JC, Truelove E: Detecting oral cancer: A new technique and case reports. *Dent Today* 2006, 25(10):94-97.

[176] Farah CS, McIntosh L, Georgiou A, McCullough MJ: Efficacy of tissue autofluorescence imaging (VELScope) in the visualization of oral mucosal lesions. *Head Neck* 2012, 34(6):856-862.

[177] Bhatia N, Matias MA, Farah CS: Assessment of a decision making protocol to improve the efficacy of VELscope in general dental practice: A prospective evaluation. *Oral Oncol* 2014.

[178] McIntosh L, McCullough MJ, Farah CS: The assessment of diffused light illumination and acetic acid rinse (Microlux/DL) in the visualisation of oral mucosal lesions. *Oral Oncol* 2009, 45(12):e227-231.

[179] Pazouki S, Chisholm DM, Adi MM, Carmichael G, Farquharson M, Ogden GR, Schor SL, Schor AM: The association between tumour progression and vascularity in the oral mucosa. *J Pathol* 1997, 183(1):39-43.

[180] Raica M, Cimpean AM, Ribatti D: Angiogenesis in pre-malignant conditions. *Eur J Cancer* 2009, 45(11):1924-1934.

[181] Shetty DC, Ahuja P, Taneja DK, Singh Rathore A, Chhina S, Ahuja US, Kumar K, Ahuja A, Rastogi P: Relevance of tumor angiogenesis patterns as a diagnostic value and prognostic indicator in oral precancer and cancer. *Vascular Health and Risk Management* 2011, 7(1):41-47.

[182] Wong Kee Song LM, Adler DG, Conway JD, Diehl DL, Farraye FA, Kantsevoy SV, Kwon R, Mamula P, Rodriguez B, Shah RJ *et al*: Narrow band imaging and multiband imaging. *Gastrointest Endosc* 2008, 67(4):581-589.

[183] Gono K, Yamazaki K, Doguchi N, Nonami T, Obi T, Yamaguchi M, Ohyama N, Machida H, Sano Y, Yoshida S *et al*: Endoscopic observation of tissue by narrowband illumination. *Opt Rev* 2003, 10(4):211-215.

[184] Takano JH, Yakushiji T, Kamiyama I, Nomura T, Katakura A, Takano N, Shibahara T: Detecting early oral cancer: narrowband imaging system observation of the oral mucosa microvasculature. *International journal of oral and maxillofacial surgery* 2010, 39(3):208-213.

[185] Tan NC, Herd MK, Brennan PA, Puxeddu R: The role of narrow band imaging in early detection of head and neck cancer. *Br J Oral Maxillofac Surg* 2012, 50(2):132-136.

[186] Fujii S, Yamazaki M, Muto M, Ochiai A: Microvascular irregularities are associated with composition of squamous epithelial lesions and correlate with subepithelial invasion of superficial-type pharyngeal squamous cell carcinoma. *Histopathology* 2010, 56(4):510-522.

[187] Katada C, Nakayama M, Tanabe S, Naruke A, Koizumi W, Masaki T, Okamoto M, Saigenji K: Narrow band imaging for detecting superficial oral squamous cell carcinoma: a report of two cases. *Laryngoscope* 2007, 117(9):1596-1599.

[188] Tan NCW, Mellor T, Brennan PA, Puxeddu R: Use of narrow band imaging guidance in the management of oral erythroplakia. *Br J Oral Maxillofac Surg* 2011, 49(6):488-490.

[189] Yang SW, Lee YS, Chang LC, Chien HP, Chen TA: Clinical appraisal of endoscopy with narrow-band imaging system in the evaluation and management of homogeneous oral leukoplakia. *ORL J Otorhinolaryngol Relat Spec* 2012, 74(2):102-109.

[190] Gono K, Obi T, Yamaguchi M, Ohyama N, Machida H, Sano Y, Yoshida S, Hamamoto Y, Endo T: Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J Biomed Opt* 2004, 9(3):568-577.

[191] Muto M, Nakane M, Katada C, Sano Y, Ohtsu A, Esumi H, Ebihara S, Yoshida S: Squamous cell carcinoma in situ at oropharyngeal and hypopharyngeal mucosal sites. *Cancer* 2004, 101(6):1375-1381.

[192] Muto M, Katada C, Sano Y, Yoshida S: Narrow band imaging: A new diagnostic approach to visualize angiogenesis in superficial neoplasia. *Clinical Gastroenterology and Hepatology* 2005, 3(7 SUPPL.):S16-S20.

[193] Yoshida T, Inoue H, Usui S, Satodate H, Fukami N, Kudo SE: Narrow-band imaging system with magnifying endoscopy for superficial esophageal lesions. *Gastrointest Endosc* 2004, 59(2):288-295.

[194] Matsumura M, Uedo N, Ishihara R, Iishi H, Fujii T, Tomita Y: A case of intraepithelial neoplasia in the oropharynx detected by endoscopic screening with narrow-band imaging videoendoscopy. *Gastrointest Endosc* 2008, 68(1):146-147.

[195] Lin YC, Wang WH: Narrow-band imaging for detecting early recurrent nasopharyngeal carcinoma. *Head Neck* 2011, 33(4):591-594.

[196] Piazza C: Is narrow band imaging the ideal screening tool for mucosal head and neck cancer? *Oral Oncol* 2011, 47(5):313.

[197] Orita Y, Kawabata K, Mitani H, Fukushima H, Tanaka S, Yoshimoto S, Yamamoto N: Can narrow-band imaging be used to determine the surgical margin of superficial hypopharyngeal cancer? *Acta medica Okayama* 2008, 62(3):205-208.

[198] Watanabe A, Taniguchi M, Tsujie H, Hosokawa M, Fujita M, Sasaki S: The value of narrow band imaging for early detection of laryngeal cancer. *European Archives of Oto-Rhino-Laryngology* 2009, 266(7):1017-1023.

[199] Watanabe A, Taniguchi M, Tsujie H, Hosokawa M, Fujita M, Sasaki S: The value of narrow band imaging endoscope for early head and neck cancer. *Otolaryngology-Head and Neck Surgery* 2008, 138(4):446-451.

[200] Igarashi Y, Okano N, Ito K, Suzuki T, Mimura T: Effectiveness of peroral cholangio-scopy and narrow band imaging for endoscopically diagnosing the bile duct cancer. *Dig Endosc* 2009, 21 Suppl 1:S101-102.

[201] Itoi T, Sofuni A, Itokawa F, Tsuchiya T, Kurihara T: Evaluation of peroral videocho-langioscopy using narrow-band imaging for diagnosis of intraductal papillary neo-plasm of the bile duct. *Dig Endosc* 2009, 21 Suppl 1:S103-107.

[202] Itoi T, Tsuji S, Sofuni A, Itokawa F, Kurihara T, Tsuchiya T, Ishii K, Ikeuchi N, Igara-shi M, Gotoda T *et al*: A novel approach emphasizing preoperative margin enhance-ment of tumor of the major duodenal papilla with narrow-band imaging in comparison to indigo carmine chromoendoscopy (with videos). *Gastrointest Endosc* 2009, 69(1):136-141.

[203] Kiyotoki S, Nishikawa J, Satake M, Fukagawa Y, Shirai Y, Hamabe K, Saito M, Oka-moto T, Sakaida I: Usefulness of magnifying endoscopy with narrow-band imaging for determining gastric tumor margin. *J Gastroenterol Hepatol* 2010, 25(10):1636-1641.

[204] Nagahama T, Yao K, Maki S, Yasaka M, Takaki Y, Matsui T, Tanabe H, Iwashita A, Ota A: Usefulness of magnifying endoscopy with narrow-band imaging for deter-mining the horizontal extent of early gastric cancer when there is an unclear margin by chromoendoscopy (with video). *Gastrointest Endosc* 2011, 74(6):1259-1267.

[205] Muto M, Horimatsu T, Ezoe Y, Morita S, Miyamoto S: Improving visualization tech-niques by narrow band imaging and magnification endoscopy. *Journal of Gastroenter-ology and Hepatology (Australia)* 2009, 24(8):1333-1346.

[206] Piazza C, Dessouky O, Peretti G, Cocco D, De Benedetto L, Nicolai P: Narrow-band imaging: a new tool for evaluation of head and neck squamous cell carcinomas. Re-view of the literature. *Acta otorhinolaryngologica Italica : organo ufficiale della Societa ital-iana di otorinolaringologia e chirurgia cervico-facciale* 2008, 28(2):49-54.

[207] Muto M, Minashi K, Yano T, Saito Y, Oda I, Nonaka S, Omori T, Sugiura H, Goda K, Kaise M *et al*: Early Detection of Superficial Squamous Cell Carcinoma in the Head and Neck Region and Esophagus by Narrow Band Imaging: A Multicenter Random-ized Controlled Trial. *Journal of Clinical Oncology* 2010, 28(9):1566-1572.

[208] Piazza C, Cocco D, Del Bon F, Mangili S, Nicolai P, Majorana A, Bolzoni Villaret A, Peretti G: Narrow band imaging and high definition television in evaluation of oral

and oropharyngeal squamous cell cancer: a prospective study. *Oral Oncol* 2010, 46(4): 307-310.

[209] Piazza C, Cocco D, Del Bon F, Mangili S, Nicolai P, Peretti G: Narrow band imaging and high definition television in the endoscopic evaluation of upper aero-digestive tract cancer. *Acta otorhinolaryngologica Italica : organo ufficiale della Societa italiana di otorinolaringologia e chirurgia cervico-facciale* 2011, 31(2):70-75.

[210] Fielding D, Agnew J, Wright D, Hodge R: Autofluorescence improves pretreatment mucosal assessment in head and neck cancer patients. *Otolaryngol Head Neck Surg* 2010, 142(3):S20-S26.

[211] Nguyen P, Bashirzadeh F, Hodge R, Agnew J, Farah CS, Duhig E, Clarke B, Perry-Keene J, Botros D, Masters IB *et al*: High specificity of combined narrow band imaging and autofluorescence mucosal assessment of patients with head and neck cancer. *Head Neck* 2013, 35(5):619-625.

[212] Vu AN, Farah CS: Efficacy of narrow band imaging for detection and surveillance of potentially malignant and malignant lesions in the oral cavity and oropharynx: A systematic review. *Oral Oncol* 2014.

[213] Yang SW, Lee YS, Chang LC, Chien HP, Chen TA: Light sources used in evaluating oral leukoplakia: broadband white light versus narrowband imaging. *International journal of oral and maxillofacial surgery* 2013, 42(6):693-701.

[214] Roblyer D, Richards-Kortum R, Sokolov K, El-Naggar AK, Williams MD, Kurachi C, Gillenwater AM: Multispectral optical imaging device for in vivo detection of oral neoplasia. *J Biomed Opt* 2008, 13(2):024019.

[215] Chi C, Du Y, Ye J, Kou D, Qiu J, Wang J, Tian J, Chen X: Intraoperative imaging-guided cancer surgery: from current fluorescence molecular imaging methods to future multi-modality imaging technology. *Theranostics* 2014, 4(11):1072-1084.

[216] Dammann F, Bootz F, Cohnen M, Hassfeld S, Tatagiba M, Kosling S: Diagnostic imaging modalities in head and neck disease. *Deutsches Arzteblatt international* 2014, 111(23-24):417-423.

[217] Shah AT, Demory Beckler M, Walsh AJ, Jones WP, Pohlmann PR, Skala MC: Optical metabolic imaging of treatment response in human head and neck squamous cell carcinoma. *PLoS One* 2014, 9(3):e90746.

[218] Nguyen QT, Tsien RY: Fluorescence-guided surgery with live molecular navigation--a new cutting edge. *Nat Rev Cancer* 2013, 13(9):653-662.

[219] Kircher MF, Willmann JK: Molecular body imaging: MR imaging, CT, and US. Part I. Principles. *Radiology* 2012, 263(3):633-643.

[220] Elias DR, Thorek DLJ, Chen AK, Czupryna J, Tsourkas A: In vivo imaging of cancer biomarkers using activatable molecular probes. *Cancer Biomarkers* 2008, 4(6):287-305.

[221] Vogelstein B, Kinzler KW: Cancer genes and the pathways they control. *Nature Medicine* 2004, 10(8):789-799.

[222] Hoeben BA, Bussink J, Troost EG, Oyen WJ, Kaanders JH: Molecular PET imaging for biology-guided adaptive radiotherapy of head and neck cancer. *Acta oncologica (Stockholm, Sweden)* 2013, 52(7):1257-1271.

[223] Hoeben BA, Starmans MH, Leijenaar RT, Dubois LJ, van der Kogel AJ, Kaanders JH, Boutros PC, Lambin P, Bussink J: Systematic analysis of 18F-FDG PET and metabolism, proliferation and hypoxia markers for classification of head and neck tumors. *BMC cancer* 2014, 14:130.

[224] Loja MN, Luo Z, Greg Farwell D, Luu QC, Donald PJ, Amott D, Truong AQ, Gandour-Edwards RF, Nitin N: Optical molecular imaging detects changes in extracellular pH with the development of head and neck cancer. *Int J Cancer* 2013, 132(7): 1613-1623.

[225] Bakker Schut TC, Witjes MJ, Sterenborg HJ, Speelman OC, Roodenburg JL, Marple ET, Bruining HA, Puppels GJ: In vivo detection of dysplastic tissue by Raman spectroscopy. *Anal Chem* 2000, 72(24):6010-6018.

[226] Kendall C, Stone N, Shepherd N, Geboes K, Warren B, Bennett R, Barr H: Raman spectroscopy, a potential tool for the objective identification and classification of neoplasia in Barrett's oesophagus. *J Pathol* 2003, 200(5):602-609.

[227] Shim MG, Song LM, Marcon NE, Wilson BC: In vivo near-infrared Raman spectroscopy: demonstration of feasibility during clinical gastrointestinal endoscopy. *Photochem Photobiol* 2000, 72(1):146-150.

[228] Molckovsky A, Song LM, Shim MG, Marcon NE, Wilson BC: Diagnostic potential of near-infrared Raman spectroscopy in the colon: differentiating adenomatous from hyperplastic polyps. *Gastrointest Endosc* 2003, 57(3):396-402.

[229] Haka AS, Volynskaya Z, Gardecki JA, Nazemi J, Lyons J, Hicks D, Fitzmaurice M, Dasari RR, Crowe JP, Feld MS: In vivo margin assessment during partial mastectomy breast surgery using raman spectroscopy. *Cancer Res* 2006, 66(6):3317-3322.

[230] Stone N, Stavroulaki P, Kendall C, Birchall M, Barr H: Raman spectroscopy for early detection of laryngeal malignancy: preliminary results. *Laryngoscope* 2000, 110(10 Pt 1):1756-1763.

[231] Lau DP, Huang Z, Lui H, Anderson DW, Berean K, Morrison MD, Shen L, Zeng H: Raman spectroscopy for optical diagnosis in the larynx: preliminary findings. *Lasers Surg Med* 2005, 37(3):192-200.

[232] Keller MD, Wilson RH, Mycek MA, Mahadevan-Jansen A: Monte Carlo model of spatially offset Raman spectroscopy for breast tumor margin analysis. *Appl Spectrosc* 2010, 64(6):607-614.

[233] Ellis DI, Cowcher DP, Ashton L, O'Hagan S, Goodacre R: Illuminating disease and enlightening biomedicine: Raman spectroscopy as a diagnostic tool. *Analyst* 2013, 138(14):3871-3884.

[234] Tollefson M, Magera J, Sebo T, Cohen J, Drauch A, Maier J, Frank I: Raman spectral imaging of prostate cancer: can Raman molecular imaging be used to augment standard histopathology? *BJU Int* 2010, 106(4):484-488.

[235] Crow P, Uff JS, Farmer JA, Wright MP, Stone N: The use of Raman spectroscopy to identify and characterize transitional cell carcinoma in vitro. *BJU Int* 2004, 93(9): 1232-1236.

[236] Johnson JT, Branstetter BF: PET/CT in head and neck oncology: State-of-the-art 2013. *Laryngoscope* 2014, 124(4):913-915.

[237] Partovi S, Kohan A, Rubbert C, Vercher-Conejero JL, Gaeta C, Yuh R, Zipp L, Herrmann KA, Robbin MR, Lee Z *et al*: Clinical oncologic applications of PET/MRI: a new horizon. *American journal of nuclear medicine and molecular imaging* 2014, 4(2):202-212.

[238] Al-Nabhani KZ, Syed R, Michopoulou S, Alkalbani J, Afaq A, Panagiotidis E, O'Meara C, Groves A, Ell P, Bomanji J: Qualitative and quantitative comparison of PET/CT and PET/MR imaging in clinical practice. *J Nucl Med* 2014, 55(1):88-94.

[239] Kubiessa K, Purz S, Gawlitza M, Kuhn A, Fuchs J, Steinhoff KG, Boehm A, Sabri O, Kluge R, Kahn T *et al*: Initial clinical results of simultaneous F-18-FDG PET/MRI in comparison to F-18-FDG PET/CT in patients with head and neck cancer. *Eur J Nucl Med Mol Imaging* 2014, 41(4):639-648.

[240] Kuhn FP, Hullner M, Mader CE, Kastrinidis N, Huber GF, von Schulthess GK, Kollias S, Veit-Haibach P: Contrast-enhanced PET/MR imaging versus contrast-enhanced PET/CT in head and neck cancer: how much MR information is needed? *J Nucl Med* 2014, 55(4):551-558.

[241] Partovi S, Kohan A, Vercher-Conejero JL, Rubbert C, Margevicius S, Schluchter MD, Gaeta C, Faulhaber P, Robbin MR: Qualitative and quantitative performance of 18F-FDG-PET/MRI versus 18F-FDG-PET/CT in patients with head and neck cancer. *AJNR American journal of neuroradiology* 2014.

[242] Queiroz M, Hullner M, Kuhn F, Huber G, Meerwein C, Kollias S, von Schulthess G, Veit-Haibach P: PET/MRI and PET/CT in follow-up of head and neck cancer patients. *Eur J Nucl Med Mol Imaging* 2014, 41(6):1066-1075.

[243] Varoquaux A, Rager O, Poncet A, Delattre BMA, Ratib O, Becker CD, Dulguerov P, Dulguerov N, Zaidi H, Becker M: Detection and quantification of focal uptake in head and neck tumours: F-18-FDG PET/MR versus PET/CT. *Eur J Nucl Med Mol Imaging* 2014, 41(3):462-475.

[244] Kanda T, Kitajima K, Suenaga Y, Konishi J, Sasaki R, Morimoto K, Saito M, Otsuki N, Nibu K, Sugimura K: Value of retrospective image fusion of F-18-FDG PET and MRI

for preoperative staging of head and neck cancer: Comparison with PET/CT and contrast-enhanced neck MRI. *Eur J Radiol* 2013, 82(11):2005-2010.

[245]   Lee G, I H, Kim SJ, Jeong YJ, Kim IJ, Pak K, Park DY, Kim GH: Clinical Implication of PET/MR Imaging in Preoperative Esophageal Cancer Staging: Comparison with PET/CT, Endoscopic Ultrasonography, and CT. *J Nucl Med* 2014.

[246]   Evangelista L, Cervino AR, Chondrogiannis S, Marzola MC, Maffione AM, Colletti PM, Muzzio PC, Rubello D: Comparison between anatomical cross-sectional imaging and F-18-FDG PET/CT in the staging, restaging, treatment response, and long-term surveillance of squamous cell head and neck cancer: a systematic literature overview. *Nucl Med Commun* 2014, 35(2):123-134.

[247]   Arias F, Chicata V, Garcia-Velloso MJ, Asin G, Uzcanga M, Eito C, Quilez I, Viudez A, Saenz J, Hernandez I *et al*: Impact of initial FDG PET/CT in the management plan of patients with locally advanced head and neck cancer. *Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico* 2014.

[248]   Adkins D, Ley J, Dehdashti F, Siegel MJ, Wildes TM, Michel L, Trinkaus K, Siegel BA: A prospective trial comparing FDG-PET/CT and CT to assess tumor response to cetuximab in patients with incurable squamous cell carcinoma of the head and neck. *Cancer medicine* 2014.

[249]   Garcheva M, Zlatareva D, Gocheva L: Positron emission tomography combined with computed tomography for diagnosis of synchronous tumors. *Klinicka onkologie : casopis Ceske a Slovenske onkologicke spolecnosti* 2014, 27(4):283-286.

[250]   Bhatnagar P, Subesinghe M, Patel C, Prestwich R, Scarsbrook AF: Functional imaging for radiation treatment planning, response assessment, and adaptive therapy in head and neck cancer. *Radiographics : a review publication of the Radiological Society of North America, Inc* 2013, 33(7):1909-1929.

[251]   Bussink J, van Herpen CML, Kaanders JHAM, Oyen WJG: PET-CT for response assessment and treatment adaptation in head and neck cancer. *Lancet Oncol* 2010, 11(7): 661-669.

[252]   Nordsmark M, Bentzen SM, Rudat V, Brizel D, Lartigau E, Stadler P, Becker A, Adam M, Molls M, Dunst J *et al*: Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiother Oncol* 2005, 77(1):18-24.

[253]   Eschmann SM, Paulsen F, Reimold M, Dittmann H, Welz S, Reischl G, Machulla HJ, Bares R: Prognostic impact of hypoxia imaging with F-18-misonidazole PET in non-small cell lung cancer and head and neck cancer before radiotherapy. *J Nucl Med* 2005, 46(2):253-260.

[254]   Rischin D, Hicks RJ, Fisher R, Binns D, Corry J, Porceddu S, Peters LJ: Prognostic significance of [F-18]-misonidazole positron emission tomography-detected tumor hy-

poxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with or without tirapazamine: A substudy of Trans-Tasman Radiation Oncology Group study 98.02. *J Clin Oncol* 2006, 24(13):2098-2104.

[255]   Thorwarth D, Eschmann S-M, Holzner F, Paulsen F, Alber M: Combined uptake of [(18)F]FDG and [(18)F]FMISO correlates with radiation therapy outcome in head-and-neck cancer patients. *Radiother Oncol* 2006, 80(2):151-156.

[256]   Zimny M, Gagel B, DiMartino E, Hamacher K, Coenen HH, Westhofen M, Eble M, Buell U, Reinartz P: FDG - a marker of tumour hypoxia? A comparison with [F-18] fluoromisonidazole and pO(2)-polarography in metastatic head and neck cancer. *Eur J Nucl Med Mol Imaging* 2006, 33(12):1426-1431.

[257]   Hendrickson K, Phillips M, Smith W, Peterson L, Krohn K, Rajendran J: Hypoxia imaging with [F-18] FMISO-PET in head and neck cancer: Potential for guiding intensity modulated radiation therapy in overcoming hypoxia-induced treatment resistance. *Radiother Oncol* 2011, 101(3):369-375.

[258]   Lee NY, Mechalakos JG, Nehmeh S, Lin Z, Squire OD, Cai S, Chan K, Zanzonico PB, Greco C, Ling CC *et al*: Fluorine-18-labeled fluoromisonidazole positron emission and computed tomography-guided intensity-modulated radiotherapy for head and neck cancer: A feasibility study. *Int J Radiat Oncol Biol Phys* 2008, 70(1):2-13.

[259]   Rajendran JG, Hendrickson KRG, Spence AM, Muzi M, Krohn KA, Mankoff DA: Hypoxia imaging-directed radiation treatment planning. *Eur J Nucl Med Mol Imaging* 2006, 33 Suppl 1:44-53.

[260]   Thorwarth D, Eschmann S-M, Paulsen F, Alber M: Hypoxia dose painting by numbers: A planning study. *Int J Radiat Oncol Biol Phys* 2007, 68(1):291-300.

[261]   Nehmeh SA, Lee NY, Schroder H, Squire O, Zanzonico PB, Erdi YE, Greco C, Mageras G, Pham HS, Larson SM *et al*: Reproducibility of intratumor distribution of F-18-fluoromisonidazole in head and neck cancer. *Int J Radiat Oncol Biol Phys* 2008, 70(1): 235-242.

[262]   Lin Z, Mechalakos J, Nehmeh S, Schoder H, Lee N, Humm J, Ling CC: The influence of changes in tumor hypoxia on dose-painting treatment plans based on (18)F-FMISO positron emission tomography. *Int J Radiat Oncol Biol Phys* 2008, 70(4):1219-1228.

[263]   Wang W, Lee NY, Georgi J-C, Narayanan M, Guillem J, Schoeder H, Humm JL: Pharmacokinetic analysis of hypoxia F-18-fluoromisonidazole dynamic PET in head and neck cancer. *J Nucl Med* 2010, 51(1):37-45.

[264]   Piert M, Machulla HJ, Picchio M, Reischl G, Ziegler S, Kumar P, Wester HJ, Beck R, McEwan AJB, Wiebe LI *et al*: Hypoxia-specific tumor imaging with F-18-fluoroazomycin arabinoside. *J Nucl Med* 2005, 46(1):106-113.

[265] Gronroos T, Eskola I, Lehtio K, Minn H, Marjamaki P, Bergman J, Haaparanta M, Forsback S, Solin O: Pharmacokinetics of [F-18]FETNIM: A potential hypoxia marker for PET. *J Nucl Med* 2001, 42(9):1397-1404.

[266] Vavere AL, Lewis JS: Cu-ATSM: A radiopharmaceutical for the PET imaging of hypoxia. *Dalton Trans* 2007(43):4893-4902.

[267] Minagawa Y, Shizukuishi K, Koike I, Horiuchi C, Watanuki K, Hata M, Omura M, Odagiri K, Tohnai I, Inoue T *et al*: Assessment of tumor hypoxia by Cu-62-ATSM PET/CT as a predictor of response in head and neck cancer: a pilot study. *Ann Nucl Med* 2011, 25(5):339-345.

[268] Chao KSC, Bosch WR, Mutic S, Lewis JS, Dehdashti F, Mintun MA, Dempsey JF, Perez CA, Purdy JA, Welch MJ: A novel approach to overcome hypoxic tumor resistance: Cu-ATSM-guided intensity-modulated radiation therapy. *Int J Radiat Oncol Biol Phys* 2001, 49(4):1171-1182.

[269] Weber WA: Monitoring tumor response to therapy with F-18-FLT PET. *J Nucl Med* 2010, 51(6):841-844.

[270] Rasey JS, Grierson JR, Wiens LW, Kolb PD, Schwartz JL: Validation of FLT uptake as a measure of thymidine kinase-1 activity in A549 carcinoma cells. *J Nucl Med* 2002, 43(9):1210-1217.

[271] Troost EGC, Bussink J, Hoffmann AL, Boerman OC, Oyen WJG, Kaanders JHAM: F-18-FLT PET/CT for early response monitoring and dose escalation in oropharyngeal tumors. *J Nucl Med* 2010, 51(6):866-874.

[272] Troost EGC, Vogel WV, Merkx MAW, Slootweg PJ, Marres HAM, Peeters WJM, Bussink J, van der Kogel AJ, Oyen WJG, Kaanders JHAM: F-18-FLT PET does not discriminate between reactive and metastatic lymph nodes in primary head and neck cancer patients. *J Nucl Med* 2007, 48(5):726-735.

[273] Cotter TG: Apoptosis and cancer: the genesis of a research field. *Nat Rev Cancer* 2009, 9(7):501-507.

[274] Hoebers FJP, Kartachova M, de Bois J, van den Brekel MWM, van Tinteren H, van Herk M, Rasch CRN, Olmos RAV, Verheij M: Tc-99m Hynic-rh-Annexin V scintigraphy for in vivo imaging of apoptosis in patients with head and neck cancer treated with chemoradiotherapy. *Eur J Nucl Med Mol Imaging* 2008, 35(3):509-518.

[275] Hoglund J, Shirvan A, Antoni G, Gustavsson S-A, Langstrom B, Ringheim A, Sorensen J, Ben-Ami M, Ziv I: F-18-ML-10, a PET tracer for apoptosis: first human study. *J Nucl Med* 2011, 52(5):720-725.

[276] Hoffman RM: Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis. *Biochim Biophys Acta* 1984, 738(1-2):49-87.

[277] Leskinenkallio S, Nagren K, Lehikoinen P, Ruotsalainen U, Teras M, Joensuu H: Carbon-11-methionine and PET is an effective method to image head and neck cancer. *J Nucl Med* 1992, 33(5):691-695.

[278] Lindholm P, Leskinenkallio S, Minn H, Bergman J, Haaparanta M, Lehikoinen P, Nagren K, Ruotsalainen U, Teras M, Joensuu H: Comparison of fluorine-18-fluorodeoxyglucose and carbon-11-methionine in head and neck cancer. *J Nucl Med* 1993, 34(10):1711-1716.

[279] Lindholm P, Leskinenkallio S, Grenman R, Lehikoinen P, Nagren K, Teras M, Ruotsalainen U, Joensuu H: Evaluation of response to radiotherapy in dead-end neck-cancer by positron emission tomography and [C-11] methionine. *Int J Radiat Oncol Biol Phys* 1995, 32(3):787-794.

[280] Chesnay E, Babin E, Constans JM, Agostini D, Bequignon A, Regeasse A, Sobrio F, Moreau S: Early response to chemotherapy in hypopharyngeal cancer: Assessment with C-11-methionine PET, correlation with morphologic response, and clinical outcome. *J Nucl Med* 2003, 44(4):526-532.

[281] Nuutinen J, Jyrkkio S, Lehikoinen P, Lindholm P, Minn H: Evaluation of early response to radiotherapy in head and neck cancer measured with [C-11]methionine-positron emission tomography. *Radiother Oncol* 1999, 52(3):225-232.

[282] Balogova S, Perie S, Kerrou K, Grahek D, Montravers F, Angelard B, Susini B, El Chater P, St Guily JL, Talbot JN: Prospective comparison of FDG and FET PET/CT in patients with head and neck squamous cell carcinoma. *Mol Imaging Biol* 2008, 10(6): 364-373.

[283] Haerle SK, Fischer DR, Schmid DT, Ahmad N, Huber GF, Buck A: F-18-FET PET/CT in advanced head and neck squamous cell carcinoma: an intra-individual comparison with F-18-FDG PET/CT. *Mol Imaging Biol* 2011, 13(5):1036-1042.

[284] Pauleit D, Zimmermann A, Stoffels G, Bauer D, Risse J, Fluss MO, Hamacher K, Coenen HH, Langen KJ: F-18-FET PET compared with F-18-FDG PET and CT in patients with head and neck cancer. *J Nucl Med* 2006, 47(2):256-261.

[285] Wester HJ, Herz M, Weber W, Heiss P, Senekowitsch-Schmidtke R, Schwaiger M, Stocklin G: Synthesis and radiopharmacology of O-(2-[F-18]fluoroethyl)-L-tyrosine for tumor imaging. *J Nucl Med* 1999, 40(1):205-212.

[286] Roivainen A, Forsback S, Gronroos T, Lehikoinen P, Kahkonen M, Sutinen E, Minn H: Blood metabolism of [methyl-C-11]choline; implications for in vivo imaging with positron emission tomography. *Eur J Nucl Med* 2000, 27(1):25-32.

[287] Khan N, Oriuchi N, Ninomiya H, Higuchi T, Kamada H, Endo K: Positron emission tomographic imaging with C-11-choline in differential diagnosis of head and neck tumors: comparison with F-18-FDG PET. *Ann Nucl Med* 2004, 18(5):409-417.

[288] Ito K, Yokoyama J, Kubota K, Morooka M, Shiibashi M, Matsuda H: F-18-FDG versus C-11-choline PET/CT for the imaging of advanced head and neck cancer after com-

bined intra-arterial chemotherapy and radiotherapy: the time period during which PET/CT can reliably detect non-recurrence. *Eur J Nucl Med Mol Imaging* 2010, 37(7): 1318-1327.

[289] Chen YJ, Wu SC, Chen CY, Tzou SC, Cheng TL, Huang YF, Yuan SS, Wang YM: Peptide-based MRI contrast agent and near-infrared fluorescent probe for intratumoral legumain detection. *Biomaterials* 2014, 35(1):304-315.

[290] Luo T, Huang P, Gao G, Shen G, Fu S, Cui D, Zhou C, Ren Q: Mesoporous silica-coated gold nanorods with embedded indocyanine green for dual mode X-ray CT and NIR fluorescence imaging. *Opt Express* 2011, 19(18):17030-17039.

[291] Peloso A, Franchi E, Canepa MC, Barbieri L, Briani L, Ferrario J, Bianco C, Quaretti P, Brugnatelli S, Dionigi P *et al*: Combined use of intraoperative ultrasound and indocyanine green fluorescence imaging to detect liver metastases from colorectal cancer. *HPB (Oxford)* 2013, 15(12):928-934.

[292] Jin XJ, Zhou L, Zhao AG: Mutants of p53 gene presence in laryngeal carcinoma and adjacent histopathologically normal tissue. *Orl-Journal for Oto-Rhino-Laryngology and Its Related Specialties* 2000, 62(3):140-142.

[293] van der Toorn PPG, Veltman JA, Bot FJ, de Jong JMA, Manni JJ, Ramaekers FCS, Hopman AHN: Mapping of resection margins of oral cancer for p53 overexpression and chromosome instability to detect residual (pre)malignant cells. *J Pathol* 2001, 193(1):66-72.

[294] Shin DM, Charuruks N, Lippman SM, Lee JJ, Ro JY, Hong WK, Hittelman WN: p53 protein accumulation and genomic instability in head and neck multistep tumorigenesis. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2001, 10(6):603-609.

[295] Szukala K, Brieger J, Bruch K, Biczysko W, Wierzbicka M, Szyfter W, Szyfter K: Loss of heterozygosity on chromosome arm 13q in larynx cancer patients: analysis of tumor, margin and clinically unchanged mucosa. *Medical science monitor : international medical journal of experimental and clinical research* 2004, 10(6):Cr233-240.

[296] Shaw RJ, Hall GL, Woolgar JA, Lowe D, Rogers SN, Field JK, Liloglou T, Risk JM: Quantitative methylation analysis of resection margins and lymph nodes in oral squamous cell carcinoma. *Br J Oral Maxillofac Surg* 2007, 45(8):617-622.

[297] Barrera JE, Ai H, Pan ZX, Meyers AD, Varella-Garcia M: Malignancy detection by molecular cytogenetics in clinically normal mucosa adjacent to head and neck tumors. *Arch Otolaryngol Head Neck Surg* 1998, 124(8):847-851.

[298] Voravud N, Shin DM, Ro JY, Lee JS, Hong WK, Hittelman WN: Increased polysomies of chromosome-7 and chromosome-17 during head and neck multistage tumorigenesis. *Cancer Res* 1993, 53(12):2874-2883.

[299] Fabricius EM, Gurr U, Wildner GP: Telomerase activity levels in the surgical margin and tumour distant tissue of the squamous cell carcinoma of the head-and-neck. *Anal Cell Pathol* 2002, 24(1):25-39.

[300] Graveland AP, de Maaker M, Braakhuis BJ, de Bree R, Eerenstein SE, Bloemena E, Leemans CR, Brakenhoff RH: Molecular detection of minimal residual cancer in surgical margins of head and neck cancer patients. *Cell Oncol* 2009, 31(4):317-328.

[301] Dasgupta S, Koch R, Westra WH, Califano JA, Ha PK, Sidransky D, Koch WM: Mitochondrial DNA mutation in normal margins and tumors of recurrent head and neck squamous cell carcinoma patients. *Cancer Prev Res (Phila)* 2010, 3(9):1205-1211.

[302] Huang X, Pateromichelakis S, Hills A, Sherriff M, Lyons A, Langdon J, Odell E, Morgan P, Harrison J, Partridge M: p53 mutations in deep tissues are more strongly associated with recurrence than mutation-positive mucosal margins. *Clin Cancer Res* 2007, 13(20):6099-6106.

[303] Ogbureke KU, Weinberger PM, Looney SW, Li L, Fisher LW: Expressions of matrix metalloproteinase-9 (MMP-9), dentin sialophosphoprotein (DSPP), and osteopontin (OPN) at histologically negative surgical margins may predict recurrence of oral squamous cell carcinoma. *Oncotarget* 2012, 3(3):286-298.

[304] Reis PP, Waldron L, Perez-Ordonez B, Pintilie M, Galloni NN, Xuan Y, Cervigne NK, Warner GC, Makitie AA, Simpson C *et al*: A gene signature in histologically normal surgical margins is predictive of oral carcinoma recurrence. *BMC cancer* 2011, 11:437.

[305] Montebugnoli L, Gissi DB, Badiali G, Marchetti C, Cervellati F, Farnedi A, Foschini MP: Ki-67 from clinically and histologically "normal" distant mucosa as prognostic marker in early-stage (T1-T2N0) oral squamous cell carcinoma: a prospective study. *Journal of oral and maxillofacial surgery : official journal of the American Association of Oral and Maxillofacial Surgeons* 2011, 69(10):2579-2584.

[306] Temam S, Casiraghi O, Lahaye JB, Bosq J, Zhou X, Julieron M, Mamelle G, Lee JJ, Mao L, Luboinski B *et al*: Tetranucleotide microsatellite instability in surgical margins for prediction of local recurrence of head and neck squamous cell carcinoma. *Clin Cancer Res* 2004, 10(12):4022-4028.

[307] Handschel J, Oz D, Pomjanski N, Depprich R, Ommerborn MA, Braunstein S, Kuebler NR, Meyer U, Boecking A: Additional use of DNA-image cytometry improves the assessment of resection margins. J Oral Pathol Med 2007, 36(8):472-475.

[308] Zhao H, Ren J, Zhuo X, Ye H, Zou J, Liu S: Prognostic significance of Survivin and CD44v6 in laryngeal cancer surgical margins. Journal of cancer research and clinical oncology 2008, 134(10):1051-1058.

[309] Schaaij-Visser TB, Graveland AP, Gauci S, Braakhuis BJ, Buijze M, Heck AJ, Kuik DJ, Bloemena E, Leemans CR, Slijper M et al: Differential Proteomics Identifies Protein Biomarkers That Predict Local Relapse of Head and Neck Squamous Cell Carcinomas. Clin Cancer Res 2009, 15(24):7666-7675.