TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

TRANSLOCATOR PROTEIN AS A BIOMARKER IN HEAD AND NECK CANCER

Sanni Tuominen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1857 | MEDICA – ODONTOLOGICA | TURKU 2025

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

TRANSLOCATOR PROTEIN AS A BIOMARKER IN HEAD AND NECK CANCER

Sanni Tuominen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1857 | MEDICA – ODONTOLOGICA | TURKU 2025

University of Turku

Faculty of Medicine
Department of Clinical Medicine
Clinical Physiology and Nuclear Medicine
Turku Doctoral Programme of Molecular Medicine
Turku PET Centre
MediCity Research Laboratory
FICAN West

Supervised by

Adjunct Professor Tove Grönroos
Turku PET Centre
University of Turku
Turku, Finland

Adjunct Professor Maria Sundvall
Institute of Biomedicine
University of Turku
Department of Oncology
Turku University Hospital
Turku, Finland

Reviewed by

Professor Linda Marklund
Department of Clinical Research,
Invention and Technology
Karolinska Institutet
Stockholm, Sweden

Associate Professor Ahmed Al-Samadi
Institute of Dentistry
School of Medicine
Faculty of Health Sciences
University of Eastern Finland
Joensuu, Finland

Opponent

Professor Esther Troost
Department of Radiotherapy and Radiation Oncology
Faculty of Medicine and University Hospital Carl Gustav Carus
Technische Universität Dresden
Dresden, Germany

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-952-02-0058-9 (PRINT)
ISBN 978-952-02-0059-6 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2025

To my family and friends

UNIVERSITY OF TURKU
Faculty of Medicine
Department of Clinical Medicine
Clinical Physiology and Nuclear Medicine
SANNI TUOMINEN: Translocator protein as a biomarker in head and neck cancer
Doctoral Dissertation, 209 pp.
Turku Doctoral Programme of Molecular Medicine
April 2025

ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) consists of heterogeneous tumours originating from the head and neck region. Although advances in the treatment of HNSCC have been made during the last decade, cancer recurrence and treatment resistance remain challenges in HNSCC. Currently, human papilloma virus (HPV), and its surrogate marker p16 for oropharyngeal cancer and Epstein–Barr virus (EBV) for nasopharyngeal cancer are the only biomarkers widely used in clinics. Thus, there is an urgent need for biomarkers to predict the prognosis and treatment response of HNSCC.

Translocator protein (TSPO) is a small protein located on the outer mitochondrial membrane. It has been suggested that it participates in several cellular functions, but its exact role remains unclear. Studies have shown an association between high TSPO expression and worse prognosis in several types of cancer.

The aim of this thesis was to evaluate the prognostic and predictive potential of TSPO in HNSCC. TSPO expression was determined in a large population-based tissue microarray (TMA) and The Cancer Genome Atlas (TCGA) patient cohorts, and possible associations with clinicopathological information were analysed. In addition, the role of TSPO in oxidative metabolism and DNA damage response was studied in HNSCC cells. Finally, the specificity of the TSPO-PET tracer [^{18}F]F-DPA was evaluated in HNSCC xenografts and cell lines.

Lower TSPO tumour expression was associated with worse survival in HNSCC, particularly in patients with p16-positive oropharyngeal cancer. Furthermore, lower TSPO expression was associated with worse survival in patients receiving radiotherapy. Low TSPO expression was associated with reduced mitochondrial respiration and DNA damage response in FaDu cells. The [^{18}F]F-DPA uptake was specific in HNSCC xenografts and cells and increased after radiotherapy.

The results of this thesis indicate that TSPO can be used as a prognostic biomarker of HNSCC and, mechanistically, may participate in the radiotherapy-induced DNA damage response via the regulation of oxidative phosphorylation. Further investigation in the usability of TSPO-PET imaging for predicting radiotherapy response and clinical value of the association between low TSPO expression and worse outcomes in p16-positive oropharyngeal cancer are warranted.

KEYWORDS: TSPO; head and neck squamous cell carcinoma; biomarker; tissue microarray; PET; radiotherapy; oxidative phosphorylation; DNA damage response

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Kliininen fysiologia ja isotooppilääketiede
SANNI TUOMINEN: Translokaattoriproteiini biomarkkerina pään ja kaulan alueen syövissä
Väitöskirja, 209 s.
Molekyylilääketieteen tohtoriohjelma
Huhtikuu 2025

TIIVISTELMÄ

Pään ja kaulan alueen levyepiteelisyövät ovat heterogeeninen joukko pään ja kaulan alueen kasvaimia. Vaikka kyseisten kasvainten hoidot ovat kehittyneet viimeisen vuosikymmenen aikana, syövän uusiutuminen ja hoitoresistenssi ovat yhä haasteita. Ihmisen papilloomavirus ja sen korvike p16 suunielun syövissä ja Epstein-Barr virus nenänielun syövissä ovat ainoat biomarkkerit, joita käytetään laajasti eri klinikoissa. Uusien biomarkkerien löytäminen pään ja kaulan alueen syöpien ennusteen ja hoitovasteen arvioimiseen onkin ensiarvoisen tärkeää.

Translokaattoriproteiini (TSPO) on mitokondrion ulkokalvolla sijaitseva proteiini, jonka on ehdotettu osallistuvan moniin solun toimintoihin. TSPOn korkean ilmentymisen on osoitettu liittyvän potilaan huonoon ennusteeseen useissa syövissä.

Tässä väitöskirjassa tutkittiin TSPOn kykyä toimia ennusteellisena biomarkkerina pään ja kaulan alueen syövissä väestöpohjaista kudosmikrosirua ja TCGAn potilaskohorttia käyttäen. Lisäksi tutkittiin TSPOn toiminnallista roolia oksidatiivisen fosforylaation ja DNA-vauriovasteen säätelyssä pään ja kaulan alueen syöpäsoluissa. Lopuksi määritettiin TSPO-PET merkkiaineen, [^{18}F]F-DPAn, spesifisyys pään ja kaulan alueen kokeellisissa kasvaimissa ja soluissa.

Matalampi TSPOn ilmentyminen liittyi potilaan huonompaan ennusteeseen etenkin p16-positiivisisa suunielun syövissä. Lisäksi matala TSPOn ilmentyminen liittyi huonompaan ennusteeseen pelkästään sädehoidetuilla potilailla. Matala TSPOn ilmentyminen liittyi matalampaan oksidatiiviseen aineenvaihduntaan ja DNA-vaurion vasteeseen. [^{18}F]F-DPAn kertymä oli spesifistä pään ja kaulan alueen syöpien kokeellisissa kasvaimissa ja soluissa ja kertymä nousi sädehoidon jälkeen.

Tutkimuksen tulokset osoittavat, että TSPOlla on potentiaalia toimia ennusteellisena biomarkkerina pään ja kaulan alueen syövissä. TSPO saattaa säädellä sädehoidon aiheuttamaa DNA-vauriovastetta oksidatiivisen fosforylaation säätelyn välityksellä. Sädehoitovasteen ennustaminen PET-kuvantamisella TSPO-merkkiainetta hyödyntäen vaatii lisätutkimuksia. TSPOn yhteys huonompaan ennusteeseen p16-positiivisissa suunielun syövissä saattaa olla kliinisesti merkitsevä löydös ja vaatii jatkotutkimuksia.

AVAINSANAT: TSPO; pään ja kaulan alueen levyepiteelisyöpä; biomarkkeri; kudosmikrosiru; PET; sädehoito; oksidatiivinen fosforylaatio; DNA-vauriovaste

Table of Contents

Abbreviations

[^{18}F]FDG	2-deoxy-2-[^{18}F]fluoro-D-glucose
[^{18}F]F-DPA	N,N-Diethyl-2-(2-(4-[^{18}F]fluorophenyl)-5,7-dimethylpyrazolo[1,5-α]pyrimidine-3-yl)-acetamide
ADP	Adenosine diphosphate
AJCC	American Joint Committee on Cancer
AKT	Protein kinase B, v-Akt murine thymoma viral oncogene homolog 1
ANOVA	Analysis of variance
ANT	Adenine nucleotide transporter
ARF	ADP ribosylation factor
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
ATR	Ataxia telangiectasia and Rad3-related
CDK	Cyclin-dependent kinase
CDKN2A	Cyclin-dependent kinase inhibitor 2A
Chk1	Checkpoint kinase 1
CI	Confidence interval
CNS	Central nervous system
CPTAC	Clinical Proteomics Tumor Analysis Consortium
CRAC	Cholesterol recognition amino acid consensus sequence
CT	Computed tomography
CTC	Circulating tumour cell
CSC	Cancer stem cell
DBI	Diazepam binding inhibitor
DFS	Disease-free survival
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSB	Double-strand break
DSS	Disease-specific survival
EBV	Epstein–Barr virus
EGFR	Epidermal growth factor receptor
EOS	End of synthesis

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
γH2Ax	Phosphorylated H2A histone family member X
HIF-1	Hypoxia inducible factor 1
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
HR	Hazard ratio
IHC	Immunohistochemistry
LSD	Least significant difference
MPT	Mitochondrial permeability transition
MPTP	Mitochondrial permeability transition pore
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
OCR	Oxygen consumption rate
ODN	Octadecaneuropeptide
OS	Overall survival
OXPHOS	Oxidative phosphorylation
p16	$p16^{INK4a}$, cyclin-dependent kinase inhibitor 2A
PARP1	Poly [ADP-ribose] polymerase 1
PBR	Peripheral benzodiazepine receptor
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PET	Positron emission tomography
PFS	Progression-free survival
PHH3	Phospho-histone H3
PI3K	Phosphatidylinositol 3-kinase
pRb	Retinoblastoma protein
RNA	Ribonucleic acid
ROI	Region of interest
ROS	Reactive oxygen species
RPLP0	Ribosomal protein lateral stalk subunit P0
RT	Radiotherapy
RT-qPCR	Real-time quantitative PCR
SD	Standard deviation
siRNA	Small-interfering RNA
StAR	Steroidogenic acute regulatory protein
T/B	Tumour-to-blood
T/P	Tumour-to-plasma
TAM	Tumour-associated macrophage
TBP	TATA-binding protein

TCGA	The Cancer Genome Atlas
TLC	Thin-layer chromatography
TME	Tumour microenvironment
TNF-α	Tumour necrosis factor α
TNM	Tumour-node-metastasis
TP53	Tumour protein P53
TSPO	Translocator protein
TTN	Triakontatetraneuropeptide
UICC	International Union Against Cancer
UV	Ultraviolet
v	Volume
VDAC	Voltage-dependent anion channel
VOI	Volume of interest

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Tuominen S, Nissi L, Kukkula A, Routila J, Huusko T, Leivo I, Minn H, Irjala H, Löyttyniemi E, Ventelä S, Sundvall M, Grönroos TJ. TSPO is a potential independent prognostic factor associated with cellular respiration and p16 in head and neck squamous cell carcinoma. *Frontiers in Oncology*, 2023; 13: 1298333.

II Tuominen S, Ketonen P, Nissi L, Auer K, Suilamo S, Ventelä S, Sundvall M, Grönroos TJ. TSPO modulates DNA damage response to radiotherapy in head and neck squamous cell carcinoma. *Manuscript*, 2024.

III Tuominen S, Keller T, Petruk N, López-Picón F, Eichin D, Löyttyniemi E, Verhassel A, Rajander J, Sandholm J, Tuomela J, Grönroos TJ. Evaluation of [^{18}F]F-DPA as a target for TSPO in head and neck cancer under normal conditions and after radiotherapy. *European Journal of Nuclear Medicine and Molecular Imaging*, 2021; 48: 1312–1326.

1 Introduction

Translocator protein (TSPO) is a small 18-kDa protein located on the outer mitochondrial membrane (Braestrup & Squires, 1977; Squires & Braestrup, 1977; Betlazar et al., 2020). Although the functional role of TSPO has been studied for decades, studies have reported contradictory results; therefore, its function has remained unclear (Bonsack & Sukumari-Ramesh, 2018). High TSPO expression is associated with aggressive phenotype in many cancers (Katz et al., 1988; Hardwick et al., 1999; Maaser et al., 2002; Galiègue et al., 2004; Bhoola et al., 2018). However, the role of TSPO in head and neck squamous cell carcinoma (HNSCC) has not been extensively studied. In fact, only one study had been published at the beginning of this thesis, reporting that high TSPO tumour expression was associated with poor survival in a relatively small cohort of patients with oral cancer (Nagler et al., 2010).

As TSPO is highly expressed in neurological disorders, its use in positron emission tomography (PET) has been widely studied and most TSPO-PET tracers have been developed to image neurological disorders (Zhang et al. 2021). However the use of TSPO-PET tracers for cancer imaging has recently gained increased interest (Vasdev et al., 2013; Zheng et al., 2016; de Souza et al., 2022).

HNSCC is the sixth most common cancer worldwide, and its 5-year survival rate varies from less than 50% to up to 90% (Chow, 2020; Johnson et al., 2020; Mody et al., 2021; Bray et al., 2024). HNSCC is mainly treated with surgery or radiotherapy with or without chemotherapy (Chow, 2020; Johnson et al., 2020). Although several improvements have been achieved in the treatment of HNSCC, cancer recurrence and treatment resistance remain common (Alsahafi et al., 2019; Johnson et al., 2020). Arduous treatments can also cause many harmful side effects. Thus, biomarkers that can predict treatment response or prognosis in HNSCC would be highly valuable. Human papilloma virus (HPV) and its surrogate marker p16, which is common in oropharyngeal cancer, and Epstein–Barr virus (EBV), prevalent in nasopharyngeal cancer, are the only biomarkers widely used in clinics (Kang et al., 2015). Several promising biomarkers are being studied, but none have yet been approved for clinical use (Budach & Tinhofer, 2019; Eberly et al., 2024).

In this thesis, the prognostic potential of TSPO was evaluated in two HNSCC cohorts–a large population-based tissue microarray (TMA) cohort and in a publicly

available The Cancer Genome Atlas (TCGA) cohort. Furthermore, *in silico* analyses were conducted to determine the functional role of TSPO in HNSCC. Based on our *in silico* findings, we then studied the role of TSPO in oxidative metabolism and DNA damage response in HNSCC cells. Finally, the specificity of the TSPO-PET tracer [^{18}F]F-DPA in HNSCC xenografts and cells was evaluated. An overview of the main aims of each study is represented in **Figure 1**.

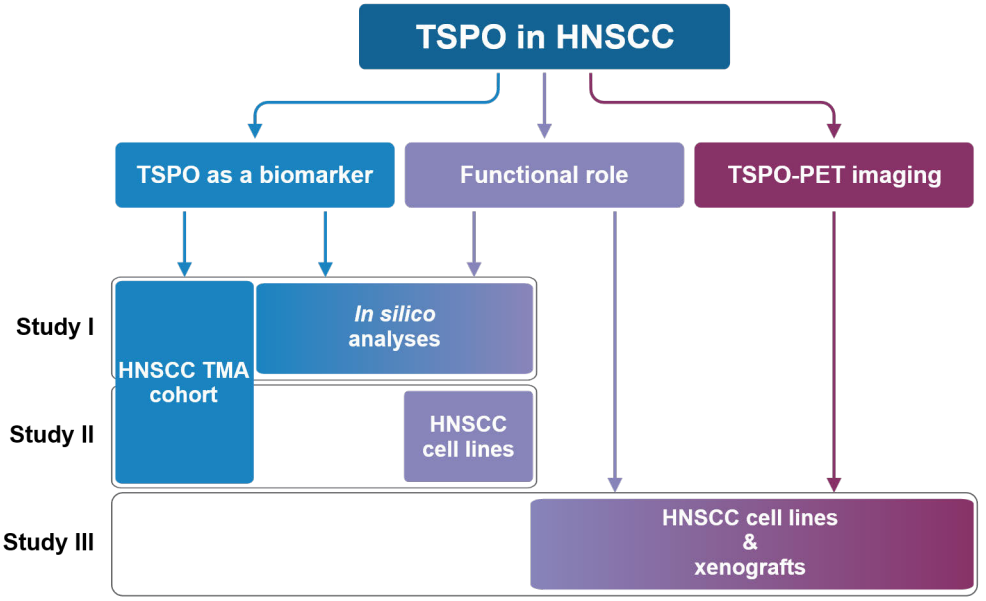

Figure 1. Main aims of each study. Created with BioRender.com.

2 Review of the Literature

2.1 Head and neck squamous cell carcinoma (HNSCC)

HNSCC is a heterogenous cancer consisting of tumours originating from the head and neck area (Chow, 2020; Mody et al., 2021). It is the sixth most common cancer worldwide (Chow, 2020; Bray et a., 2024), and towards 2050, the number of new HNSCC cases is estimated to increase (Ferlay et al., 2024).

Survival outcomes of patients with HNSCC vary significantly, depending on several factors, such as the time of diagnosis and tumour site. Patients diagnosed with early-stage HNSCC have 5-year survival rates ranging from 70% to up to 90%. However, patients with late-stage HNSCC have less than 50% survival rates (Johnson et al., 2020).

HNSCC is more common in men than in women (Barsouk et al., 2023). The incidence of HNSCC also differs by geographical region; for example HNSCC tumours in the oral cavity are more common in Southern and Central Asia and some areas in Oceania than in other continents (Miranda-Filho & Bray, 2020). On the other hand, the number of HPV-positive tumours located in the oropharynx has been increasing in North America and Europe (Gillison et al., 2015).

2.1.1 Anatomical sites and symptoms

HNSCC tumours can be divided according to the primary site of the lesion (**Figure 2**). The tumours are generally located in the oral cavity, oropharynx, hypopharynx, nasopharynx, or larynx.

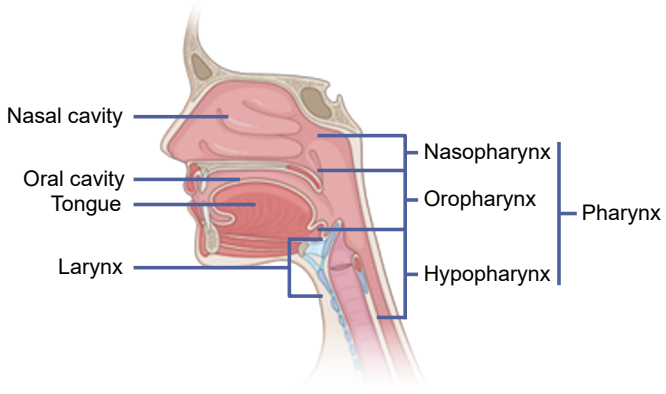

Figure 2. The most common HNSCC sites. Created with BioRender.com.

2.1.1.1 Oral cavity

Squamous cell carcinoma of the oral cavity includes tumours originating from the lips, mobile tongue, floor of the mouth, buccal mucosa, upper and lower alveolar ridge, retromolar trigone, and hard palate (Chen & Myers, 2001; Johnson et al., 2020). However, lip cancer differs pathologically and aetiologically from other oral cancers, and is thus considered as a separate entity (Vigneswaran & Williams, 2014). Cancer of the oral cavity can develop from different potentially malignant lesions, including leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis (Montero & Patel, 2015). Leukoplakia is described as white patches or plagues that are not characterized as any other disease and increase the risk of developing malignant lesions (Kramer et al., 1978; Warnakulasuriya et al., 2007). It is often associated with alcohol and tobacco use (Montero & Patel, 2015). Erythroplakia has the highest risk of malignant transformation and is described as a bright red patch that cannot be characterised as any other disease. Lichen planus is an immune condition that causes hyperkeratosis, erythema (redness of mucous membranes), and possibly ulceration, whereas oral submucous fibrosis is a chronic disorder associated with the presence of fibrous lines in the mucosa and considered a potentially malignant disorder (Warnakulasuriya et al., 2007).

Early-stage oral cancer is often asymptomatic or otherwise difficult to notice. However, pain can be an early symptom in certain areas, such as the tongue or the floor of the mouth (Bagan et al., 2010). Later, changes in the oral mucosa can be seen (e.g. leukoplakia). Progressed oral cancer causes bleeding in the oral area, loosening of teeth, difficulty wearing dentures, difficulties in swallowing (dysphagia), slurred speech (dysarthria), pain when swallowing (odynophagia), and development of a neck mass (Neville & Day, 2002). Although examination of the

oral cavity is easy, a significant number of oral cancers are detected at an advanced stage (Chamoli et al., 2021). Moreover, there has been a noticeable rise in incidence rates, particularly among younger individuals. However, these patients lack the common risk factors, such as tobacco and alcohol use, leaving the cause of the increased rates unclear (Hussein et al., 2017; Gormley et al., 2022).

2.1.1.2 Oropharynx

Oropharyngeal cancer can be divided according to the tumour site in the base of the tongue, tonsillar region, soft palate, uvula, and posterior and lateral pharyngeal walls (Huber & Tantiwongkosi, 2014). Oropharyngeal cancer is a relatively rare disease. However, the number of HPV-positive oropharyngeal cancers has been increasing in the recent decades (see section 2.1.2 for details) (Gillison et al., 2015). Symptoms for early oropharyngeal cancer are often mistaken for something else. These symptoms include sensation of a foreign body, nasal regurgitation, odynophagia, and ear pain (otalgia) (Licitra et al., 2002). Later, a mass, nodule, or non-healable ulcer is present, and sometimes colour changes in the oropharyngeal area. More advanced cancer causes pain, dysphagia, inflammation in the ear (otitis), weight loss, fixation, and lockjaw (trismus) (Huber & Tantiwongkosi, 2014). Metastasis to the regional lymph nodes in the neck is frequently observed even in early-stage oropharyngeal cancer (Shimizu et al., 2006) and is often the initial symptom in HPV-positive oropharyngeal cancer (McIlwain et al., 2014).

2.1.1.3 Hypopharynx

Hypopharyngeal cancer subsites are located in the pyriform sinuses, lateral and posterior pharyngeal walls, and postcricoid region (Garneau et al., 2018). Hypopharyngeal cancer is uncommon in developed countries and is often associated with heavy alcohol and tobacco consumption (Bradley, 2019). Hypopharyngeal cancer can be asymptomatic until metastasis or invasion to surrounding tissues occurs, or symptoms may be misinterpreted for something else, such as reflux (Garneau et al., 2018). The most common symptoms are dysphagia, neck mass, hoarseness, and sore throat (Hall et al., 2008).

2.1.1.4 Larynx

Laryngeal cancer is common in Europe, particularly in men (Licitra et al., 2003). The larynx can be divided into three subsites: supraglottis, glottis, and epiglottis. The supraglottis can be further divided into five subsites: suprahyoid epiglottis, infrahyoid epiglottis, false vocal folds, arytenoids, and aryepiglottic folds. The

glottis consists of the vocal cords and the rima glottis, i.e. the space between vocal cords (Steuer et al., 2017). One of the most common symptoms of laryngeal cancer is hoarseness, and depending on the tumour location, it is a late or early symptom. Other symptoms include dysphagia, coughing, and the sensation of a foreign body (Licitra et al., 2003).

2.1.1.5 Nasopharynx

Nasopharyngeal cancer is a biologically and aetiologically distinct cancer from other HNSCCs (Bruce et al., 2015) and is often associated with EBV infection (Chen et al., 2019) (see section 2.1.2 for details). It is uncommon, and most cases are diagnosed in East and Southeast Asia. Most often the tumour is located in the pharyngeal recess (fossa of Rosenmüller). Typical symptoms of nasopharyngeal cancer include nose bleeding (epistaxis), unilateral nasal obstruction, conductive hearing loss, and cranial nerve palsies (loss of feeling or paralysis). In addition, an unusual mass in the cervical area is a general symptom (Chen et al., 2019).

2.1.2 Risk factors

The most common high-risk factors for HNSCC are tobacco and heavy alcohol use (Blot et al., 1988). The longer the smoking history, the higher the smoking frequency, and the number of packs smoked the higher the risk of cancer (Hashibe et al., 2007); however, the risk decreases after smoking cessation (Blot et al., 1988). In addition, the use of other tobacco-related or smokeless tobacco products, such as betel nuts, increases the risk of HNSCC (Secretan et al., 2009). Although relatively low alcohol consumption by itself is not a clear risk factor for HNSCC (Blot et al., 1988), the combination of alcohol and tobacco use is associated with multiplicative risk (Hashibe et al., 2007).

HPV is also a risk factor for oropharyngeal cancer. HPVs are small double-stranded DNA viruses that exist as extrachromosomal elements or episomes in infected cells (Gillison et al., 1999; Moody & Laimins, 2010). HPV is primarily transmitted via oral sex and infects the basal cells of the squamous epithelium (Gillison et al., 1999; Shah et al., 2017). HPV-positive HNSCCs usually develop in the oropharyngeal area. Most HPV-positive tumours are HPV type 16, followed by HPV type 18 with a lower occurrence rate (Kreimer et al., 2005). The incidence of HPV-positive oropharyngeal cancer has been steadily increasing in recent decades (Gillison et al., 2015).

The viral genome contains early (E) genes, which are mainly required for regulatory functions, DNA replication and activation of lytic cycle genes, and late (L) genes, which are required to produce structural proteins (zur Hausen, 1996). Of

the early genes, E5, E6, and E7 are risk genes associated with cancer development. The E5 protein regulates the activity signalling pathways of growth factor receptors and acts together with the E6 and E7 proteins (DiMaio & Mattoon, 2001; Moody & Laimins, 2010). E7 binds to retinoblastoma protein (pRb) causing its degradation and leading to the activation of E2F-mediated transcription of genes needed for progression to S phase in the cell cycle (McLaughlin-Drubin & Münger, 2009; Moody & Laimins, 2010). This, in turn, leads to p16 overexpression (Grønhøj Larsen et al., 2014), which is associated with better prognosis in oropharyngeal cancer (Langendijk & Psyrri, 2010). Thus, p16 is used as a surrogate marker for HPV (Grønhøj Larsen et al., 2014). In contrast, the E6 protein increases the degradation rate of p53 (Yim & Park, 2005). Together, these functions lead to the transformation of normal cells into malignant cells and thus cancer development (Moody & Laimins, 2010).

The most common risk factor in nasopharyngeal cancer is the EBV virus, which is a type of herpes virus (Chen et al., 2019). EBV infection is relatively common, primarily targets B lymphocytes, and causes mononucleosis (Papesch & Watkins, 2001). However, latent EBV gene expression is associated with virus-related tumours and can activate later e.g. due to immune suppression. It is not exactly known when EBV infection occurs during tumorigenesis, but it causes genomic instability and oncogenic gene expression (Bruce et al., 2015; Li et al., 2023).

2.1.3 Molecular mechanisms driving HNSCC development

In addition to genetic alterations caused by HPV or EBV infection (see section 2.1.2 for details), other epigenetic and genetic changes participate in HNSCC development (Johnson et al., 2020). Approximately 80% of HNSCC cases are HPV-negative and most of these cases are characterised with high chromosome instability. The rest of the HNSCC cases have low chromosome instability, meaning a low number of genetic changes (Leemans et al., 2011). Typically, HNSCC tumorigenesis consists of several genetic alterations, and the number of alterations increases throughout HNSCC tumorigenesis (Pai & Westra, 2009).

The first step in HNSCC progression is the formation of benign hyperplasia (i.e. increased cell production), which is often associated with a loss of heterozygosity on chromosome 9p21 (Pai & Westra 2009). This chromosome region has the tumour suppressor genes cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and ADP ribosylation factor (*ARF*) (Johnson et al., 2020). *CDKN2A* encodes $p16^{INK4A}$ (also known as p16), an inhibitor of cyclin D1–CDK4 and cyclin D1–CDK6 complexes and regulates senescence, cell cycle, and differentiation (Leemans et al., 2011). *ARF* encodes p14, which is a stabiliser of p53 (Johnson et al., 2020).

The next step in tumorigenesis is the progression from hyperplasia to dysplasia (i.e. presence of abnormal cells), which is often related to chromosomal loss in chromosomes 3p21 and 17p13 (Pai & Westra 2009; Johnson et al. 2020). Chromosome 17p13 contains the tumour suppressor gene tumour protein P53 (*TP53*), which is also the most mutated gene in HNSCC (Stransky et al., 2011). *TP53* encodes p53, which is important in cell cycle regulation and apoptosis (Pai & Westra, 2009).

Progression from dysplasia to carcinoma *in situ* (i.e. abnormal cells in their original formation place) is related to the loss of chromosomal regions 11q13, 13q21, and 14q32. Finally, chromosomal loss of 6p, 8p, 8q, and 10q23 is associated with the progression from *in situ* to invasive carcinoma (Pai & Westra, 2009; Johnson et al., 2020).

In addition to genetic changes described above, overexpression of human epidermal growth factor receptor (EGFR) and mutations in the phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR signalling pathway are common in HNSCC (Johnson et al., 2020). EGFR is overexpressed in most HNSCCs and EGFR amplifications have been described in approximately 15% of HPV-negative HNSCCs (Lawrence et al., 2015; Rehmani & Issaeva, 2020). Binding of ligands to EGFR causes its tyrosine kinase activation, which in turn activates signalling pathways (e.g. MAPK pathway and PI3K-AKT pathway). EGFR signalling regulates hallmark properties of cancer including proliferation, survival, invasion, and angiogenesis (Kalyankrishna & Grandis, 2006). The PI3K-AKT-mTOR signalling pathway, which is important in the regulation of cell survival, growth, invasion, and homeostasis, is the most frequently mutated pathway in both HPV-negative and -positive HNSCC (Lawrence et al., 2015; Wang et al., 2017).

In 2015, the genomic characterisation of 279 patients with HNSCC performed by the TCGA also identified several new mutations related to its development, including mutations targeting receptor tyrosine kinases FGFR1-3 and IGF1R in HPV-negative HNSCC (Lawrence et al., 2015). Mutations in the *TP53* and *CDKN2A* genes are long-known factors in HPV-negative HNSCC development, and this study showed that nearly all HNSCCs caused by smoking had deletions in *TP53* and inactivation of CDKN2A. Furthermore, new mutations in *PIK3CA*, *TRAF3*, and *E2F1* were identified in HPV-positive HNSCCs. In addition, Huang *et al.* (2021) studied comprehensively proteogenomic alterations in 108 HPV-negative HNSCC cases and identified three different molecular subtypes of HNSCC based on potential sensitivity to CDK inhibitors, anti-EGFR antibody therapy, or immunotherapy.

Patients with HNSCC often have a dysregulated immune system and are immune evasive. Moreover, patients with HNSCC have lower lymphocyte counts, impaired tumour-infiltrating lymphocyte function, and an increased proportion of suppressive regulatory T cells (T_{regs}), all of which are associated with HNSCC progression

(Ferris, 2015; Moskovitz et al., 2018; Chen et al., 2020). T cells are part of the adaptive immune system and can be categorised into $CD4^+$ and $CD8^+$ subgroups. In addition to an increased proportion of T_{regs}, Th17 cells are often increased in HNSCC. Downregulation of human leukocyte antigen expression enables HNSCCs to become immune evasive because T cells can no longer detect cancer cells (Chen et al., 2020). In addition to T cell dysfunctionality, an increased number of tumour-associated macrophages (TAMs) is present in the tumour microenvironment. TAMs are divided into M1 macrophages, which mediate pro-inflammatory and anti-tumour responses, and M2 macrophages, which have an immunosuppressive role (Ferris, 2015; Chen et al., 2020). Myeloid-derived suppressor cells may also promote HNSCC growth by inhibiting activated T cells (Chen et al., 2020).

2.1.4 Diagnosis and staging

The diagnosis of HNSCC, depending on the tumour location, begins with the clinician by visual inspection of the ear, nose, throat, or oral area, palpation, laryngoscopy, or nasopharyngoscopy (Neville & Day, 2002; Licitra et al., 2003; Bruce et al., 2015; Garneau et al., 2018). In case of suspicious findings, biopsy or, in case of a cervical neck mass, fine needle aspiration is performed (Johnson et al., 2020). Histological analysis and immunohistochemical staining of the biopsy are performed to determine the diagnosis and differentiation state of the squamous cells. In addition, possible HPV status is analysed, most often with p16 immunohistochemistry (IHC), in patients with oropharyngeal cancer (O'Sullivan et al., 2016; Johnson et al., 2020). The detection of p16 using IHC is both affordable and straightforward, making it a commonly chosen method for diagnosing HPV (Thomas & Primeaux, 2012). Several other methods for diagnosing HPV exist, such as HPV DNA *in situ* hybridisation and detection of E6 and E7 messenger RNA (mRNA) (Johnson et al., 2020). However, a recent large multicentre study showed that p16 status does not always correlate with HPV status in patients with oropharyngeal cancer. In fact, patients who were p16-negative but HPV-positive had significantly worse survival outcomes than those who were both p16-positive and HPV-positive (Mehanna et al., 2023). Thus, in addition to p16 analysis, HPV status should be confirmed with a definitive detection method (Craig et al., 2019).

After diagnosis, the cancer is staged according to the International Union Against Cancer/American Joint Committee on Cancer (UICC/AJCC) tumour-node-metastasis (TNM) staging system (Lydiatt et al., 2017; Zanoni et al., 2019). Contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) is performed to study the primary tumour site in more detail (Mody et al., 2021). PET and CT imaging are often performed for staging, as well as to identify possible metastases.

In TNM staging, T stands for the size and depth of the primary tumour, whereas N indicates the presence and size of regional lymph nodes, and M indicates the presence of distant metastases (Huang & O'Sullivan, 2017). These classifications are used for staging the HNSCC tumour into seven categories, where a higher stage indicates a more aggressive tumour and worse prognosis (van der Schroeff & Baatenburg de Jong, 2009). The TNM staging differs for each primary tumour site, and changes are made regularly to increase the accuracy of the staging, e.g. HPV status was introduced in the 8th version of the AJCC Cancer Staging Manual (O'Sullivan et al., 2016; Amin et al., 2017; Lydiatt et al., 2017). **Table 1** represents a simplified TNM classification.

Table 1. Simplified TNM classification. Based on https://www.cancer.gov/about-cancer/diagnosis-staging/staging (accessed: 23.12.2024).

PRIMARY TUMOUR (T)	**EXPLANATION**
TX	Primary tumour cannot be measured.
T0	Primary tumour cannot be found.
T1–T4	The size and/or extent of the primary tumour. A higher number following the 'T' indicates a larger tumour or growth into nearby tissues. The 'T' classifications may be further detailed with subdivisions for additional specificity.
REGIONAL LYMPH NODES (N)	**EXPLANATION**
NX	Cancer in nearby lymph nodes cannot be measured.
N0	No cancer in nearby lymph nodes.
N1–N3	The number and location of lymph nodes with cancer. A higher number following the 'N' signifies a greater number of cancerous lymph nodes.
DISTANT METASTASIS (M)	**EXPLANATION**
MX	Metastasis cannot be measured.
M0	Cancer has not spread to other parts of the body.
M1	Cancer has spread to other parts of the body.
STAGE	**EXPLANATION**
0	Abnormal cells are present but have not invaded nearby tissue (i.e. carcinoma *in situ*).
I–III	Cancer is present. A higher number indicates a larger tumour and greater spread into nearby tissues.
IV	The cancer has metastasised to distant parts of the body.

2.1.5 Treatment and its adverse effects

The treatment of HNSCC is determined according to the tumour site, TNM stage, disease characteristics, and patient's preference (Marur & Forastiere, 2016; Chow, 2020; Johnson et al., 2020). Patients with early-stage (stage I or II) HNSCC (approximately 30%–40% of diagnosed HNSCCs) are usually treated with surgery or radiotherapy, with or without chemotherapy. For oral cavity and paranasal sinus cancers, surgery is often the preferred option. However, radiotherapy with or without chemotherapy is more common for nasopharyngeal cancer (Pfister et al., 2020). Oropharyngeal cancer is conventionally treated with a combination of surgery, radiotherapy, and chemotherapy (Chow, 2020). Although HPV-positive oropharyngeal cancer has better survival rates compared with HPV-negative cancer (Ang et al., 2010), treatment deintensification can lead to undertreatment and worse survival (Cheraghlou et al., 2018). Nevertheless, possibilities for treatment deintensification are studied. Transoral laser microsurgery was introduced by Strong and Jako in 1972 for early laryngeal cancer and was further developed by Steiner *et al.*, who introduced the minimally invasive transoral laser microsurgery (reviewed in Jäckel et al., 2007) that is broadly used for laryngeal and hypopharyngeal cancer (Chow, 2020). Radiotherapy is also commonly used for laryngeal cancer (Chow, 2020).

Approximately 60% of diagnosed HNSCC cases are high-stage (stage III or IV) diseases (Chow, 2020). When making treatment decisions, treatment morbidity and toxicity, its effect on the preservation of function, as well as the size and site of the tumour, age, performance status, and coexisting conditions, are considered (Chow, 2020; Pfister et al., 2020). Advanced oral cancer is generally treated with surgery followed by radiotherapy with or without chemotherapy (Mody et al., 2021). Surgery is the preferred treatment for advanced hypopharyngeal cancer (Cramer et al., 2019) and is often followed by radiotherapy or chemoradiotherapy (Johnson et al., 2020). In addition, for advanced laryngeal tumours, surgery is primarily performed when the lesion causes poor functionality. Otherwise chemoradiotherapy is the primary treatment option (Mody et al., 2021).

Both primary HNSCC and recurrent disease can metastasise. Metastases in the cervical lymph nodes, which are often detected during primary disease, are treated with surgery, radiotherapy, or a combination of both treatments. Disease recurrence or distant metastasis is common if the primary tumour was diagnosed at an advanced stage. Often surgery and radiotherapy are not possible treatment options for a relapsed tumour or metastases; thus, systemic therapy is the only treatment option. Systemic therapy includes treatment with cisplatin-based regimens often combined with 5-fluorouracil, programmed death 1 (PD-1)–targeting antibodies, EGFR–targeting antibodies and taxanes (Mody et al., 2021).

2.1.5.1 Adverse effects

All available treatment options for HNSCC have several acute and long-term adverse effects. Conventional open surgery can have adverse effects that are not related to the tumour site, such as haemorrhage, dysphagia and infection (Baehring & McCorkle, 2012). Adverse effects, such as nerve damages around the tumour area, bone exposure, airway obstruction, and carotid rupture, are possible depending on the site of resection and some have a relatively high mortality (Kerawala, 2010; Baehring & McCorkle, 2012). In addition, surgery may decrease a patient's quality of life due to disfigurement, dental problems, speech problems, and voice changes, among others (Baehring & McCorkle, 2012).

Radiotherapy is associated with several acute and long-term complications. Some of the most common acute complications are mucositis, radiation dermatitis, pain, and oropharyngeal candidiasis (Sroussi et al., 2017). Some long-term complications include mucosal pain, permanently dry mouth, and dental problems (Brook, 2020). These complications significantly affect patient quality of life.

In addition, chemotherapy, such as high-dose cisplatin, causes short-term adverse effects, including nausea, vomiting, cytopenia, infections, and altered taste. Furthermore, chemotherapy can have irreversible adverse effects, such as ototoxicity, neurotoxicity, and renal toxicity (Porceddu et al., 2019).

2.1.5.2 Treatment resistance

Although several treatment options exist for HNSCC, treatment resistance remains a significant problem. Numerous mechanisms affect resistance to radiotherapy and chemotherapy.

Radioresistance can form either intrinsic resistance, which develops due to phenotypic and genetic changes in the cancer cell caused by irradiation, or extrinsic resistance, which develops when the tumour microenvironment protects the cancer cell (Huang & Zhou, 2020). Irradiation causes both direct DNA damage by ionisation and indirect DNA damage by increasing reactive oxygen species (ROS) generation (Kim et al., 2019). Typical irradiation-induced DNA damages includes single-strand breaks, double-strand breaks (DSBs), crosslinks, and base and sugar damage (Huang & Zhou, 2020). Mostly non-DSB damage occurs after irradiation and it is relatively rapidly and easily repaired. In contrast, DSB damage is more complex and its repair is less efficient; thus, DSB damage is the main cause of irradiation-induced cancer cell death (Kim et al., 2019; Huang & Zhou, 2020).

DSBs induce DNA damage repair via homologous recombination or non-homologous end joining (Yamamoto et al., 2016). Furthermore, irradiation triggers cell cycle checkpoints to activate and arrest the cell cycle until DNA damage is repaired (Huang & Zhou, 2020). Imbalances in the DNA repair pathway in cancer

cells is one of the main causes of radioresistance. The ataxia telangiectasia mutated protein (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) are both important factors in DNA damage repair, as well as in the regulation of cell cycle checkpoints (Yamamoto et al., 2016). ATM and ATR are considered the master regulators of DNA DSB repair; thus, studies have focused on using inhibitors targeting these proteins to sensitise cancer cells to irradiation (Huang & Zhou, 2020). Mutations in *TP53* are common in cancers, including HNSCC, and they have been associated with poor radiotherapy response (Alsner et al., 2001).

Another important factor in radioresistance in HNSCC is the hypoxic areas in the tumour. Hypoxia, i.e. reduced oxygen availability in tissues (Gray et al., 1953), promotes radioresistance by decreasing ROS levels in the tumour after radiotherapy, leading to reduced DNA damage in cancer cells (Hill et al., 2022). A hypoxic environment in the tumour arises due to dysregulation of new blood vessel formation (angiogenesis), leading to disorganised and non-functional vasculature (Vaupel, 2004). Moreover, rapid tumour growth causes insufficient vasculature formation, further promoting the formation of a hypoxic environment (Chen et al., 2023). Hypoxia inducible factor 1 (HIF-1) (Semenza & Wang, 1992) is the master regulator of oxygen sensing in cells (Jaakkola et al., 2001). It consists of two subunits, HIF-1α and HIF-1β (Wang & Semenza, 1995). Under normal oxygen levels, HIF-1α is constantly ubiquitinated for proteasomal degradation. However, during hypoxia, HIF-1α ubiquitination is suppressed, enabling it to dimerise with HIF-1β. The dimer enters into the nucleus and binds to the hypoxia response element, which is responsible for regulating several downstream target genes participating in, e.g. apoptosis, angiogenesis, and cell division (Semenza, 2001). It has been proposed that HIF-1 affects radiotherapy response via its ability to regulate apoptosis, metabolism, proliferation, and angiogenesis (reviewed in Moeller & Dewhirst, 2006). However, there is no widely used method to overcome tumour hypoxia and radioresistance, although several trials and studies have focused on this challenge and are currently ongoing (reviewed in Hill et al., 2022).

As previously mentioned in section 2.1.2, EGFR is overexpressed in most HNSCC cases. Furthermore, EGFR overexpression may be associated with radioresistance, as locoregional relapse after radiotherapy is more common in patients with high EGFR tumour expression (Hutchinson et al., 2020). EGFR may participate in radiotherapy-induced DSB repair by forming a complex with DNA-dependent kinase (DNA-PK) (Dittmann et al., 2005). Clinical trials have reported that treatment with the EGFR inhibitor cetuximab leads to better radiotherapy response than radiotherapy alone, but it does not outperform cisplatin (reviewed in Hutchinson et al., 2020). Nevertheless, cetuximab, together with radiotherapy, is an alternative option for treating locally advanced HNSCCs in patients not tolerating cisplatin.

A more recent proposed factor in radioresistance is cancer stem cells (CSCs), which are self-renewing and differentiating cancer cells (Yamamoto et al., 2016). CSCs have enhanced DNA repair capabilities caused by irradiation (Reid et al., 2017).

Finally, defects in apoptotic pathways, such as increased expression of pro-survival Bcl-2 family proteins induce radioresistance. Mutations in apoptosis-associated genes allow cancer cells to resist cell death caused by irradiation (Okada & Mak, 2004).

The same factors that cause radioresistance in HNSCC are associated with chemotherapy resistance. These include changes in apoptosis signalling, CTCs, tumour microenvironment, and DNA damage response (Ortiz-Cuaran et al., 2021). Other important factors are epigenetic modifications, which are changes in the chemical structure of DNA that do not change the DNA sequence (Gauss et al., 2024). Such epigenetic alterations can occur, for example, through DNA hypermethylation, chromatin remodelling, and histone modification (Ortiz-Cuaran et al., 2021; Gauss et al., 2024). Changes in several different signalling pathways, such as the RAS-MAPK and PI3K-AKT-mTOR pathways, also participate in chemotherapy resistance (Ortiz-Cuaran et al., 2021).

2.1.6 Biomarkers of HNSCC

Several slightly different definitions of cancer biomarkers exist. According to the National Cancer Institute in the United States, a biomarker is 'A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition.' (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/biomarker, accessed: 8.7.2024).

Although several different biomarkers have been proposed for HNSCC, only a few have been validated and used in clinics. These biomarkers include HPV and EBV for oropharyngeal cancer and nasopharyngeal cancer, respectively (Economopoulou et al., 2019). In addition, a combined positive score, i.e. the sum of all (tumour cells, macrophages, and lymphocytes) PD-L1–stained cells divided by the total number of viable tumour cells and multiplied by 100 (Kulangara et al., 2019), is another biomarker used in clinics to guide the use of checkpoint inhibitors in some instances (Magnes et al., 2021).

2.1.6.1 Biomarkers currently used in clinics

In addition to HPV (and its surrogate marker p16) being a risk factor for oropharyngeal cancer (see section 2.1.2 for details), it is used as a biomarker because

of the association between HPV and better overall survival (OS), disease-free survival (DFS) and recurrence-free survival in oropharyngeal cancer (Lindquist et al., 2007; Sedaghat et al., 2009; Attner et al., 2011; Kühn et al., 2021). HPV-associated oropharyngeal cancer is more common in younger patients (Kühn et al., 2021) and is often poorly differentiated (Fakhry et al., 2008). p16 is used as a surrogate marker for HPV, as its increased expression is associated with HPV infection (see section 2.1.2 for details) and can be cost-effectively and easily detected using IHC (see section 2.1.4 for details). However, as discussed in the section 2.1.4, p16 status does not always align with HPV status. Although HPV-positive oropharyngeal cancer responds better to radiotherapy (Lindel et al., 2001), the biological mechanisms are still not understood (Tawk et al., 2021). Several studies using HNSCC cell lines have proposed that a better radiotherapy response could be due to p16 regulating the homologous recombination DNA repair pathway by inhibiting RAD51 binding (Dok et al., 2014) or regulating DNA DSB repair via prolonged cell cycle G2 phase arrest (Kimple et al., 2013; Rieckmann et al., 2013).

Similar to HPV being both a risk factor and biomarker for oropharyngeal cancer, EBV is a risk factor (see section 2.1.2 for details) and biomarker for nasopharyngeal cancer (Kang et al., 2015). High EBV DNA levels are associated with advanced disease stage, poor radiotherapy response (Lo et al., 1999), and poor survival (Lo et al., 2000).

PD-L1 and PD-1 are expressed primarily in macrophages, other immune cells, and tumour cells. PD-L1 regulates the immune tolerance response in the tumour microenvironment via T cell activation, proliferation, and cytotoxic secretion, enabling the tumour cells to escape anti-tumoural responses (Han et al., 2020). PD-L1 expression itself has shown contradictory results as a predictive survival biomarker in HNSCC, as some studies have shown that high PD-L1 expression is associated with poor survival (Lin et al., 2015; Ngamphaiboon et al., 2019), while other studies have not shown such an association (Yang et al., 2018). Nevertheless, patients with HNSCC who have high PD-L1 expression benefit from checkpoint inhibitors (Magnes et al., 2021).

2.1.6.2 Novel biomarkers

In addition to currently used biomarkers in clinics, several other candidates have been studied (Budach & Tinhofer, 2019; Economopoulou et al., 2019). However, none of these putative biomarkers have entered routine clinical use. Studies have focused on identifying either liquid biopsy-based or tissue-based tumour biomarkers (Budach & Tinhofer, 2019).

Several hypoxia-associated markers have been studied in recent decades (Budach & Tinhofer, 2019). Tumour hypoxia is associated with worse radiotherapy

response (Gray et al., 1953, see section 2.1.5.2 for details), and it is a prognostic factor in HNSCC (Nordsmark et al., 1996, 2005; Hill et al., 2022). Several other studies have shown a correlation between hypoxia markers, such as HIF-1α, and worse clinicopathological characteristics. Furthermore, the expression of these markers is often associated with worse locoregional control (Swartz et al., 2015).

A promising and widely studied liquid biopsy-based biomarker is the neutrophil-to-lymphocyte ratio, with an increased ratio being associated with poor OS and DFS in all HNSCC sites (Mascarella et al., 2018; Tham et al., 2018). Haematologic cells, especially neutrophils, are associated with tumour-promoting activities (Shen et al., 2014). However, there is a need for further studies to include neutrophil-to-lymphocyte ratio as a biomarker for clinical use (Mascarella et al., 2018).

CTCs have been associated with treatment resistance, but they are also promising liquid biopsy biomarkers (Kong & Birkeland, 2021). CTCs are associated with worse OS, disease-specific survival (DSS), and progression-free survival (PFS), as well as with advanced tumour stage in HNSCC (Sun et al., 2017). Furthermore, CTCs have been associated with regional lymph node metastasis (Hristozova et al., 2011).

Both PIK3CA and EGFR, which are important factors in the molecular mechanisms of HNSCC tumorigenesis (see section 2.1.3), are potential biomarkers of HNSCC. A higher number of mutations in the PI3K pathway are associated with more advanced tumours (Lui et al., 2013; Budach & Tinhofer, 2019), and increased EGFR expression is associated with worse survival in HNSCC (Zhu et al., 2013).

MicroRNAs (miRNAs) are promising biomarkers that have been studied both as liquid- and tissue-based biomarkers, and several clinical trials are currently ongoing. Depending on the specific miRNA, high or low expression levels are associated with poor prognosis, metastatic potential, or cancer growth, among others (reviewed in Thomaidou et al., 2022).

In addition to the above-mentioned promising biomarkers, several other possible targets have been suggested to be promising biomarkers (Dahiya & Dhankhar, 2016). Although several studies have been conducted and both preclinical and clinical trials are currently ongoing, there is an urgent need for new biomarkers for HNSCC (Kang et al., 2015).

2.2 TSPO

TSPO was identified by Braestrup and Squires in 1977 while they were trying to find binding sites of benzodiazepine in the central nervous system (CNS) (see also Squires & Braestrup, 1977). TSPO was first known as a peripheral-type benzodiazepine receptor (PBR) due to its higher abundance in other tissues compared with the brain, in contrast to the central benzodiazepine receptors in the CNS (Braestrup & Squires, 1977; Möhler & Okada, 1977; Squires & Braestrup,

1977). Several years later, Papadopoulos *et al*. (2006) suggested a new name, TSPO, to better describe its function. Although TSPO has been studied for years, its exact structure, interaction with other proteins, and functional role still remain unclear.

2.2.1 Structure

TSPO is located in the 22q13.3 chromosome band of the human genome (Riond et al., 1991; Chang et al., 1992). The gene encodes a protein comprising 196 amino acids (Sprengel et al., 1989; Parola & Laird, 1991) with a molecular weight of approximately 18 kDa (Doble et al., 1987; Antkiewicz-Michaluk et al., 1988; Sprengel et al., 1989). TSPO is an evolutionally conserved protein with highly similar amino acid sequences between different species (Riond et al., 1991; Yeliseev et al., 1997). TSPO has five outer mitochondrial membrane-spanning alpha-helices with an amino-terminal domain (N-terminus) pointing towards the interior of the outer mitochondrial membrane and a carboxyl-terminal domain (C-terminus) in the cell cytoplasm (Bernassau et al., 1993; Joseph-Liauzun et al., 1998; Murail et al., 2008; Jaremko et al., 2014; Guo et al., 2015). TSPO has a cholesterol recognition amino acid consensus sequence (CRAC) that binds cholesterol (Li & Papadopoulos, 1998; Li et al., 2001; Jamin et al., 2005).

Studies on bacterial and mammalian TSPO have found that, depending on its functional purpose, TSPO can exist as a monomer (Lacapère et al., 2001; Hinsen et al., 2015; Jaipuria et al., 2017), dimer (Yeliseev et al., 1997; Boujrad et al., 1996; Delavoie et al., 2003; Li et al., 2015; Jaipuria et al., 2017; Zeng et al., 2018), or polymer (Papadopoulos et al. 1994; Hinsen et al. 2015). Furthermore, when no high-affinity ligands are bound to TSPO, its tertiary structure is flexible (Jaremko et al., 2015).

TSPO has been proposed to interact with several proteins. McEnery *et al.* (1992) isolated TSPO together with the 32-kDa voltage-dependent anion channel 1 (VDAC1) and adenine nucleotide transporter (ANT), suggesting an interaction between TSPO and these proteins. Other early studies indicated that diazepam binding inhibitor (DBI), also known as ACBD1 and ACBP, binds to TSPO and activates steroidogenesis (Papadopoulos et al., 1992; Boujrad et al., 1993), but is thus more often thought to act as a TSPO ligand instead of a protein–protein interaction. Additionally, there is evidence of an interaction between TSPO and steroidogenic acute regulatory protein (StAR) when Hauet *et al.* (2005) showed that treating Leydig tumour cells with TSPO antisense oligodeoxynucleotides inhibited steroidogenesis and StAR expression. In addition, West *et al.* (2001) demonstrated an interaction between these proteins, but later found that such an interaction did not exist (Bogan et al., 2007). TSPO has also been suggested to interact with CYP11A1 and ATAD3A (He et al., 2007; Rone et al., 2012), PAP7 (Liu et al., 2003), PRAX-1

(Galiègue et al., 1999), CPK (Dolder et al., 2001; Meng et al., 2020), and pk10 (Blahos et al., 1995).

2.2.2 Subcellular localisation

The main cellular localisation of TSPO occurs on the outer mitochondrial membrane (Anholt 1986; Bribes et al. 2004). However, TSPO is also present in the nuclear (Schoemaker & Nickolson, 1983; Mukherjee & Das, 1989; Hardwick et al., 1999), microsomal (Schoemaker & Nickolson, 1983), and cytosolic (Mukherjee & Das, 1989) parts of cells. Moreover, TSPO localises to the plasma membranes (O'Beirne & Williams, 1988; Oke et al., 1992; Woods & Williams, 1996) of cells and in the membranes of erythrocytes (Olson et al., 1988). In addition, TSPO is expressed in haematopoietic cells (Marangos et al., 1982; Canat et al., 1993).

TSPO is expressed at different sites throughout the body, especially in the adrenal glands. Low levels of TSPO are expressed in the brain, liver, and intestines (Braestrup & Squires, 1977; Marangos et al., 1982; Anholt et al., 1985; De Souza et al., 1985; Kurumaji & Toru, 1996; Gavish et al., 1999). In some organs, TSPO is distributed unevenly. For example, TSPO is mainly located in the adrenal cortex of the adrenal glands (De Souza et al., 1985), the thick ascending limb of the loop of Henle and convoluted and collecting tubules in the kidneys (Butlen, 1984; Gehlert et al., 1985), and interstitial tissue in the testes (De Souza et al., 1985). In the CNS, low TSPO expression levels are found in astrocytes, glial cells, and ependymal cells (Schoemaker & Nickolson, 1983; Gehlert et al., 1985; Benavides et al., 1990; Kuhlmann & Guilarte, 2000).

2.2.3 Function

TSPO has been proposed to participate in many cellular functions, such as cholesterol import, apoptosis, oxidative phosphorylation (OXPHOS), ROS homeostasis, and immune response (**Figure 3**). However, the exact role of TSPO in these functions is unclear.

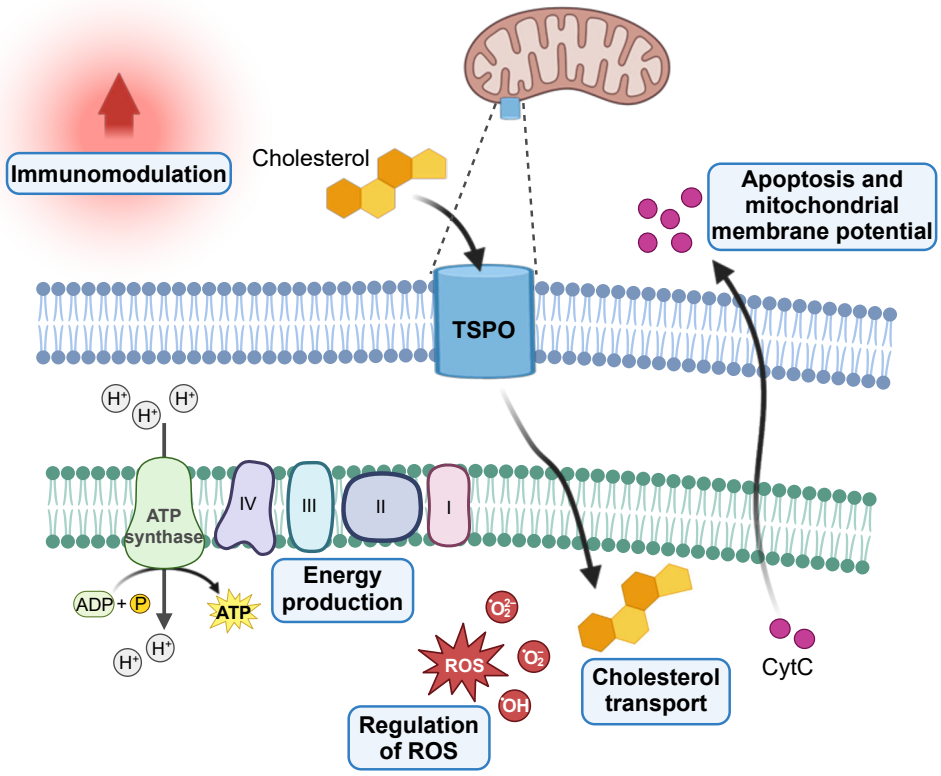

Figure 3. The proposed functions of TSPO. Inspired by Figure 2 by Notter *et al*. (2018) and Figure 1 by Dimitrova-Shumkovska *et al*. (2020). CytC = Cytochrome C. Created with Biorender.com.

2.2.3.1 Steroidogenesis

Steroidogenesis is the most studied and well-known function of TSPO. TSPO binds cholesterol at nanomolar affinity to its CRAC motif and transports it into mitochondria (Li & Papadopoulos, 1998; Li et al., 2001). This binding drives the structure of TSPO towards the monomer form (Jaipuria et al., 2017). The imported cholesterol is then used for steroid hormone production. The role of TSPO in steroidogenesis was first demonstrated by treating cells with TSPO ligands or DBI, which stimulated cholesterol import into mitochondria and increased steroid production (Besman et al., 1989; Mukhin et al., 1989; Ritta & Calandra, 1989; Krueger & Papadopoulos, 1990; Papadopoulos et al., 1990; Panagopoulos et al., 1991). Subsequent studies using TSPO-knockdown and -knockout cells further supported the role of TSPO in steroidogenesis (Kelly-Hershkovitz et al., 1998; Hauet et al., 2005). In addition, targeted disruption of TSPO resulted in abnormal steroidogenesis in cells (Papadopoulos et al., 1997a), and a TSPO-knockout mouse

model was embryonic lethal; thus, demonstrating the importance of TSPO in embryonic development (Papadopoulos et al., 1997b). Supporting findings were observed when lower TSPO levels were measured in the adrenal glands and testes after hypophysectomy (removal of the pituitary gland) in rats (Anholt et al., 1985). The pituitary gland is required for the production of adrenocorticotropic hormone (ACTH), which induces steroidogenesis in the adrenal glands, and TSPO has been proposed as an important regulator in this process (Panagopoulos et al., 1991).

Although several studies showed a significant role of TSPO in steroidogenesis, the premise was questioned by Cavallaro *et al*. in 1993. They showed that after hypophysectomy and administration of ACTH, the increase in TSPO expression was not directly associated with increased steroidogenesis. In fact, during the last decade, studies using TSPO-knockout mice and cells began to question the general opinion that TSPO is a crucial factor in steroidogenesis. In 2014, Morohaku *et al*. (2014) showed that conditional knockout of TSPO in testicular Leydig cells in mice did not affect steroidogenesis. Furthermore, another study by the same group (Tu et al., 2016) showed that steroidogenesis in Leydig tumour cells of TSPO-knockout mice was no different compared with cells in control mice. In addition, no differences in steroidogenesis were observed after administration of the TSPO-specific ligand PK11195. Wang *et al*. (2016) showed that global TSPO-knockout in mice had no effect on viability, indicating that TSPO is not crucial in steroidogenesis. Banati *et al*. (2014) reported similar results showing no changes in steroidogenesis in global TSPO-knockout mice. Milenkovic *et al*. (2019) reported that TSPO-knockout microglial cells have only low levels of steroidogenesis, and TSPO does not affect it. While studying the role of TSPO in the regulation of mitochondrial permeability transition pore (MPTP), Šileikytė *et al*. (2014) found that TSPO-deficiency did not affect mitochondrial function, indirectly supporting the hypothesis that TSPO is not necessary for steroidogenesis.

After the first studies questioning the role of TSPO in steroidogenesis, several publications have indicated that TSPO indeed plays a crucial role in this process. Fan *et al*. (2015) showed elevated embryonic lethality in global TSPO-knockout mice and obstructed steroidogenesis. In other studies by the same group, Owen *et al*. (2017) reported that global TSPO-knockout rats exhibit impaired steroidogenesis, whereas Fan *et al*. (2017) demonstrated that TSPO-knockout Leydig cells had disturbed steroidogenesis. In a more recent study by Fan and Papadopoulos (2020), global TSPO-knockout mice showed delayed preimplantation embryo development and dysregulated steroidogenesis. Several recent studies by the same group and others further support the important role of TSPO in steroidogenesis (Bader et al., 2019; Chung et al., 2020; Barron et al., 2021; Farhan et al., 2021; Garza et al., 2022).

Taken together, TSPO presumably has a role in steroidogenesis, but other regulatory pathways may compensate for its loss; thus, TSPO is not critical for

normal steroidogenesis. However, TSPO may have a significant role in steroidogenesis in specific organs and tissues, whereas in other organs, TSPO is not needed at all. Most of the studies investigating the role of TSPO in steroidogenesis have been conducted using cells and tissues with high levels of steroidogenesis. Moreover, these studies have used different methods to generate TSPO-silenced or TSPO-knockout cell lines and mouse models. The use of different mouse models may also explain some of the contradictory results.

2.2.3.2 Energy production

Another recently proposed function of TSPO is a regulatory role in energy production. Although the first studies showing decreased oxygen consumption after treatment with TSPO ligands in rat kidney mitochondria (Hirsch et al., 1989) and mouse neuroblastoma cells (Larcher et al., 1989) were conducted decades ago, research had focused more on steroidogenesis. Several years later, studies have shown that in mice and zebrafish, the administration of TSPO ligands PK11195 and Ro5-4864 reduce glucose levels, and PK11195 also affects other energy-producing processes, such as fatty acid beta-oxidation and oxidative metabolism (Gut, 2015).

Banati *et al*. (2014) reported reduced oxygen consumption and adenosine triphosphate (ATP) production in TSPO-deficient microglial cells. Similar results were obtained by Bader *et al*. (2019), Milenkovic *et al*. (2019), and Fairley *et al*. (2023). Zhao *et al*. (2016) showed reduced oxygen consumption rates (OCRs) in TSPO-deficient fibroblasts, and Liu *et al*. (2017) reported increased oxidative metabolism in TSPO-overexpressing leukaemia cells. Furthermore, treatment with the TSPO ligand etifoxine increased oxidative metabolism in rat brain mitochondria (Palzur et al., 2021). In addition, other studies have shown increased oxidative metabolism in neuroblastoma and microglial cells following ligand administration (Bader et al., 2019; Lejri et al., 2019; Grimm et al., 2020).

However, Šileikytė *et al*. (2014) reported no changes in mitochondrial function in TSPO-deficient liver cells. A study by Tu *et al*. (2016) showed increased fatty acid oxidation levels in TSPO-deficient steroidogenic cells; however, they did not observe any differences in oxygen consumption.

In summary, TSPO likely participates in the regulation of oxidative metabolism. Studies with TSPO-deficient microglial cells indicate that TSPO positively regulates oxidative metabolism in the CNS. TSPO ligand administration has also been reported to increase energy production in microglial cells, implying increased TSPO activity after ligand binding. However, contradictory results been reported in non-CNS cells and tissues, indicating that TSPO exerts a negative regulatory role in metabolism or no role at all. Thus, it would be worthwhile to study this in several non-CNS tissues using TSPO-knockout cell lines and mouse models.

2.2.3.3 TSPO in ROS regulation

The role of TSPO in ROS regulation was first studied approximately 30 years ago. The first evidence was shown in haematopoietic cells where TSPO abundance increased after H_2O_2 treatment (Carayon et al., 1996). In addition, TSPO overexpression in leukaemia cells increased their resistance to ROS-induced cellular damage. A study by Stoebner *et al*. (2001) found that TSPO overexpression in leukaemia cells reduced UV-induced ROS production. Further evidence supporting the regulatory role of TSPO in ROS production showed that TSPO overexpression decreased tumour necrosis factor α (TNF-α)-induced ROS levels (Joo et al., 2012) and TSPO silencing increased ROS levels in endothelial cells (Joo et al., 2015). In addition, treatment with TSPO ligands increased ROS levels in microglial cells (Jayakumar et al., 2002; Choi et al., 2011), as well as in astrocytes and neurones (Jayakumar et al., 2002). Moreover, TSPO-deficiency increased ROS levels in steroidogenic cells (Tu et al., 2016) and glioblastoma cells (Fu et al., 2020).

However, there is also contradictory evidence showing that the administration of PK11195 reduced ROS levels caused by cobalt chloride administration, which is used to induce hypoxia-like conditions, in glioblastoma cells (Zeno et al., 2009). In addition, another study showed increased ROS levels in TSPO-overexpressed and decreased ROS levels in TSPO-downexpressed mouse embryonic fibroblasts and canine mammary cancer cells (Gatliff et al., 2014).

Altogether, most studies focusing on the role of TSPO in ROS regulation have been conducted with neural cells and a few cancer cell lines. Studies thus far have mainly reported that TSPO reduces ROS levels. The above-mentioned study (Zeno et al., 2009) reporting contradictory results was conducted by chemically inducing hypoxia with cobalt chloride, which does not necessarily reflect changes in ROS homeostasis caused by a naturally formed hypoxic environment.

2.2.3.4 Apoptosis and mitochondrial membrane potential

Another controversial function of TSPO that has been studied for approximately 30 years is its role in apoptosis. The first studies showed that TSPO ligands induced mitochondrial permeability transition (MPT) and apoptosis. Pastorino *et al*. (1994) showed that TSPO ligands protoporphyrin IX, diazepam, and PK11195 induced MPT and led to cell death in rat hepatocytes treated with rotenone, an electron transport inhibitor. This apoptotic effect was inhibited by adding cyclosporin A, an inhibitor of MPT. Later, the same group showed that TNF-α–induced cell death, which could be induced by TSPO ligands, was prevented by inhibiting MPT with cyclosporin A (Pastorino et al., 1996). Moreover, it was reported that PK11195 treatment induces apoptosis by disrupting mitochondrial membrane stability in mouse thymocytes, T cell hybridoma cells, B cell leukaemia cells, and T

lymphoblastic leukaemia cells (Hirsch et al., 1989). A different study showed that the TSPO ligands PK11195, diazepam, and clonazepam reduced mitochondrial transmembrane potential, leading to apoptosis in thymocytes (Tanimoto et al., 1999). More recent studies have reported that TSPO ligands induce MPT in a dose-dependent manner in cardiac cells (Chelli et al., 2001), induce apoptosis by activating caspase-3 in colorectal cancer cells (Maaser et al., 2001), and disrupt the mitochondrial membrane potential leading to MPTP opening (Fischer et al., 2001). Levin *et al*. (2005) showed that TSPO knockdown reduced apoptosis induced by an antineoplastic aether lipid (erucylphosphocholine) in glioma cells. In addition, TSPO knockdown decreased the apoptosis of adrenergic Leydig cells (Weisinger et al., 2004) and human colorectal cancer cells (Shoukrun et al., 2008).

However, the TSPO ligands PK11195 and Ro5-4864, also inhibited erucylphosphocholine-induced apoptosis in a concentration-dependent manner (Kugler et al., 2008). An older study showed that the TSPO ligand Ro5-4864 had an antiapoptotic effect on TSPO-inserted leukaemia cells by inhibiting the TNF-α–induced apoptosis (Bono et al., 1999). However, PK11195 exerted proapoptotic effects by reversing the effect of Ro5-4864.

TSPO is believed to regulate apoptosis via MPT regulation. In recent years, several studies have questioned the role of TSPO in apoptosis altogether. Šileikytė *et al*. (2014) showed that MPTP functions normally without TSPO in mouse mitochondria. Other studies questioning the role of TSPO in apoptosis have focused on VDAC1 and ANT, which are believed to form the MPTP with TSPO. These studies have demonstrated that MPTP functions relatively normally in ANT-deficient hepatocytes, but some of its regulatory functions, such as regulation by ANT ligands, are disrupted (Kokoszka et al., 2004). Another study showed that VDAC1- and VDAC3-deficiency in mitochondria did not affect apoptosis caused by MPT and Bcl-2 (Baines et al., 2007). One hypothesis is that instead of VDAC, ANT, and TSPO forming the MPTP and regulate apoptosis, ATP synthase dimers form it (Giorgio et al., 2013).

2.2.3.5 Immunomodulation

Several studies have investigated the role of TSPO in immunomodulation and have mainly focused on neuroinflammation. TSPO is upregulated after injury or during inflammation (Guilarte et al., 1995; Gavish et al., 1999; Wilms et al., 2003; Notter et al., 2018), and it is also upregulated in several neurological disorders, such as Alzheimer's disease (Owen et al., 1983; Papadopoulos et al., 2006) and Huntington's disease (Schoemaker et al., 1982; Pavese et al., 2006). It has been proposed that TSPO functions as a negative regulator by weakening the nuclear factor-κB activation in neuroinflammation (Bae et al., 2014). Other studies have shown that

treating microglial cells with PK11195 inhibited lipopolysaccharide-induced cyclooxygenase-2, TNF-α, and intracellular Ca^{2+} responses (Choi et al., 2002), and reduced microglial inflammatory response and neural cell death in rat striatum injected with quinolinic acid, a glutamate subtype N-methyl-D-aspartate (NMDA) receptor agonist (Ryu et al., 2005). However, the exact molecular background of the anti-inflammatory role of TSPO remains unclear (Notter et al., 2018).

2.2.4 Ligands

Many endogenous (**Table 2**) and synthetic ligands have been proposed for TSPO. Except for cholesterol that binds to the CRAC motif (Li & Papadopoulos, 1998; Li et al., 2001; Jamin et al., 2005), all ligands have been proposed to bind to different sites in the transmembrane helices (Jaremko et al., 2014; Graeber & Korkhov, 2019).

Table 2. Endogenous TSPO ligands.

NAME	SYSTEMATIC NAME
Cholesterol	-
Deuteroporphyrin IX	Dimethyl 3,7,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate
Diazepam binding inhibitor (DBI)	-
Hemin	Chloro(protoporphyrinato)iron(III)
Mesoporphyrin IX	7,12-Diethyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid dihydrochloride
Octadecaneuropeptide (ODN)	-
Phospholipase A2	Phosphatidylcholine 2-acylhydrolase
Protoporphyrin IX	3,7,12,17-Tetramethyl-8,13-divinyl-2,18-porphinedipropionic acid
Triakontatetraneuropeptide (TTN)	-

One of the most studied endogenous TSPO ligands is cholesterol, which TSPO binds via its CRAC motif (Li & Papadopoulos, 1998). In addition, the binding of protoporphyrin IX, mesoporphyrin IX, deuteroporphyrin IX, and hemin to TSPO has been studied for several years (Snyder et al., 1987; Verma et al., 1987). Another intensively studied TSPO ligand is DBI (Alho et al., 1988; Bovolin et al., 1990). Other proposed endogenous TSPO ligands are phospholipase A2 (Mantione et al., 1988), TTN (Berkovich et al., 1990; Veenman & Gavish, 2006), and ODN (Ferrero et al., 1986; Tonon et al., 1990).

TSPO has many synthetic ligands, and most of them belong to isoquinoline carboxamides, quinoline carboxamides, phenoxyphenyl acetamides, benzodiazepines,

benzothiazepines, imidazopyridine acetamides, pyrazolopyrimidine acetamides, arylindol acetamides, or phenylindolylglyoxylamides (Rupprecht et al., 2010). Examples of synthetic TSPO ligands are presented in **Table 3**.

Table 3. Examples of synthetic TSPO ligands (based on Rupprecht *et al*., 2010).

LIGAND CLASS	EXAMPLE(S)
Alkaloid	Vinpocetine
Arylindol acetamide	FGIN-1-27
Benzodiazepine	Diazepam, Ro5-4864
Benzothiazepine	THIA-66
Benzoxazepine	OXA-17f
Benzoxazine	Etifoxine
Imidazopyridine acetamide	Alpidem
Isoquinoline carboxamide	PK11195
Phenoxyphenyl acetamide	PBR01, FMDAA1106
Phenylindolyl glyoxylamide	IND-18
Phenylpurine acetamide	Emapunil
Pyrazolo-pyrimidine acetamide	DPA-714, PYRA-3j
Pyridazinoindole acetamide	SSR180575
Quinoline carboxamide	VC198M

One of the oldest synthetic TSPO ligands is the TSPO antagonist PK11195 (1-(2-chlorophenyl)-n-methyl-n-(1-methylpropyl)-3-isoquinolinecarboxamide), which has been used as a ligand to study the functional role of TSPO and as a radioligand to study its binding to TSPO (Le Fur et al., 1983a; Le Fur et al., 1983b). The other classical synthetic ligands are Ro5-4864 and 4-chlorodiazepam (Schoemaker et al., 1981; Le Fur et al., 1983c). Both ligands bind to TSPO with nanomolar affinity (Le Fur et al., 1983c).

2.2.5 TSPO in cancer

The role of TSPO in cancer has been studied for the past three decades. Although several findings have been reported, the exact role of TSPO in cancer is not understood. Because increased TSPO expression is associated with neuroinflammatory conditions, the role of TSPO in cancer has been extensively studied in glioblastoma. TSPO expression is increased in glioblastoma and is associated with increased cell proliferation and tumorgenicity (Miettinen et al., 1995; Veenman et al., 2004). Furthermore, TSPO may play an important role in the

hallmarks of cancer in glioblastoma, such as its regulatory role in angiogenesis and apoptosis (reviewed in Ammer et al., 2020). However, TSPO has also been studied in other cancers, although not as much as in glioblastoma.

2.2.5.1 Preclinical studies

Several preclinical studies have focused on investigating the role of TSPO in breast cancer (reviewed in Wongso et al., 2024). First studies reported an association between increased TSPO expression and breast cancer aggressiveness (Hardwick et al., 1999; Beinlich et al., 2000; Hardwick et al., 2002) and increased binding potential of TSPO ligands [^{3}H]PK11195 (Hardwick et al. 1999) and [^{3}H]Ro-4864 (Beinlich et al., 2000). TSPO localisation has been reported to differ between aggressive and nonaggressive breast cancer cells, showing higher localisation in the nucleus in aggressive cancer (Hardwick et al., 1999). Increased TSPO expression in breast cancer cells may also be associated with elevated cholesterol uptake into nucleus (Hardwick et al. 1999), enhanced cell proliferation (Carmel et al., 1999; Hardwick et al., 1999; Beinlich et al., 2000; Li et al., 2007), reduced apoptosis (Li et al., 2007), and increased number of mitochondria (Beinlich et al., 2000). Increased TSPO expression was associated with amplified cell proliferation in the outer layer of a 3D breast cancer cell culture model and promoted survival in the lumen, indicating an antiapoptotic role of TSPO in breast cancer. Furthermore, combining the TSPO ligands PK11195 or Ro5-4864 with lonidamine, a mitochondria-targeting drug that inhibits metabolism, increased cell death in aggressive breast cancer cells (Wu & Gallo, 2013). Zheng *et al*. (2011) determined TSPO expression in patient-derived breast cancer cells, macrophages, and breast cancer xenografts and found that TSPO expression within tumours to be affected by non-cancerous cells.

Higher TSPO expression has also been reported in prostate cancer xenografts compared with healthy prostate (Batra & Alenfall, 1994; Perrone et al., 2016; Tantawy et al., 2018; Mattner et al., 2021) and in colorectal cancer cells, where TSPO was found to be localised in the perinuclear area of mitochondria (Ricken et al., 1998; Ostuni et al., 2007). In addition, treatment with TSPO ligands induced cell cycle arrest and apoptosis in both prostate (Fafalios et al., 2009) and colorectal cancer cells (Maaser et al., 2001). Furthermore, TSPO ligands were shown to affect the intracellular Ca^{2+} levels in colorectal cancer cells (Ostuni et al., 2007).

The role of TSPO has also been determined in other cancers, but these studies have been scarce. High TSPO expression is associated with increased proliferation, migration, and metastasis in hepatocellular cancer cells and xenografts (Pan et al., 2023; Zhang et al., 2023). Furthermore, TSPO has been proposed to regulate ROS production and mitochondrial membrane potential in hepatocellular cancer (Pan et al., 2023). TSPO expression has also been reported to be increased in murine

pancreatic cancer (Cohen et al., 2020). *TSPO* silencing was shown to decrease cell proliferation and migration in oesophageal cancer cells (Chen et al., 2017). In thyroid cancer, PK11195 treatment or *TSPO* silencing had no effect on cell proliferation and migration (Klubo-Gwiezdzinska et al., 2012). In contrast, TSPO ligands protected lung cancer cells from cobalt chloride-induced cellular damage (Zeineh et al., 2020). Furthermore, a possible connection between cigarette smoke and reduced TSPO ligand binding potential has been proposed in lung cancer (Nagler et al., 2015) and pre-treatment with TSPO ligands reduced cigarette smoke-induced TSPO-related processes, such as apoptosis (Zeineh et al., 2021).

Altogether, these findings suggest that TSPO has an oncogenic role in breast, prostate, colorectal, hepatocellular, oesophageal, and pancreatic cancer. Moreover, TSPO function may be inhibited with ligands, leading to reduced cancer cell proliferation and increased apoptosis. However, TSPO ligands have also been shown to protect lung cancer cells from apoptosis, indicating cancer-dependent responses to ligands.

2.2.5.2 Studies using human samples

In breast cancer, higher TSPO levels were observed in aggressive metastatic tumour biopsies compared with healthy breast tissue and non-invasive breast tumours (Hardwick et al., 1999). Another study reported that higher TSPO expression correlated with aggressive breast cancer phenotype and worse DFS in patients with lymph-node negative invasive breast cancer (Galiègue et al., 2004). However, no significant differences were reported in *TSPO* mRNA expression between breast cancer and healthy breast tissue (Bhoola et al., 2018).

In prostate cancer, both *TSPO* mRNA and TSPO protein expression was increased in prostate cancer compared with healthy tissue (Fafalios et al., 2009; Bhoola et al., 2018). Moreover, high TSPO expression was associated with worse survival and aggressive cancer phenotype (Fafalios et al., 2009).

In colon cancer, increased levels of TSPO binding sites with [^{3}H]PK11195 were observed compared with the normal colon (Katz et al., 1988, 1990), indicating higher TSPO levels in colon cancer. In addition, increased *TSPO* mRNA expression was detected in colon cancer compared with healthy tissue (Königsrainer et al., 2007). High TSPO expression is also associated with worse survival in stage III colorectal cancer (Maaser et al., 2002). However, another study showed that *TSPO* mRNA expression was decreased in high-grade colon cancer compared with healthy tissue (Bhoola et al., 2018).

Increased TSPO expression and ligand binding were observed in liver cancer compared with healthy tissue (Venturini et al., 1999); similarly, an association was

found between high TSPO expression and worse OS and PFS survival in hepatocellular carcinoma (Zhang et al. 2023).

In oesophageal cancer, higher TSPO tumour expression was observed compared with normal tissue, and higher expression was associated with worse survival (Chen et al., 2017). Moreover, studies have reported increased TSPO expression in pancreatic (Cohen et al., 2020), specific thyroid (Klubo-Gwiezdzinska et al., 2012), and gastric cancers (Bhoola et al., 2018). However, TSPO expression was lower in lung cancer compared with healthy tissue (Bhoola et al., 2018).

Results with patient samples propose an oncogenic role of TSPO in breast, prostate, colon, liver, oesophageal, pancreatic, and gastric cancers. In contrast, TSPO expression was reported to be lower in lung cancer, indicating a tumour suppressor role. However, some contradictory findings exist related to the mRNA expression between tumour and healthy tissues.

2.2.5.3 TSPO in HNSCC

Few studies have focused on the role of TSPO in HNSCC. Miyakawa *et al*. (1998) and Reis *et al*. (2002) showed that mutations in human chromosome 22q13 were associated with the development of oral cancer and disease progression. *TSPO* is located in chromosome 22q13; thus, these findings were the first possible indications of a tumour suppressor role of TSPO in oral cancer.

The first publication of TSPO in HNSCC was by Nagler *et al*. in 2010, who reported that high TSPO levels analysed by IHC were associated with worse survival in a relatively small (n = 69) cohort of patients with oral cancer. In addition, TSPO binding capacity studied using [^{3}H]PK11195 binding in cells showed lower TSPO binding after cigarette smoke exposure. Similar results were found for the cellular fraction of saliva from smoking patients compared with the non-smoking patients, and they also found lower TSPO binding in patients with oral cancer compared with healthy controls. Gavish *et al*. (2017) demonstrated that there are two distinct binding sites (high affinity and low affinity) for TSPO in oral cancer cells. Furthermore, exposure to cigarette smoke decreased the binding potential of the high affinity binding sites of TSPO. Nagler *et al*. (2010) and Gavish *et al*. (2017) proposed that these findings indicate that lower TSPO binding predisposes patients to oral cancer and that TSPO ligands help inhibit the reduced TSPO binding potential caused by cigarette smoke; thus, these findings could be used for improved therapy of oral cancers. However, no other studies have focused on the role of TSPO in HNSCC.

2.3 Positron emission tomography

PET is a non-invasive imaging method that is used in a wide range of applications. It is based on the spontaneous positron emission of short-lived radionuclides in which the number of protons in the nucleus is higher than the number of neutrons. If an atom is uncharged, the number of protons and electrons in the atom is equal. An excessive number of protons or electrons causes the atom to be unstable and it undergoes radioactive decay (Cherry & Dahlbom, 2006). There are different ways of radioactive decay, and one of them is positron emission (β^+ decay). Positron emission occurs when the stability of an atom is achieved by converting a proton into a neutron, and a neutrino is emitted:

$$ {}^{1}_{1}p \rightarrow {}^{1}_{0}n + {}^{0}_{1}e^{+} + v $$

where p is a proton, n is a neutron, e^+ is a positron, and v is a neutrino. The positron must lose its kinetic energy by interacting with surrounding matter before annihilation occurs (Cherry & Dahlbom, 2006). The flight length of a proton depends on its kinetic energy and the density of the environment (Granov et al., 2013). Eventually, the positron combines with the electron, annihilation occurs, and the energy is released as electromagnetic radiation of two same-energy (511 keV) γ-photons emitted approximately 180° apart from each other (Cherry & Dahlbom, 2006).

PET imaging takes advantage of positron emission and is used to detect the two emitted γ-photons and locate the point of annihilation (**Figure 4**). A PET scanner consists of a ring-shaped gantry with photon-detecting scintillation detectors consisting of inorganic crystals. Detectors opposite to each other detect the emitted photons, and the annihilation point can be located on the line (coincidence line) between these two detections. Annihilation does not always occur between these two detections, and PET uses coincidence windows (Granov et al., 2013). In addition to true coincidences, other coincidences need to be considered in PET imaging, such as random, scattered, and multiple coincidences. The detectors emit visible light when a photon is detected. After data collection and correction, images are constructed using mathematical algorithms (Cherry & Dahlbom, 2006).

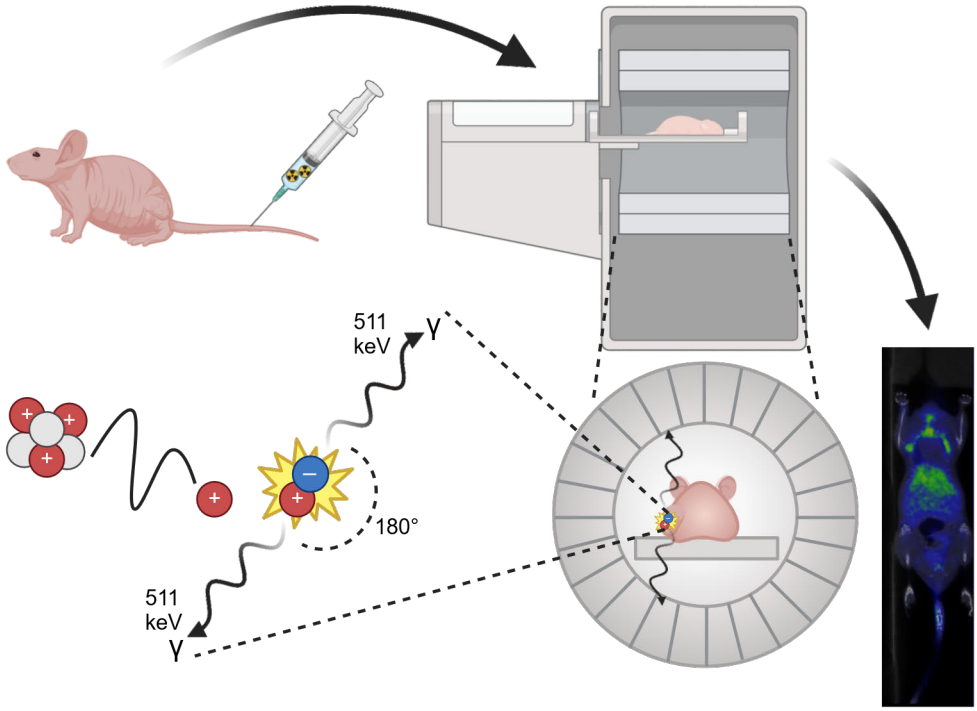

Figure 4. Principles of PET and PET imaging. Created with Biorender.com.

2.3.1 PET imaging of HNSCC

2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) is the most used tracer in oncological PET imaging (combined with CT). [^{18}F]FDG is a glucose analogue and its use in cancer imaging is based on the Warburg effect (Warburg, 1956), i.e. the increased glycolysis in tumours, even in the presence of oxygen. [^{18}F]FDG differs from glucose by its chemical structure (the hydroxyl group at the C-2 position is replaced by ^{18}F). [^{18}F]FDG is taken into cells by glucose transporters but cannot be further metabolised in the glycolytic pathway after it is phosphorylated by hexokinase to [^{18}F]FDG-6-phosphate; therefore, it is trapped in the cell (Basu et al., 2014). Thus, cells with a higher glycolytic rate accumulate more of the phosphorylated tracer, creating a contrast in tracer uptake between tumour and surrounding tissues (Couturier et al., 2004). However, inflammation and infection can increase the [^{18}F]FDG uptake, leading to false-positive findings (Omami et al., 2014). [^{18}F]FDG is mainly used to detect unknown primary tumours (Rusthoven et al., 2004) and nodal and distant metastases (Hohenstein et al., 2020). In addition, [^{18}F]FDG is used to assess treatment response (Cacicedo et al., 2016; Hohenstein et al., 2020).

Several studies have focused on evaluating the use of [^{18}F]FDG for treatment planning, especially in tumour delineation and dose optimisation in radiotherapy treatment planning (reviewed in Cacicedo et al., 2016), and [^{18}F]FDG-PET imaging is increasingly used routinely or in experimental settings in HNSCC. [^{18}F]FDG PET is often performed after radiotherapy to assess treatment response and detect residual tumour or cancer recurrence (Helsen et al., 2018).

In addition to [^{18}F]FDG, several other tracers have been evaluated in HNSCC, such as [^{11}C]Methionine, [^{18}F]Methionine, and a thymidine analogue [^{18}F]FLT, which target amino acid metabolism and determine the proliferative status of the tumour (Wedman et al., 2013). Several tracers targeting tumour hypoxia have been evaluated in recent decades (reviewed in Mokoala & Sathekge, 2024), of which [^{18}F]fluoromisonidazole (FMISO) is the most studied (Rasey et al., 1996). New tracers targeting the fibroblast activation protein have recently been evaluated in several cancers, including HNSCC, showing high sensitivity to and specificity against tumour cells (Giesel et al., 2019).

2.3.2 PET imaging of TSPO

TSPO expression is higher during neuroinflammation than during normal conditions (Werry et al., 2019). The expression of TSPO is high in activated microglia and is associated with microglial activation caused by brain injury or neural inflammation. Therefore, TSPO-PET imaging has mainly focused on imaging neuroinflammatory conditions (Zhang et al., 2021).

2.3.2.1 TSPO-PET tracers

The first TSPO-PET tracer, [^{11}C]PK11195, was developed in 1984 by a French company (Charbonneau et al., 1986; Zhang et al., 2021). Later, it was shown that this tracer's enantiomer, [^{11}C](R)PK11195, has superior binding affinity (Shah et al., 1994). Another first-generation tracer is [^{11}C]Ro5-4864 (Watkins et al., 1988); however, it did not have increased uptake in areas with high levels of TSPO (Junck et al., 1989). In addition, these first-generation TSPO-PET tracers have several limitations, such as low specific binding, high lipophilicity, and short isotope half-life (^{11}C half-life $t_{1/2}$ = 20.4 min) (Zhang et al., 2021; Singh et al., 2022).

Several other TSPO-PET tracers with higher specific binding and lower lipophilicity have been developed, and these are called second-generation tracers (Schweitzer et al., 2010; Zhang et al., 2021). There are more than 50 second-generation tracers (reviewed in Schweitzer et al., 2010; Zhang et al., 2021) and include [^{11}C]DPA-713 and [^{18}F]F-DPA. [^{18}F]F-DPA is a fluorine-18 labelled (^{18}F $t_{1/2}$ = 109.8 min) pyrazolopyrimidine radiotracer with high specificity, low lipophilicity,

and high stability (Keller et al., 2017). However, the problem with these radiotracers is their sensitivity to the single nucleotide polymorphism (SNP) rs6971, which causes an amino acid substitution from alanine to threonine (Ala147Thr) in the human *TSPO* gene, leading to decreased binding (Owen et al., 2011; Mizrahi et al., 2012).

To solve this problem, new third-generation TSPO-PET radiotracers have been developed. [^{18}F]GE-180 is a well-studied tracer, showing promising results in preclinical imaging (Wadsworth et al., 2012; Dickens et al., 2014) and human studies (Fan et al., 2016; Feeney et al., 2016). However, it was reported that [^{18}F]GE-180 had low brain uptake in humans (Zanotti-Fregonara et al., 2018). Another new-generation tracer, [^{11}C]ER176, showed promising results in *in vitro* experiments and showed high binding affinity for all three rs6971 genotypes (Zanotti-Fregonara et al., 2018). However, *in vivo* studies showed sensitivity to the SNP (Ikawa et al., 2017).

Although [^{11}C](R)PK11195 is a first generation TSPO-PET tracer, most studies have used this tracer (Viviano et al., 2022). In fact, several neurological disorders have been studied with this tracer, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and epilepsy (Kreisl et al., 2020).

2.3.2.2 TSPO-PET imaging of cancer

The primary interest in studying TSPO-PET tracers in cancer has been gliomas, as TSPO is overexpressed in glioma cancer cells and its expression is associated with cancer aggressiveness (Ammer et al., 2020). Studies have focused on evaluating the correlation between TSPO-PET tracer uptake and tumour grade or survival in post-recurrent cancer or have investigated the usefulness of TSPO-PET tracers compared with other imaging methods (Filippi et al., 2023). Su *et al*. (2015) showed that [^{11}C](R)PK11195 tracer uptake was higher in aggressive gliomas than in lower-grade gliomas, whereas Quach *et al*. (2023) reported that [^{18}F]GE180 uptake was associated with poor prognosis in an aggressive type of recurrent glioma. Albert *et al*. (2017) showed that TSPO-PET with [^{18}F]GE180 had high tumour-to-background ratios and uptake in areas that were not visible with the MRI contrast agent. The same group (Albert et al., 2023) compared the association between [^{18}F]GE180 tracer uptake and survival in patients with isocitrate dehydrogenase-wild type glioblastoma. They reported that higher tracer uptake was associated with worse survival, indicating the possible prognostic potential of TSPO-PET imaging in glioblastoma.

In addition to using TSPO-PET imaging for neuroinflammation and gliomas, some studies have focused on the use of TSPO-PET tracers in cancer imaging. TSPO expression is increased in breast cancer and has thus been one of the most studied

cancers of TSPO-PET tracers. The TSPO-PET tracer [^{18}F]DPA-714 binds specifically to breast cancer xenografts *ex vivo* and exhibits higher uptake than muscle tissue (Zheng et al., 2011). However, another study focusing on the same tracer reported that the [^{18}F]DPA-714 uptake *in vivo* was not significantly higher than that of muscle tissue in breast cancer xenografts (Zheng et al., 2016). Vasdev *et al*. (2013) showed that the TSPO-PET tracer [^{18}F]FEPPA can be used to visualise breast cancer xenograft tumours, although the uptake was lower than that of nearby muscle tissue and was at the same level as that of blood circulation. In addition, they showed that the uptake was unspecific, as no reduction in tracer uptake was observed after blocking TSPO with non-labelled FEPPA. Another study showed that [^{11}C](R)-PK11195 tracer uptake was associated with tumour inflammation in mouse breast cancer models (de Souza et al., 2022).

Few studies have evaluated the use of TSPO-PET tracers in lung, colorectal, and prostate cancers. One study showed that TSPO-PET tracer ^{18}F-PBR06 uptake was low in lung tumour tissue but high in inflammatory areas of the body and could thus be used to differentiate between peripheral lung cancer and inflammation nodules (Zhang et al., 2019). The use of [^{18}F]FEPPA, which was the same tracer that was studied in breast cancer, was also investigated in colorectal cancer. It was shown that the autoradiography uptake of [^{18}F]FEPPA was higher in colorectal cancer than in healthy tissue and could thus be a potential tracer for colorectal cancer imaging (Berroterán-Infante et al., 2018). In prostate cancer mouse models, the uptake of [^{18}F]VUIIS1008 had high tumour-to-background ratios (Tantawy et al., 2018).

In addition to studying the use of TSPO-PET tracers to image tumours, there has been studies focusing on the imaging of inflammatory cells in tumours, e.g. the imaging of TAMs in pancreatic cancer (Lanfranca et al., 2018).

3 Aims

Several promising biomarkers for HNSCC have been studied, but only HPV in oropharyngeal cancer and EBV in nasopharyngeal cancer are widely used in clinics. Furthermore, treatment resistance has remained a problem in HNSCC. TSPO has been suggested to participate in many cellular functions, which are also important factors in treatment resistance in HNSCC. Thus, the aim of this thesis was to evaluate the prognostic and functional role of TSPO in HNSCC. Moreover, the feasibility and specificity of TSPO-PET imaging in HNSCC were determined.

The main aims of this thesis were:

Aim 1:

- To determine TSPO expression in different clinicopathological subgroups of HNSCC
- To evaluate the potential of TSPO as a prognostic biomarker in HNSCC

Aim 2:

- To determine the association between TSPO tumour expression and survival in subgroups defined by treatment modality
- To study the functional role of TSPO after radiotherapy and cisplatin treatment

Aim 3:

- To evaluate the specificity of [^{18}F]F-DPA-PET imaging of TSPO in HNSCC

4 Materials and Methods

TSPO protein and mRNA expression and its prognostic potential were evaluated in a population-based TMA and publicly available TCGA HNSCC patient cohorts (**I**). The association between TSPO expression and survival in patient subgroups defined by treatment modality was determined (**II**). The role of TSPO in DNA damage response and oxidative metabolism was investigated using HNSCC cells (**II**). The specificity of [^{18}F]F-DPA, a TSPO-PET tracer, was studied in HNSCC cells and xenografts (**III**).The materials and methods used in the original publications (**I**, **III**) and the manuscript (**II**) are listed in **Table 4**.

Table 4. Materials and methods used in this thesis.

MATERIAL/METHOD	PURPOSE	STUDIES USING THE METHOD
[^{18}F]F-DPA biodistribution in mice	Study the [^{18}F]F-DPA uptake into organs and tissues in mice	III
[^{18}F]F-DPA uptake in cells	Evaluate [^{18}F]F-DPA uptake in cells	III
Cell lines and culture conditions	TSPO *in vitro* studies	II, III
DNA damage response	Study the role of TSPO in DNA damage response *in vitro*	II
***Ex vivo* tumour autoradiography**	Evaluate [^{18}F]F-DPA uptake in HNSCC xenograft tumour sections	III
HNSCC-TMA cohort	Study the prognostic role of TSPO in patients with HNSCC	I, II
Immunohistochemistry (IHC)	Study the expression of different proteins in tumour sections	I, II, III
***In silico* analysis**	Study the prognostic and functional role of TSPO in patients with HNSCC	I
Irradiation of cells and xenografts	Study the role of TSPO in radiotherapy response	II, III
Measurement of mitochondrial respiration	Measure the mitochondrial respiration in HNSCC cells	II
OXPHOS measurement	Study the role of TSPO in OXPHOS regulation *in vitro*	II
PET/CT imaging of xenografts	Evaluate [^{18}F]F-DPA and [^{18}F]FDG uptake in xenografts	III
Proportion of monocytes and TAMs with flow cytometry	Study the immune response in xenografts	III
Radiometabolite analysis	Analyse the amount of radiometabolites and [^{18}F]F-DPA	III
Radionuclide and tracer production	Production of the PET tracers [^{18}F]F-DPA and [^{18}F]FDG	III
Real-time quantitative PCR (RT qPCR)	Measure *TSPO* mRNA levels	III
TSPO silencing in cells	Study the specificity of [^{18}F]F-DPA *in vitro* and the functional role of TSPO in HNSCC cells	II, III
Western blot	Study the expression of different proteins	II, III
Xenografts	TSPO *in vivo* studies	III

4.1 HNSCC-TMA cohort (I and II)

The population-based HNSCC cohort collected during 2005–2015 included all patients diagnosed with new HNSCC and treated at the Southwest Finland regional referral centre of Turku University Hospital (Denissoff et al., 2022; Mylly et al., 2022; Routila et al., 2022). In total, 1 033 patients were included, of which 611 patients with available samples were included in TMA for IHC staining with an antibody against TSPO. IHC staining and analysis of p16 was performed previously (Mylly et al., 2022).

OS was determined as the end-of-treatment to end-of-follow-up or death. DSS was determined as the end-of-treatment to end-of-follow-up or death from HNSCC. DFS was determined as the end-of-treatment to disease progression. Patients who had 0 survival days were excluded from the DFS analysis.

4.2 *In silico* analysis (I)

The databases used for the *in silico* analyses are shown in **Figure 5**. *TSPO* mRNA expression and clinical data of the TCGA patient cohort were acquired from the UCSC Xena database (Goldman et al., 2020). *TSPO* expression was available from 520 primary tumour samples and 44 healthy tissue samples. OS, DSS, and progression-free interval were available from 519 patients, and disease-free interval was available from 133 patients. TSPO protein expression was available from the Clinical Proteomics Tumor Analysis Consortium (CPTAC) pan-cancer HNSCC cohort (Huang et al., 2021), and the data were acquired from the LinkedOmics database (Vasaikar et al., 2018). Both TSPO protein and mRNA expression were analysed in different clinicopathological groups.

The TCGA HNSCC PanCancer Atlas patient cohort (Cancer Genome Atlas Network, 2015) was used to analyse the gene co-expression with cBioPortal (Cerami et al., 2012; Gao et al., 2013). Genes co-expressed with *TSPO* were available from 488 patients. Gene overlap analyses with the top 100 negatively or positively co-expressed genes were performed with the Molecular Signatures Database (MSigDB, v7.0) (Subramanian et al., 2005; Liberzon et al., 2011, 2016). The Hallmark (Liberzon et al., 2016), Kyoto Encyclopedia of Genes and Genomes Canonical Pathways (CP:KEGG) (https://www.kegg.jp/kegg/), and Gene Ontology Biological Process (GO:BP) (http://geneontology.org/) gene sets were selected.

The TISIDB database (Ru et al., 2019) was used to analyse the correlation between *TSPO* mRNA expression in the TCGA cohort and the abundance of immune cells in different cancer types (Charoentong et al., 2017).

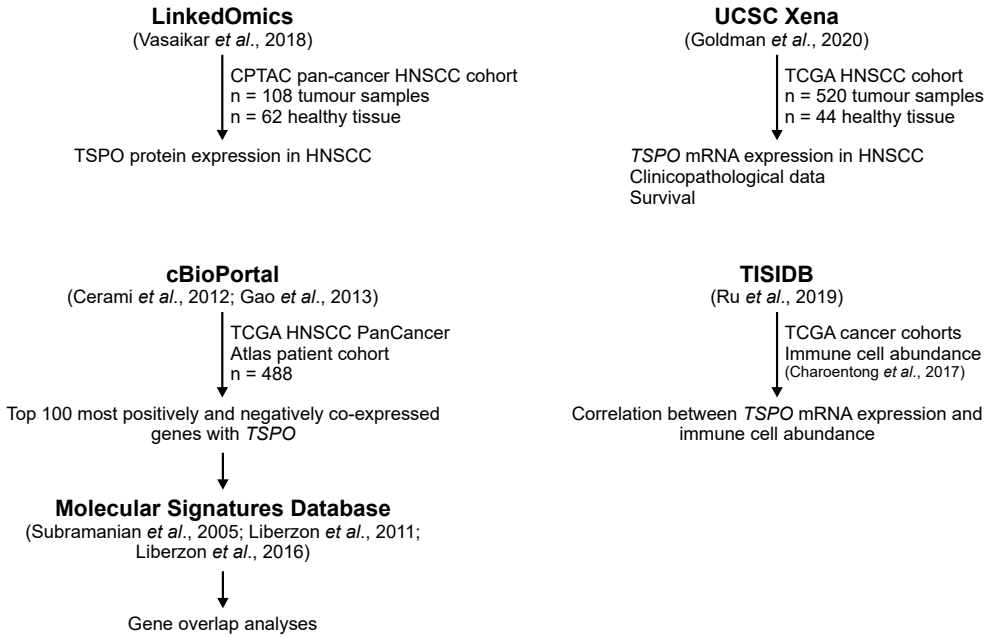

Figure 5. Databases used for *in silico* analyses.

4.3 Cell lines and culture conditions (II and III)

FaDu cells (originating from a hypopharyngeal tumour) used in study **III** were obtained from ATCC (HTB-43). Cal33 (originating from an oral tongue tumour) and FaDu cells used in study **II** were a kind gift from Prof. Anna Dubrovska (OncoRay–National Center for Radiation Research in Oncology, Medizinische Fakultät Dresden, Germany). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 1× L-glutamine, 1× 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1× Minimal Essential Medium (MEM) Non-Essential Amino Acids (NEAA), sodium pyruvate, and penicillin-streptomycin (all from Gibco Thermo Fisher). The purchased FaDu cells were identified using short tandem repeat (STR) profiling (**III**). The gifted FaDu and Cal33 cells were previously genotyped using microsatellite polymorphism analysis (**II**) (Kurth et al., 2015).

4.4 TSPO silencing in cells (II and III)

Cells were silenced using TSPO or non-targeting (NT) siRNA according to the manufacturer's protocol (Accell siRNA, Dharmacon Horizon Discovery). Briefly, cells were plated on 6-well (**II**) or 96-well (**III**) plates in normal culture medium and allowed to attach overnight. The next day, the medium was replaced with serum-free

Accell siRNA Delivery Media, and NT or TSPO siRNA was added at a final concentration of 1 μM for 72 h. Silenced cells were further plated for other experiments. Successful silencing was confirmed using Western blotting (**II**, **III**) and qPCR (**III**).

4.5 Xenografts (III)

Female nude mice (Hsd;athymic Nude-Foxn1^{nu}, age 4–6 weeks, Envigo) were housed under controlled pathogen-free environmental conditions. Cells (1×10^6) were inoculated subcutaneously into the left or right hind limb of 34 mice. When the tumour diameter was 5–6 mm, mice were stratified into non-irradiated and irradiated groups.

4.6 Irradiation of cells and xenografts (II and III)

Cells and xenografts were irradiated using a Faxitron MultiRad 350 (Faxitron Bioptics) or linear TrueBeamSTx accelerator (Varian Medical Systems). Cells were plated on either 6-well or 96-well plates, depending on the experiment, and allowed to attach overnight. Irradiations were performed once with a single 5-Gy dose (6 MV flattened beam, source-to-phantom distance 100 cm, 5 Gy/min) (**II**) or on five consecutive days (2 Gy/day) for a total dose of 10 Gy (320 kV X-rays, 10 mA current, Al 0.5 mm filter, source of surface distance 37.0 cm, 6 Gy/min) (**III**).

Xenograft-bearing mice were locally irradiated (Faxitron MultiRad 350) to the tumour area while protecting the rest of the body with a lead shield. Animals were kept anesthetised under 2.5% isoflurane while irradiating the tumours with a 5-Gy dose on two consecutive days for a total dose of 10 Gy (320 kV X-rays, 10 mA current, SnCuAl filter, source of surface distance 45.0 cm, 1 Gy/min).

4.7 DNA damage response (II)

Parental and siRNA-transfected cells were plated on 6-well plates and allowed to attach overnight. The next day, the fresh medium was changed and 10 μM of PK11195 (Sigma-Aldrich) was added prior to irradiation of a subset of parental cells. Control cells were treated with 0.1% dimethyl sulfoxide (DMSO). Cells were irradiated once with a 5-Gy dose. Control cells were not irradiated. At 1 h and 16 h after irradiation, cells were collected for Western blot analysis.

In addition, parental and siRNA-transfected cells were plated on 6-well plates and allowed to attach overnight. The next day, 0, 0.5, 1, and 3 μM of cisplatin (AbMole) was added. In addition, a subset of parental cells was treated with 10 μM of PK11195, 3 μM of cisplatin, or a combination of PK11195 and cisplatin. Control

cells were treated with 0.1% DMSO. Protein samples were collected 24 h after treatment for Western blot analysis.

4.8 Measurement of mitochondrial respiration (II)

Cells were plated on XFe96 Cell Culture Microplates (Agilent Technologies) and allowed to attach overnight following irradiation with a 5-Gy dose. Control cells were not irradiated. The next day, a Seahorse XF Cell Mito Stress Kit (Agilent Technologies) experiment was conducted according to the manufacturer's protocol. PK11195 (10 μM) was administered as an acute injection after basal measurements. Control cells were treated with 0.1% DMSO. The effect of PK11195 was measured 5 times approximately every 6 to 7 min, following 5 μM oligomycin injection, 10 μM FCCP injection, and 0.5 μM antimycin C/rotenone injection. Results were normalised to the amount of cellular DNA using CyQUANT™ Cell Proliferation Assay (Invitrogen) and the results were expressed as mean OCR (pmol/min) ± standard deviation (SD).

4.9 OXPHOS measurement (II)

The same samples prepared for DNA damage response experiments (see section 4.7) were used to study the role of TSPO in OXPHOS. The detected subunits of the OXPHOS complexes are listed in **Table 5**.

Table 5. Subunits of the OXPHOS complexes detected with Western blot.

COMPLEX	SUBUNIT	FULL NAME
I	NDUFB8	NADH:ubiquinone oxidoreductase subunit B8
II	SDHB	Succinate dehydrogenase complex iron sulfur subunit B
III	UQCRC2	Ubiquinol-cytochrome c reductase core protein 2
IV	COX II	Cyclooxygenase II
V	ATP5A	ATP synthase lipid-binding protein

4.10 Radionuclide and tracer production (III)

Two PET tracers were used in this thesis: [^{18}F]FDG and [^{18}F]F-DPA. Radionuclides and tracers were produced at the Radiopharmaceutical Chemistry Laboratory of Turku PET Centre.

[^{18}F]FDG was synthesised using the FASTlab synthesizer (GE Healthcare) as previously described (Hamacher et al., 1986). The radiochemical purity of [^{18}F]FDG

exceeded 98% in all syntheses, and the molar activity (A_m) at the end of synthesis (EOS) was > 100 GBq/μmol.

N,N-Diethyl-2-(2-(4-[^{18}F]fluorophenyl)-5,7-dimethylpyrazolo[1,5α]pyrimidine -3-yl)-acetamide ([^{18}F]F-DPA) was synthesised via two different approaches resulting in low A_m (10 GBq/ μmol at EOS) [^{18}F]F-DPA (Keller et al., 2017) and high A_m (360–900 GBq/μmol at EOS) [^{18}F]F-DPA (Keller et al., 2020).

4.11 [^{18}F]F-DPA uptake in cells (III)

The uptake of [^{18}F]F-DPA in cells was measured approximately 5 h after the last irradiation. Cells were incubated in normal culture medium containing 0.5 MBq/mL [^{18}F]F-DPA for 60 min at 37 °C (5% CO_2). To block TSPO cells were first incubated with PK11195 (10 μM) for 30 min. After incubation, the medium was changed to one containing 0.5 MBq/ mL of [^{18}F]F-DPA and 10 μM PK11195 and incubated for 60 min. After incubation, cells were washed, collected, and the number of viable cells was counted (Cellometer auto 2000, Nexcelom). The ^{18}F-radioactivity was measured using a gamma counter (2480 Wizard2, PerkinElmer), and the results were expressed as mean radioactivity/viable cells (Bq/cells) ± SD.

4.12 PET/CT imaging of xenografts (III)

PET/CT imaging of xenograft-bearing mice was performed at 1 or 2 weeks after irradiation. [^{18}F]FDG or [^{18}F]F-DPA was injected intravenously via a cannula inserted into the tail vein under 2.5% isoflurane anaesthesia. Imaging of FaDu tumours (Inveon, Siemens) was performed with [^{18}F]FDG and [^{18}F]F-DPA on two consecutive days. Cal33 xenografts were scanned only with [^{18}F]F-DPA (x- and β-cubes, Molecubes). Mice scanned with [^{18}F]FDG were kept anaesthetised for 60 min before starting a 20-min static scan. The next day, a 20-min static [^{18}F]F-DPA scan was performed 20 min after injection. The scanning time point of [^{18}F]F-DPA was chosen based on dynamic imaging data. A 10-min long CT scan was performed prior to the PET scan for attenuation correction of the PET data and for anatomical reference of the PET images.

Pre-treatment with PK11195 (1 mg, intraperitoneal administration) was performed in non-irradiated FaDu xenograft-bearing mice 30 min prior to [^{18}F]F-DPA injection to block [^{18}F]F-DPA binding to TSPO.

The imaging data were collected in list mode and reconstructed using the OSEM3D algorithm. Volume of interest (VOI) was drawn using both CT- and PET-images and analysed using the Inveon Research Workplace Image Analysis (Siemens Medical Solutions). The uptake of ^{18}F-radioactivity in tumours was calculated as a percentage of the injected dose per millilitre of tissue (% ID/mL), and

the data were corrected for the injected activity and decay. The results were expressed as mean ± SD.

4.13 *Ex vivo* tumour autoradiography (III)

To determine the intratumoural distribution of [^{18}F]F-DPA, tumours were removed from animals, frozen in chilled isopentane, cut into 20 µm sections using a cryomicrotome (Microm HM 500 OM), exposed to an imaging plate, and scanned using a Fuji Analyzer BAS5000 with a resolution of 25 µm (Fuji). The specificity of [^{18}F]F-DPA for TSPO was evaluated by pre-incubating frozen tumour sections in Tris-NaCl for 5 min at 4 °C, followed by incubation with [^{18}F]F-DPA (2 nmol/L) for 1 h at room temperature. TSPO blocking was performed by co-incubating PK11195 (1 000 nmol/L) with [^{18}F]F-DPA. Brain sections from an APP/PS1-21 transgenic Alzheimer mouse were used as positive controls. The slides were then exposed to an imaging plate and scanned using a Fuji Analyzer BAS5000. The images were analysed using AIDA (v. 4.22, Raytest).

4.14 [^{18}F]F-DPA biodistribution in mice (III)

[^{18}F]F-DPA was allowed to accumulate for 40 min before the mice were sacrificed by cardiac puncture under deep anaesthesia (4% isoflurane). Blood and tissue samples were dissected, weighed, and measured for ^{18}F-radioactivity with a gamma counter (2480 WIZARD2, PerkinElmer). The measured radioactivity was corrected for decay and background and expressed as percentage of the injected dose per gramme of tissue (% ID/g tissue) or as muscle-to-blood (M/B), tumour-to-plasma (T/P), and tumour-to-blood (T/B) ratios. The results were expressed as mean ± SD.

4.15 Radiometabolite analysis (III)

The proportion of unchanged [^{18}F]F-DPA and amount of radioactive metabolites were analysed from blood, plasma, and tumour samples of non-irradiated, irradiated, and PK11195 pre-treated FaDu tumours 40 min after [^{18}F]F-DPA injection. The blood samples were collected in Li-heparin tubes and plasma proteins were precipitated by adding 1.5 parts (v) of methanol. Tumour samples were homogenised with 1:1 (v/v) mixture of methanol and water. After centrifugation, the supernatant was spotted on a silica gel 60/Kieselguhr F254 thin-layer chromatographic (TLC) plate (Merck Millipore, art. no 1.05567). The TLC plates were developed using a 9:1 (v/v) of dichloromethane and methanol for a distance of 6 cm. The plates were exposed onto an imaging plate (Fuji BAS Imaging Plate TR2025, Fuji Photo Film Co.) for approximately two half-lives of fluorine-18, followed by scanning of the

plates using a Fuji Analyzer BAS5000 with a resolution of 50 μm. The images were processed using AIDA (v. 4.22, Raytest) and the proportion (%) of intact tracer in the total ^{18}F-radioactivity of samples was calculated.

4.16 Proportion of monocytes and TAMs (III)

Tumours were minced and digested in 3 mL Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2% HEPES, 2% FBS, 0.5 mg/mL collagenase D and 0.1 mg/mL DNAse 1 at 37 °C on a shaker for 45 min (enzymes from Roche), followed by the addition of EDTA to stop the reaction. To obtain a single cell suspension, gentleMACS C tubes were used with a gentleMACS Dissociator and the suspension was filtered. BD's FC-block was used to block the cells, followed by staining with directly conjugated antibodies and a viability dye (**Table 6**). Events were recorded on a LSR Fortessa flow-cytometer and analysed using Flowjo v10 (FlowJo LLC) and the results were expressed as percentage of total monocyte/macrophage population ± SD.

Table 6. Antibodies used for flow cytometry.

ANTIBODY	TARGET	MANUFACTURER
Fixable viability dye eFluor 780	Dead cells	eBioscience (Cat. no. 65-0865-14)
CD11b FITC	B cells, dendritic cells, granulocytes, macrophages, monocytes, neutrophils, NK cells, T cells, and Tregs	BioLegend (Cat. no. 101206)
Ly-6G PerCP-Cy5.5	Bone marrow cells	BD (Cat. no. 560602)
F4/80 APC	Mature macrophages	eBiosicence (Cat. no. 17-4801-82)
MHCII PE	B cells, dendritic cells, monocytes, macrophages, and activated T cells	BD (Cat. no. 557000)
Ly-6C-BV421	Monocyte/macrophage populations, granulocytes, endothelial cells, plasma cells, thymocytes, NK cells, and T-subsets	BD (Cat. no. 562727)

4.17 Immunohistochemistry (I–III)

The primary antibodies used in the studies are listed in **Table 7**.

TMA blocks (**I**, **II**) were made from formalin-fixed and paraffin-embedded tissue samples using TMA Grand Master (3DHISTECH) at Auria Biobank (Turku, Finland). IHC staining of TMAs against TSPO was performed using a Labvision autostainer (Thermo Fisher Scientific) at Histocore (University of Turku). Stained

sections were scanned with a Pannoramic P1000 (3DHISTECH Ltd.). Cytoplasmic TSPO staining was independently scored based on staining intensity on a scale of 0–3 by two researchers. In cases of inconsistency, an HNSCC oncology specialist was consulted to reach consensus. For statistical analyses, staining intensities were grouped as either low (scores 0 and 1) or high (scores 2 and 3).

Formalin-fixed (10% neutral-buffered formalin) and paraffin-embedded xenograft tumours (**III**) were cut into 6 µm sections. The sections were stained at Histocore using the following antibodies: TSPO, cleaved caspase-3, and phospho-histone H3 (PHH3). The stained sections were scanned using a Pannoramic 250 Flash (3DHISTECH Ltd.). All analyses were performed using scripts with QuPath (Bankhead et al., 2017). The percentage of cells from ROIs (700 µm radius) stained positively for PHH3 and cleaved caspase-3 were analysed. For TSPO, positively stained cells from the whole tumour area were analysed.

Table 7. Antibodies used for IHC.

ANTIBODY	DILUTION	MANUFACTURER
TSPO	1:5 000 (III), 1:50 000 (I, II)	Abcam (Cat. no. ab109497)
PHH3	1:100	Cell Signaling Technology (Cat. no. #9701)
Cleaved Caspase-3	1:500	Cell Signaling Technology (Cat. no. #9964)

4.18 Western blot (II and III)

Cells and xenografts were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors (all from Thermo Scientific). The protein concentration was measured using the bicinchoninic acid method (Pierce BCA Protein Assay Kit, Thermo Scientific) and equal amounts of protein were loaded and run on 4%–20% gradient gels (Mini-PROTEAN TGX Precast Protein Gels, Bio-Rad). Samples were transferred either to a PVDF (polyvinylidene difluoride) membrane (**II**) or to a nitrocellulose membrane (**III**) (all from Bio-Rad). Membranes were blocked with EveryBlot Blocking Buffer (**II**, Bio-Rad) or 5% milk (**III**) followed by overnight incubation with primary antibodies (4 °C). The primary antibodies can be found on **Table 8**. Fluorescent secondary antibody (1:2 000 in TBS-T or EveryBlot Blocking Buffer with 0.02% SDS, IRDye 800CW LI-COR Biosciences [Cat. no. 926-32213] or IRDye 680RD Secondary Antibody, LI-COR Biosciences [Cat. no. 926-68072]) was incubated for 1 h at room temperature protected from light. Protein bands were detected using an LI-COR Odyssey CLx (LI-COR Biosciences) and results analysed with Image Studio Lite v5.2 (LI-COR Biosciences). Protein expression was

calculated as fold-change relative to nontreated and/or non-irradiated parental or NT siRNA cells. The results were expressed as mean ± SD.

Table 8. Antibodies used for Western blot.

ANTIBODY	DILUTION	MANUFACTURER
Akt	1:500	Cell Signaling Technologies (Cat. no. #2920)
Phospho-Akt (pAkt)	1:1 000	Cell Signaling Technologies (Cat. no. #9271)
Chk1	1:500	Cell Signaling Technologies (Cat. no. #2360)
Phospho-Chk1 (pChk1)	1:500	Abcam (Cat. no. ab283261)
Cleaved PARP1 (cPARP1)	1:1 000	Abcam (Cat. no. ab32064)
Total OXPHOS human WB antibody cocktail	1:1 000	Abcam (Cat. no. ab110411)
TSPO	1:1 000	Abcam (Cat. no. ab109497)
Vinculin	1:1 000	Sigma-Aldrich (Cat. no. V9131)
γH2Ax	1:500	Abcam (Cat. no. ab22551)

4.19 Real-time quantitative PCR (III)

Cells were lysed in 1:1 (v/v) RLT buffer (Qiagen) and 96% ethanol. Total RNA was isolated using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. The isolated total RNA was converted into cDNA using Oligo-d(T) 18 mRNA Primer (New England Biolabs), dNTP Mix, RiboLock RNAse Inhibitor, RT Buffer, and Maxima Reverse Transcriptase (all from Thermo Scientific). For RT-qPCR, 30 ng of cDNA was used with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad). The primers used were hTSPO, hTBP, and hRPLP0 (**Table 9**).

The data analysis was performed using C_t values normalised to house-keeping genes (TBP and RPLP0). Results are shown as average fold-change expression ± SD relative to non-irradiated parental or NT siRNA cells (delta-delta C_t method).

Table 9. Primers.

PRIMER	SEQUENCE	REFERENCE	MANUFACTURER
hTSPO	N/A	N/A (commercial primer)	Bio-Rad
hTBP (forward)	5'-GAA TAT AAT CCC AAG CGG TTT G-3'	Menschikowski *et al.*, 2012	Oligomer
hTBP (reverse)	5'-ACT TCA CAT CAC AGC TCC CC-3'	Menschikowski *et al.*, 2012	Oligomer
hRPLP0 (forward)	5'-ACC TCC TTT TTC CAG GCT TT-3'	Favre *et al.*, 2011	Oligomer
hRPLP0 (reverse)	5'-CCC ACT TTG TCT CCA GTC TTG-3'	Favre *et al.*, 2011	Oligomer

4.20 Ethical aspects (I–III)

The use of human tissue samples was approved by the Institutional Review Board of the Finnish National Authority for Medicolegal Affairs (V/39706/2019), the Regional Ethics Committee of the University of Turku (51/1803/2017), and the Auria Biobank Scientific Board (AB19-6863). The authors affirm that the study was conducted following the rules of the 1975 Declaration of Helsinki, which was revised in 2013. Informed consent was waived due to the retrospective design of the study according to the Finnish Act on Secondary Use of Social and Health Data, which was effective from April 2019 (Act 552/2019). All data were collected, stored, and handled in a manner that meets the regulations of GDPR and the Secondary Use Act 552/2019.

Animals were cared for in accordance with Directives 2012/707/EU and 2014/11/EU and the European Parliament and Council for the Care and Use of Laboratory Animals. Ethical approvals (ESAVI/2329/04.10.07/2017 and ESAVI/7015/2020) for the studies were obtained from the ethics committee (Regional State Administrative Agencies in Finland).

4.21 Statistical analysis (I–III)

IBM SPSS Statistics for Windows version 28 (IBM Corp.) was used in studies **I** and **II**, GraphPad Prism version 10.1.2 was used in study **II**, and SAS System version 9.4 for Windows (SAS Institute Inc.) was used for statistical analyses in study **III**. p-values less than 0.05 (two-tailed) were considered statistically significant. p-values above 0.05 but less than 0.1 (two-tailed) were considered indicatively significant.

The Cox's proportional hazard model was used for Kaplan–Meier survival and hazard ratio (HR) with 95% confidence interval (CI) analyses, and the chi-square test of independence was used to determine the differences in the frequencies in

HNSCC subgroups (**I**). p-values from the hypergeometric distribution and false discovery rate q-values for gene overlap analyses were acquired from MSigDB (**I**). TISIDB was used to analyse the correlation (Spearman's rank correlation coefficient) between *TSPO* expression and immune cell abundance in different cancers (**I**).

Student's t-test or Welch's t-test was used to compare differences between two groups (**I**–**III**). One-way analysis of variance (ANOVA) followed by Šídák's or Bonferroni *post hoc* tests was used to compare differences between multiple groups (**I**–**III**). Two-way ANOVA followed by uncorrected Fisher's least significant difference (LSD) *post hoc* tests was performed to determine differences between groups with two factors (e.g. siRNA transfection and irradiation) (**II**, **III**). The normality assumption was checked using the Shapiro–Wilk test (**II**) or studentised residuals (**III**) when applicable.

5 Results

5.1 TSPO expression in patient cohorts

Representative images of TSPO IHC staining scored according to TMA cohort staining intensity are shown in **Figure 6**. Higher TSPO expression was associated with lower T-status ($p = 0.015$), tumour grade ($p < 0.001$), stage ($p = 0.002$), and no lymph-node metastases ($p = 0.005$) (original publication **I**, **Table 1**). Both TSPO mRNA ($p < 0.001$, original publication **I**, **Figure 6B**) and protein ($p < 0.001$, original publication **I**, **Figure 6C**) expression levels were lower in tumours compared with healthy tissue in the TCGA and CPTAC pan-cancer HNSCC cohorts. Furthermore, lower *TSPO* mRNA expression was associated with higher tumour stage (original publication **I**, **Supplementary Figure S4**).

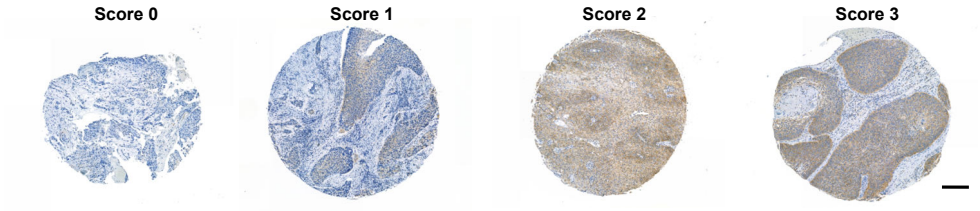

Figure 6. Representative images of TSPO IHC in the HNSCC TMA cohort. Sections were scored according to staining intensity (0–3). Scale bar 100 µm.

5.2 Association between TSPO and survival

Higher TSPO tumour expression was associated with better OS (HR: 0.678, 95% CI: 0.537–0.857, $p = 0.001$) and DSS (HR: 0.569, 95% CI: 0.429–0.756, $p < 0.001$), and an indicatively significant association (HR: 0.769, 95% CI: 0.569–1.041, $p = 0.089$) was observed between better DFS and high TSPO expression (original publication **I**, **Table 2**). In addition, TSPO was an independent prognostic marker for DSS (HR: 0.713, 95% CI: 0.524–0.968, $p = 0.030$) in all patients with HNSCC.

Site-specific survival analyses showed an indicatively significant (HR: 0.732, 95% CI: 0.512–1.048, $p = 0.089$) association between better OS and high TSPO expression in tumours originating from the oral cavity (original publication **I**, **Figure**

2A). A statistically significant (HR: 0.564, 95% CI: 0.341–0.872, p = 0.011) association was observed between OS and TSPO tumour expression in the oropharynx (original publication **I**, **Figure 2B**). Better DSS associated (HR: 0.488, 95% CI: 0.314–0.759, p = 0.001) with higher TSPO tumour expression in the oral cavity, and an indicatively significant (HR: 0.494, 95% CI: 0.218–1.120, p = 0.091) association was seen in laryngeal cancer (**I**, **Figure 7**). However, no such association was found in patients with oropharyngeal and other cancers. Moreover, no clear association was found between TSPO tumour expression and DFS in any of the tumour sites, except in laryngeal cancer where an indicatively significant (HR: 0.548, 95% CI: 0.270–1.113, p = 0.096) association was observed between higher TSPO tumour expression and better DFS (original publication **I**, **Figure 4C**).

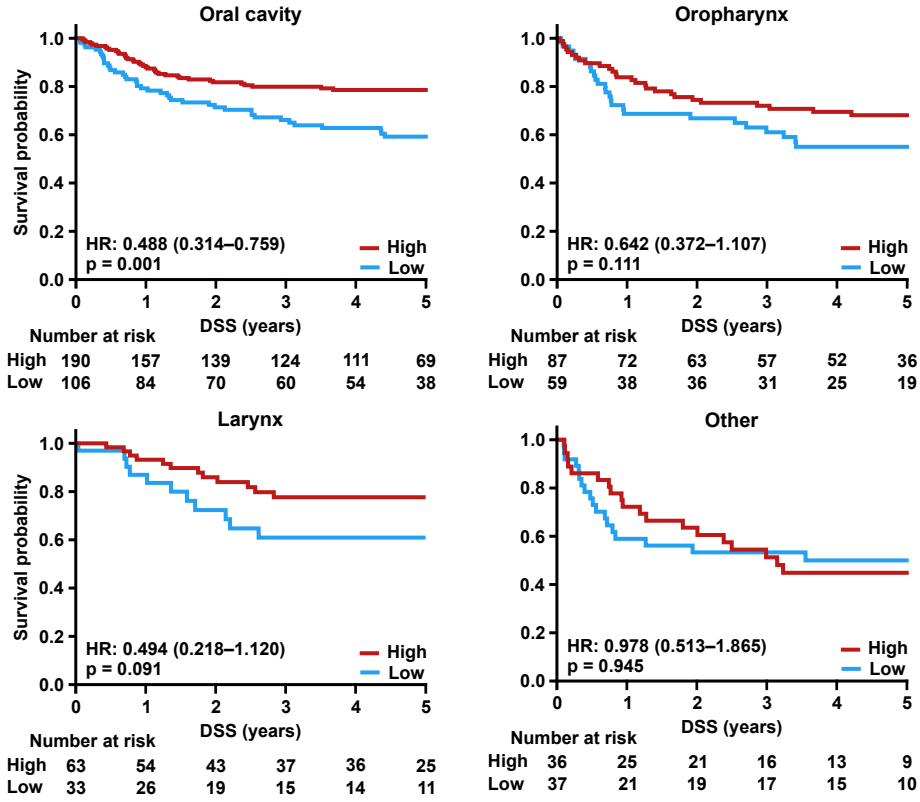

Figure 7. Site-specific prognostic trends according to HR (95% CI) for 5-year DSS divided into low and high TSPO tumour expression. Statistical significance was calculated using Cox's proportional hazard model. p-values < 0.05 were considered statistically significant.

Interestingly, no association was observed between survival and TSPO tumour expression in p16-negative oropharyngeal cancer, whereas high TSPO expression was associated with better OS (HR: 0.249, 95% CI: 0.111–0.563, p < 0.001, original publication **I**, **Figure 5A**) and DSS (HR: 0.263, 95% CI: 0.105–0.654, p = 0.004) in p16-positive oropharyngeal cancer (**I**, **Figure 8A**). No differences in DFS were found in either group. Furthermore, an indicatively significant (HR: 0.516, 95% CI: 0.259–1.030, p = 0.061) association between TSPO tumour expression and OS was observed in patients treated with radiotherapy alone (original publication **II**, **Figure 1A**). Statistical significance (HR: 0.373, 95% CI: 0.156–0.890, p = 0.026) was reached for DSS (**I**, **Figure 8B**). Such an association was not observed in patients treated with chemoradiotherapy, surgery, or a combination of surgery and radiotherapy with or without chemotherapy (original publication **II**, **Supplementary Figure S1**).

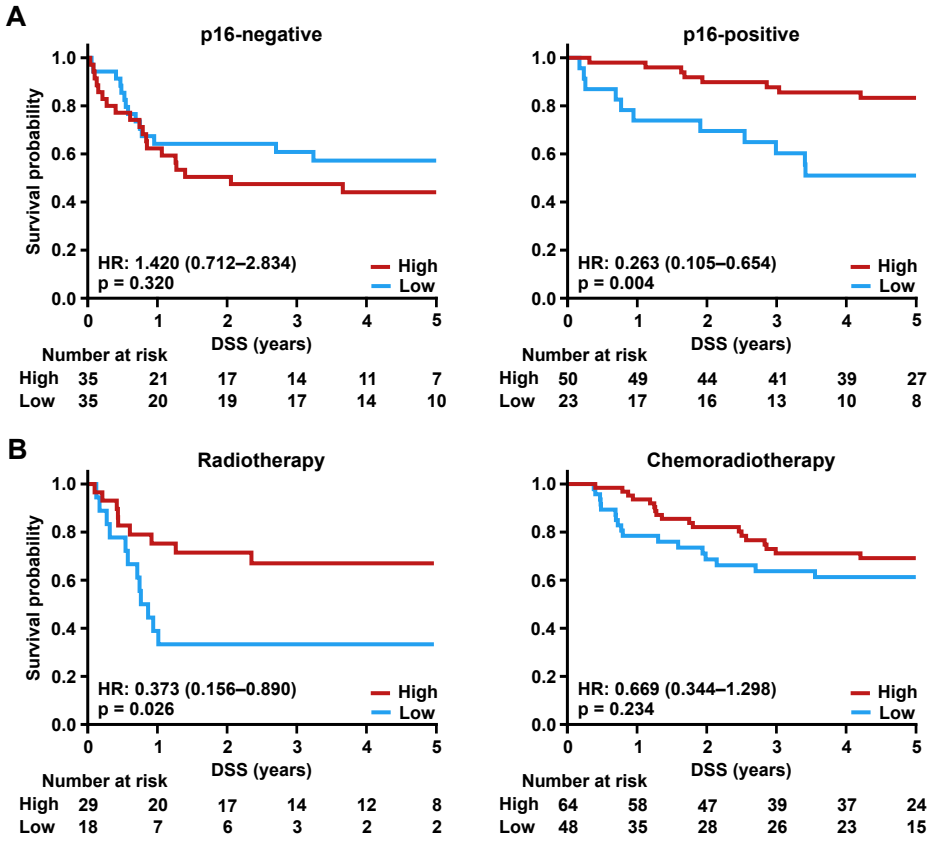

Figure 8. Prognostic trends with HR (95% CI) for 5-year DSS in (**A**) patients with p16-positive or p16-negative oropharyngeal cancer and (**B**) patients treated with radiotherapy alone or chemoradiotherapy divided into low and high TSPO tumour expression. Statistical significance was calculated using Cox's proportional hazard model. p-values < 0.05 were considered statistically significant.

TCGA HNSCC cohort analyses showed an association (HR: 0.708, 95% CI: 0.528–0.948, p = 0.021) between high TSPO expression and better OS at 3 years of follow-up, but this association was not found towards the 5-year endpoint (original publication **I**, **Figure 6A**). Site-specific survival analyses showed better OS (HR: 0.445, 95% CI: 0.234–0.845, p = 0.013) in patients with high TSPO-expressing laryngeal tumours compared with lower TSPO expression (original publication **I**, **Supplementary Figure S5E**). No differences were found in other tumour sites.

5.3 TSPO gene overlap analyses

Pathway gene overlap analyses were performed for genes strongly co-expressed (either negatively or positively) with *TSPO* (original publication **I**, **Figure 7**). For positively co-expressed genes, OXPHOS had the highest statistical significance (**I**, **Figure 9A**). In addition, pathways related to oxidative metabolism were associated with genes positively co-expressed with *TSPO*. Genes either positively or negatively co-expressed with *TSPO* were associated with DNA damage response pathways. Negatively co-expressed genes were also associated with the G2/M checkpoint. The genes associated with OXPHOS, DNA damage response, and the G2/M checkpoint are shown in **Table 10**.

Table 10. Genes associated with OXPHOS, DNA damage response, and the G2/M checkpoint.

PATHWAY	GENES
OXPHOS	*ATP5F1D*, *COX6A1*, *COX7A2*, *COX8A*, *COX4I1*, *NDUFA6*, *NDUFA7*, *NDUFA11*, *NDUFA13*, *NDUFB7*, *NDUFS7*, *POLR2F*, *SCO2*, *UQCR10*, *UQCR11*, and *UQCRQ*
DNA damage response	*ATRX*, *ATF2*, *CBL*, *EDF1*, *ERCC4*, *ERCC6L2*, *FAM168A*, *GUK1*, *NME3*, *POLR2F*, *PRKDC*, *PTPN1*, *REV3L*, *RIF1*, *SHPRH*, *SMG1*, *TAF1*, *TAOK1*, *TP53BP1*, and *VPS28*
G2/M checkpoint	*ATRX*, *CASP8AP2*, *CCNT1*, and *CDC27*

In addition to pathway overlap analyses, the correlation between *TSPO* expression and immune cell abundance in different cancers was studied using publicly available databases (original publication **I**, **Figure 8**). Correlations between *TSPO* expression and immune cell abundances were significant in many immune cell types in HNSCC. However, these correlations were clearly more prominent in other cancers, such as glioblastoma, lower grade glioma, and testicular germ cell tumours (**I**, **Figure 9B**). The clearest correlation between *TSPO* expression and immune cell abundance was found in activated CD8 T cells, $CD56^{dim}$, and $CD56^{bright}$ natural killer cells, whereas the most significant negative associations were observed

in memory B cells, type 2 T helper cells, and effector memory CD4 T cells (original publication **I**, **Supplementary Figure S6**).

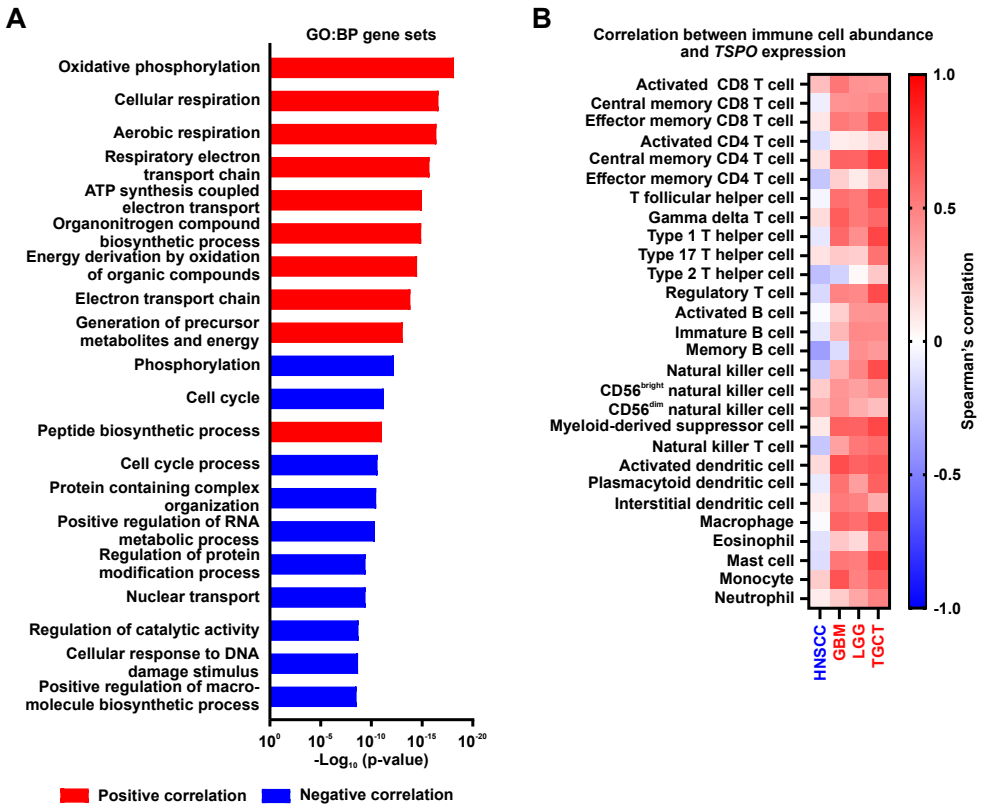

Figure 9. (**A**) Gene Ontology: Biological Processes (GO:BP) gene set pathway overlap analysis for the top 100 genes positively (red) or negatively (blue) co-expressed with *TSPO*. (**B**) Correlation between TSPO expression and immune cell abundance in HNSCC, glioblastoma multiforme (GBM), lower-grade glioma (LGG), and testicular germ cell tumours (TGCT).

5.4 TSPO in DNA damage response

An overview of DNA damage response after irradiation and cisplatin treatment is represented in **Figure 10**.

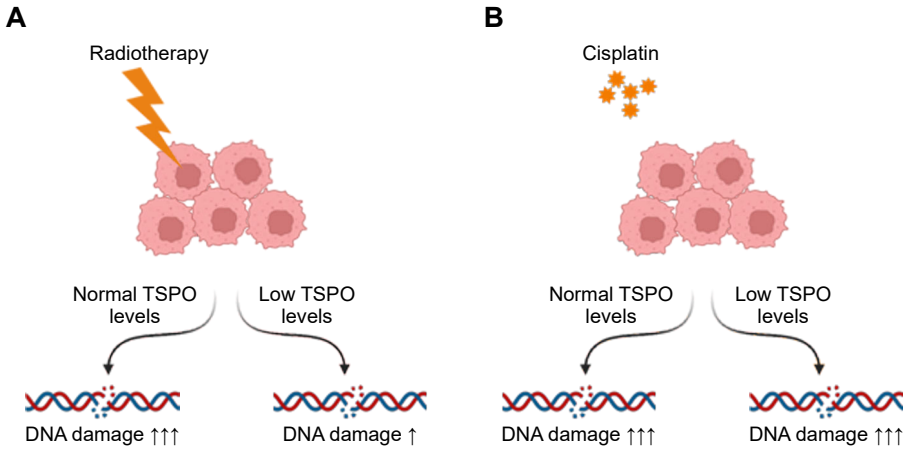

Figure 10. (**A**) Radiotherapy-reduced decrease in DNA damage response in TSPO-silenced FaDu cells. (**B**) No such a difference was observed after cisplatin treatment. Created with BioRender.com.

5.4.1 DNA damage after irradiation

TSPO expression decreased significantly (0.65 ± 0.18, p = 0.0017) 1 h after irradiation and increased (1.67 ± 0.31, p = 0.0009) 16 h after irradiation in NT siRNA FaDu cells (original publication **II**, **Figure 2C**). Higher γH2Ax expression was observed after irradiation in both NT and TSPO siRNA-transfected cells and was significantly (4.39 ± 2.08, p = 0.0066) higher 1 h after irradiation in NT siRNA cells. An indicatively significant (p = 0.069) increase in γH2Ax expression was observed in irradiated (2.61 ± 0.92) TSPO siRNA cells compared with non-irradiated (0.66 ± 0.13) cells. Moreover, an indicatively significant (p = 0.093) decrease in γH2Ax expression was observed in TSPO siRNA cells compared with NT siRNA cells. γH2Ax expression was increased in NT siRNA cells even 16 h after irradiation, whereas no difference was seen in TSPO siRNA cells. pChk1 expression was similarly higher 1 h after irradiation in both NT (3.57 ± 0.48, p = 0.0005) and TSPO siRNA (3.02 ± 0.69, p = 0.0063) FaDu cells compared with non-irradiated NT siRNA and TSPO siRNA (1.33 ± 0.75) cells, and it remained elevated 16 h after irradiation, although no significance was reached.

In Cal33 cells, TSPO expression remained stable after irradiation regardless of TSPO silencing (original publication **II**, **Figure 2D**). No clear changes were observed in γH2Ax expression after irradiation. However, pChk1 expression tended

to increase in both NT (2.93 ± 1.50, p = 0.053) and TSPO (3.06 ± 1.30, p = 0.076) siRNA cells 1 h after irradiation compared with non-irradiated NT siRNA and TSPO siRNA (1.33 ± 0.63) cells and remained higher 16 h after irradiation, although no statistical significances were reached.

PK11195 treatment had no effect on TSPO expression in parental FaDu cells (original publication **II**, **Figure 3C**). Again, γH2Ax expression increased after irradiation but was significantly (5.15 ± 3.59, p = 0.035) higher only in non-treated cells 1 h after irradiation. Similarly, PK11195 treatment had no clear effect on TSPO expression in Cal33 cells (original publication **II**, **Figure 3D**). The expression of γH2Ax and pChk1 increased after irradiation. Furthermore, a significant (2.58 ± 1.48, p = 0.038) increase in pChk1 expression was observed in non-treated cells 1 h after irradiation, which remained increased (2.76 ± 1.86, p = 0.053) 16 h after irradiation.

5.4.2 DNA damage after cisplatin treatment

Due to high variation, an indicatively significant decrease in TSPO expression was observed in NT siRNA-transfected FaDu cells after 1 μM (0.69 ± 0.012, p = 0.083) and 3 μM (0.69 ± 0.28, p = 0.084) cisplatin treatment compared with 0.5 μM treatment (2.30 ± 2.14, original publication **II**, **Figure 6B**). Both γH2Ax and pChk1 tended to increase in a dose-dependent manner. A significant increase in γH2Ax expression was observed after 3 μM cisplatin treatment (5.76 ± 1.29) compared with nontreated (p = 0.001) and 0.5 μM cisplatin-treated (1.90 ± 0.32, p = 0.0065) NT siRNA cells. Furthermore, γH2Ax expression increased significantly (5.79 ± 2.63, p = 0.0016) after 3 μM cisplatin treatment in TSPO siRNA cells compared with that in nontreated cells (1.23 ± 0.42).

In Cal33 cells, cisplatin treatment did not affect TSPO expression in NT and TSPO siRNA cells (original publication **II**, **Figure 6D**). pChk1 expression increased significantly after 3 μM cisplatin treatment in both NT (3.45 ± 1.73, p = 0.0088) and TSPO (3.60 ± 0.53, p = 0.010) siRNA cells compared with that in nontreated NT siRNA and TSPO siRNA (1.21 ± 0.29) cells. Moreover, pChk1 expression increased significantly after 0.5 μM cisplatin treatment (3.35 ± 1.20) in TSPO siRNA cells compared with nontreated TSPO siRNA cells (p = 0.023) and NT siRNA cells (1.69 ± 0.37, p = 0.022) treated with the same dose.

PK11195 treatment did not affect the expression of TSPO, γH2Ax, pChk1, cPARP1, and pAkt in FaDu cells (original publication **II**, **Figure 7B**). Again, γH2Ax increased significantly after cisplatin treatment (4.14 ± 0.88) and combination treatment with cisplatin and PK11195 (5.89 ± 1.33) compared with nontreated (p = 0.0055 and p = 0.0005, respectively) and PK11195-treated (0.70 ± 0.55, p = 0.0042 and p = 0.0004, respectively) cells.

In Cal33 cells, PK11195 alone had no effect on TSPO, γH2Ax, pChk1, cPARP1, and pAkt expression (original publication **II**, **Figure 7C**). pChk1 expression increased after cisplatin treatment and was significantly higher after combination treatment (8.14 ± 2.70) compared with the nontreated ($p = 0.0086$) and PK11195-treated (1.80 ± 0.79, $p = 0.017$) cells.

5.5 Oxidative phosphorylation

OCRs were measured after irradiation in NT and TSPO siRNA-transfected cells, as well as in parental cells with or without acute PK11195 injection. In addition, the expression subunits of OXPHOS complexes were analysed after irradiation in NT and TSPO siRNA FaDu and Cal33 cells. Moreover, a subset of parental cells was treated with PK11195.

5.5.1 Mitochondrial respiration in cells

Basal respiration (65.06 ± 8.31, $p = 0.0009$), maximal respiration (105.25 ± 19.31, $p = 0.0039$), and ATP production (50.48 ± 5.80, $p = 0.0007$) were significantly lower in TSPO-silenced FaDu cells compared with NT siRNA (89.39 ± 7.83, 146.20 ± 10.57, and 69.76 ± 4.86, respectively) cells (original publication **II**, **Figure 4A**). Basal respiration levels were significantly (76.21 ± 21.63, $p = 0.038$) lower in irradiated TSPO siRNA cells compared with NT siRNA cells (90.37 ± 8.99). Maximal respiration (124.74 ± 42.79, $p = 0.064$) and ATP production (59.68 ± 17.91, $p = 0.055$) also trended towards lower levels after irradiation in TSPO siRNA cells compared with NT siRNA cells (149.85 ± 19.78 and 69.84 ± 5.80, respectively). PK11195 alone had no effect on OCRs in either NT siRNA or TSPO siRNA FaDu cells (original publication **II**, **Figure 5B**). However, a significant ($p = 0.020$) difference in acute response was observed after irradiation in PK11195-treated (-5.62 ± 2.16 non-irradiated vs. -11.13 ± 5.05 irradiated) cells (original publication **II**, **Figure 5A**). Significantly higher basal respiration (115.84 ± 33.33, $p = 0.0034$) and ATP production (82.20 ± 28.46, $p = 0.020$) and significantly lower maximal respiration (108.44 ± 23.58, $p = 0.003$) were observed after irradiation in PK11195-treated cells compared with nontreated and non-irradiated cells (90.08 ± 9.40, 70.46 ± 9.26, and 150.12 ± 24.48, respectively).

TSPO silencing and irradiation did not affect the OCRs of Cal33 cells (original publication **II**, **Figure 4B**). However, a significant (-4.90 ± 1.31 non-irradiated vs. -8.71 ± 4.39 irradiated, $p = 0.015$) acute response was observed after irradiation in PK11195-treated cells (original publication **II**, **Figure 5A**). Significantly lower basal respiration (62.54 ± 7.22, $p = 0.0009$) and ATP production (53.94 ± 10.82, $p = 0.0002$) were measured in nonirradiated Cal33 cells after PK11195 treatment

compared with nontreated (82.04 ± 11.78 and 67.24 ± 9.83, respectively) cells (original publication **II**, **Figure 5B**).

5.5.2 OXPHOS in HNSCC cells

No effect of TSPO silencing was seen on the expression of OXPHOS complex subunits in FaDu cells (original publication **II**, **Supplementary Figure S5B**). A significant decrease in the expression of SDHB (0.76 ± 0.13, p = 0.027) and ATP5A (0.79 ± 0.16, p = 0.033) was observed 1 h after irradiation in NT siRNA-transfected FaDu cells. ATP5A expression was also decreased in irradiated TSPO siRNA-transfected cells (0.69 ± 0.056) compared with non-irradiated cells (1.04 ± 0.11, p = 0.0031). PK11195 had no effect on subunit expression (original publication **II**, **Supplementary Figure S6B**). However, a slight (0.72 ± 0.27, p = 0.061) decrease in SHDB expression was observed 1 h after irradiation in nontreated cells, which reached significance (1.12 ± 0.26 non-irradiated vs. 0.82 ± 0.095 irradiated, p = 0.048) in PK11195-treated cells. A significant decrease in ATP5A expression was also found in nontreated (0.78 ± 0.12, p = 0.035) cells 1 h after irradiation. Moreover, ATP5A expression decreased in irradiated and PK11195-treated (0.82 ± 0.22, p = 0.042) cells compared with non-irradiated cells (1.03 ± 0.10). NDUFB8 expression decreased slightly (0.67 ± 0.21, p = 0.058) in nontreated cells 16 h after irradiation.

In Cal33 cells, no clear effect of irradiation was observed on subunit expression either 1 or 16 h after irradiation (original publication **II**, **Supplementary Figure S5D**). Only an indicatively significant (p = 0.076) decrease in ATP5A expression (0.74 ± 0.042) was observed 1 h after irradiation in TSPO siRNA cells compared with non-irradiated TSPO siRNA cells (0.94 ± 0.24). Nevertheless, a slight decrease (0.75 ± 0.19, p = 0.064) in SDHB and a significant decrease (0.75 ± 0.18, p = 0.020) in COX II expression were observed in non-irradiated TSPO siRNA cells compared with NT siRNA cells. PK11195 treatment had no effect on subunit expression in non-irradiated cells (original publication **II**, **Supplementary Figure S6D**). NDUFB8 expression increased (1.24 ± 0.20, p = 0.034) in PK11195-treated cells compared with nontreated (0.94 ± 0.24) cells 16 h after irradiation. Moreover, irradiation increased (1.12 ± 0.078, p = 0.050) UQCRC2 expression in PK11195-treated cells compared with nontreated cells (0.96 ± 0.056) 16 h after irradiation. COX II expression decreased (0.72 ± 0.14, p = 0.024) in nontreated cells 16 h after irradiation and was significantly (1.05 ± 0.16, p = 0.011) higher in PK11195-treated cells compared with nontreated cells.

5.6 TSPO mRNA and protein expression after irradiation in HNSCC cells and xenografts

TSPO mRNA levels were slightly increased in fractionally irradiated FaDu cells compared with non-irradiated cells (original publication **III**, **Figure 7D**). However, no statistical differences were seen. An indicatively significant increase was observed in protein levels (1.80 ± 1.51, p = 0.078) after fractionated irradiation compared with non-irradiated cells (1.00 ± 0.46, original publication **III**, **Figure 7C**).

TSPO protein expression in FaDu tumours had large variations after irradiation compared to non-irradiated tumours (original publication **III**, **Figure 3B**). The mean TSPO protein expression was ~3.0 folds (3.02 ± 2.27) higher 1 week after irradiation and ~6.6 folds (6.64 ± 6.99) higher 2 weeks after irradiation, but no statistical significance was reached.

TSPO expression was also analysed immunohistochemically from mouse xenografts. No difference in TSPO expression was found between irradiated and non-irradiated FaDu tumours (original publication **III**, **Supplementary Figure 3A**).

5.7 [^{18}F]F-DPA uptake by HNSCC cells and xenografts

5.7.1 Uptake in cells

The [^{18}F]F-DPA uptake was significantly (0.020 ± 0.0089, p = 0.0087) higher in irradiated FaDu cells than in non-irradiated (0.013 ± 0.0039) cells (**III**, **Figure 11A**). Tracer uptake was 88% and 78% lower in non-irradiated and irradiated cells, respectively, after PK11195 pre-treatment. TSPO silencing decreased tracer uptake by 37% in non-irradiated (0.0042 ± 0.0015, p = 0.044) and 48% in irradiated (0.0042 ± 0.0010, p = 0.0007) FaDu cells compared with the corresponding control-silenced (0.0066 ± 0.0019 and 0.0081 ± 0.0019, respectively) cells (**III**, **Figure 11B**). However, no significant increase in tracer uptake was observed after irradiation. Successful TSPO silencing was analysed with Western blot (**III**, **Figure 11C**) and qPCR (original publication **III**, **Figure 8C**).

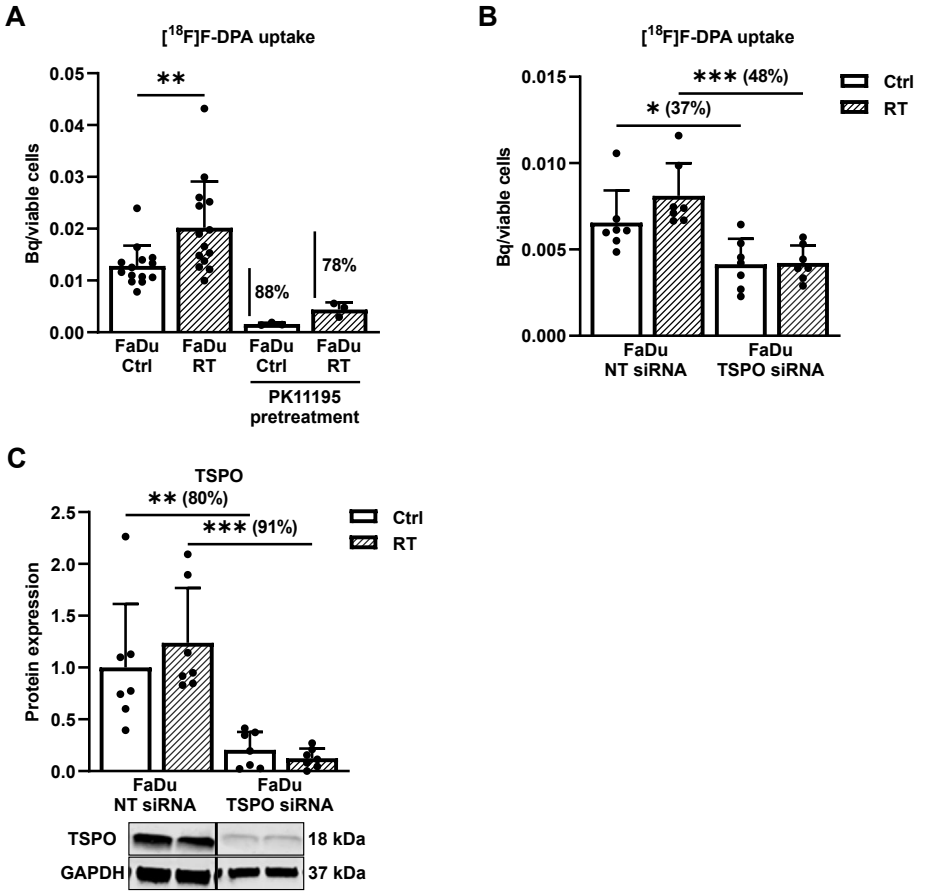

Figure 11. (**A**) [^{18}F]F-DPA uptake in non-irradiated (Ctrl) and irradiated (RT) non-treated and PK11195 pre-treated FaDu cells. (**B**) [^{18}F]F-DPA uptake by Ctrl and RT control-silenced (NT siRNA) and TSPO-silenced (TSPO siRNA) FaDu cells. Statistical significance was calculated using two-way ANOVA. (**C**) TSPO protein expression in Ctrl and RT control NT siRNA and TSPO siRNA FaDu cells. GAPDH was used as a loading control. Two-tailed Student's t-test was used to compare the uptake between Ctrl and RT nontreated cells, and two-way ANOVA was used to compare the uptake or TSPO expression in siRNA cells. p-values < 0.05 were considered statistically significant. *p < 0.05, **p < 0.01, ***p < 0.001.

5.7.2 Uptake in animals (*in vivo* and *ex vivo*)

Irradiation significantly increased *in vivo* [^{18}F]F-DPA uptake in the FaDu xenografts after both 1 (1.51 ± 0.60, p = 0.033) and 2 weeks (1.12 ± 0.17, p = 0.0024) after irradiation compared with non-irradiated (0.73 ± 0.31 and 0.64 ± 0.18, respectively) xenografts (**III**, **Figure 12A**). A similar increase, although not statistically significant (1.47 ± 0.31, p = 0.063), was observed in irradiated Cal33 xenografts compared with non-irradiated (0.91 ± 0.22) xenografts (original publication **III**,

Figure 2D). In contrast, [^{18}F]FDG uptake was not increased in FaDu xenografts 1 week after irradiation, but was significantly (1.78 ± 0.45 non-irradiated vs. 0.91 ± 0.14 irradiated, p = 0.0025) lower after 2 weeks (original publication **III**, **Figure 2B**). The [^{18}F]F-DPA uptake into tumours was fast and reached a plateau approximately 15 min after tracer injection (original publication **III**, **Figure 3A**).

In addition, *ex vivo* counting of ^{18}F-radioactivity was higher (3.26 ± 1.01, p = 0.0015) in irradiated FaDu tumours compared with non-irradiated (1.20 ± 0.70) tumours (**III**, **Figure 12B**). T/B ratios showed increased (p = 0.012) ^{18}F-radioactivity in irradiated FaDu tumours (7.40 ± 2.29) compared with non-irradiated (3.07 ± 2.08) tumours (**III**, **Figure 13A**).

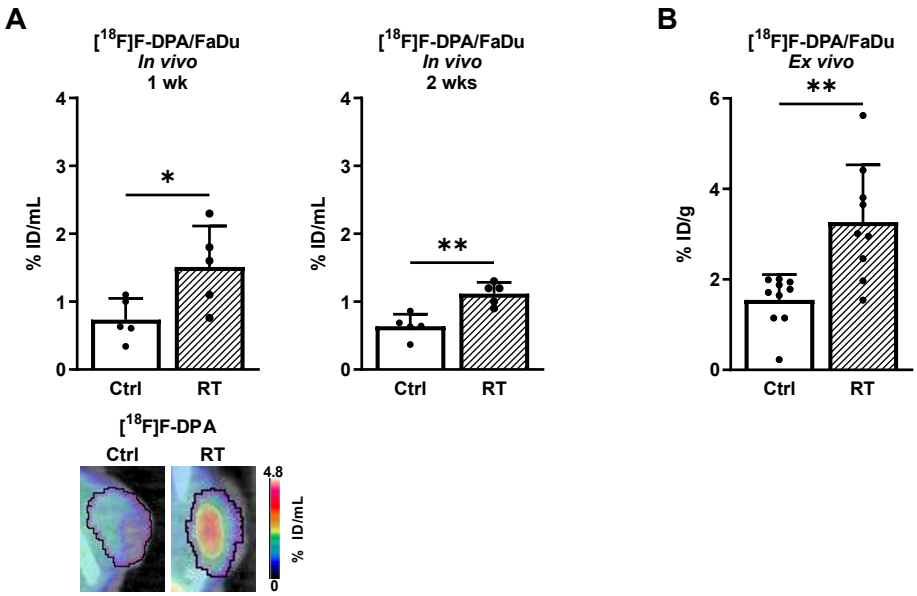

Figure 12. (**A**) [^{18}F]F-DPA uptake in non-irradiated (Ctrl) and irradiated (RT) FaDu xenografts. (**B**) *Ex vivo* ^{18}F-radioactivity counting of non-irradiated (Ctrl) and irradiated (RT) FaDu xenografts. Two-tailed Student's t-test was used for statistical analyses. p-values < 0.05 were considered statistically significant. *p < 0.05, **p < 0.01.

5.7.3 Blocking of TSPO

Significantly higher ^{18}F-radioactivity was observed in the blood (2.73 ± 0.72, p < 0.0001), plasma (4.03 ± 1.13, p < 0.0001), erythrocytes (1.88 ± 0.56, p = 0.0002), and muscle (2.06 ± 0.51, p = 0.039) of non-irradiated and PK11195 pre-treated FaDu xenografts compared with nontreated (0.47 ± 0.094, 0.19 ± 0.089, 0.67 ± 0.068, and 1.45 ± 0.56, respectively) xenografts (original publication **III**, **Figure 4B**). An indicatively significant (2.64 ± 0.54, p = 0.075) increase in [^{18}F]F-DPA uptake was

seen in PK11195 pre-treated tumours compared with nontreated tumours (1.55 ± 0.56).

PK11195 pre-treated and non-irradiated tumours had significantly lower T/B ratios (1.00 ± 0.26) compared with nontreated tumours (7.00 ± 2.47, p = 0.0003) and non-irradiated and nontreated tumours (3.65 ± 1.71, p = 0.0022) (**III**, **Figure 13A**).

Ex vivo autoradiography showed higher tracer uptake in FaDu tumours after irradiation (**III**, **Figure 13B**). Furthermore, tracer uptake was observed in *in vitro* autoradiography after [^{18}F]F-DPA incubation. Pre-treatment with PK11195 completely blocked the *in vitro* [^{18}F]F-DPA uptake.

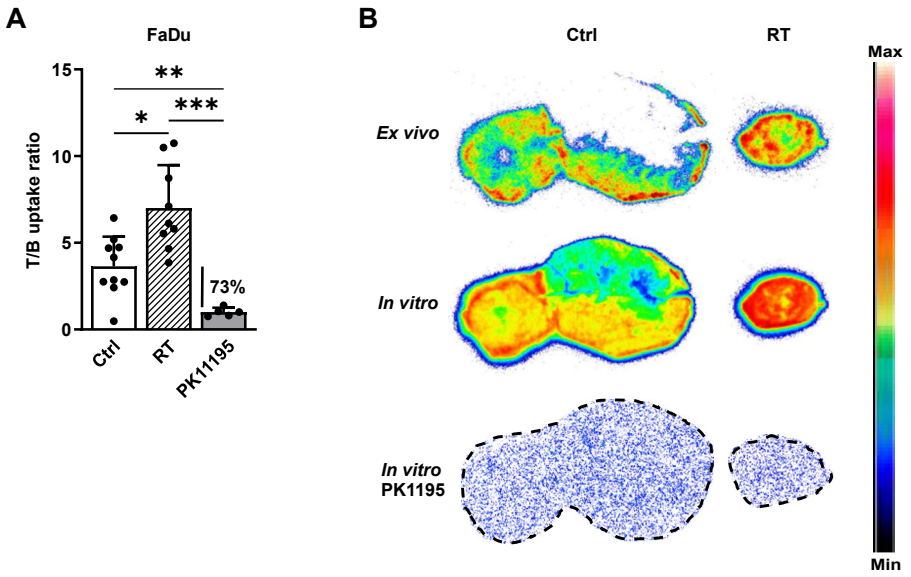

Figure 13. (**A**) T/B ratios of the ^{18}F-radioactivity in non-irradiated (Ctrl), irradiated (RT), and non-irradiated and PK11195 pre-treated FaDu tumours. (**B**) *Ex vivo* and *in vitro* autoradiography of Ctrl and RT FaDu tumours. PK11195 pre-treatment was performed by co-incubation with [^{18}F]F-DPA. One-way ANOVA was used for statistical analysis. p-values < 0.05 were considered statistically significant. *p < 0.05, **p < 0.01, ***p < 0.001.

5.7.4 Biodistribution of [^{18}F]F-DPA

Significantly higher ^{18}F-radioactivity accumulation was found in the adrenal glands (p = 0.028) and kidneys (p = 0.023) in the whole-body biodistribution of [^{18}F]F-DPA in mice with FaDu tumours that were locally irradiated (112.51 ± 8.00 and 41.78 ± 7.62, respectively) compared with non-irradiated mice (64.30 ± 23.34 and 25.06 ± 1.28, respectively) (original publication **III**, **Figure 4A**). High tracer uptake was observed in brown adipose tissue (56.60 ± 35.91) and (38.74 ± 17.94). Intermediate

uptake was observed in the lungs (22.57 ± 4.53), spleen (18.25 ± 4.30), liver (13.50 ± 0.51), and salivary glands (10.30 ± 6.00).

5.7.5 Radiometabolite analyses and autoradiography of [^{18}F]F-DPA

Five different radiometabolites were visible in plasma 40 min after [^{18}F]F-DPA injection, and these accounted, on average, for 34%, 40%, and 50% of the total radioactivity in non-irradiated, irradiated and PK11195 pre-treated mice, respectively (original publication **III**, **Figure 5A**). The same radiometabolites were found in tumours, but accounted for only 5% of the activity, and 95% of the activity originated from the unchanged tracer.

5.8 Proportion of monocytes and TAMs in xenografts

The number of monocytes increased slightly, although not statistically, in irradiated tumours compared with non-irradiated tumours (original publication **III**, **Figure 6A**). A significant (23.2 ± 5.61 non-irradiated vs. 37.75 ± 6.63 irradiated, $p = 0.029$) increase in the percentage of migratory monocytes/macrophages was observed after irradiation. The percentage of M1 (26.68 ± 6.62 non-irradiated vs. 12.95 ± 4.43 irradiated, $p = 0.029$) and M2 (24.38 ± 6.71 non-irradiated vs. 13.25 ± 6.51 irradiated, $p = 0.011$) macrophages decreased after irradiation.

6 Discussion

6.1 Prognostic role of TSPO

The prognostic role of TSPO has been previously studied in several cancers (Venturini et al., 1999; Maaser et al., 2002; Han et al., 2003; Königsrainer et al., 2007; Chen et al., 2017), showing an association between poor survival and high TSPO tumour expression. However, only one study had been published at the beginning of this thesis, reporting that high TSPO tumour expression was associated with poor survival in a small cohort (n = 69) of patients with oral cancer (Nagler et al., 2010). As HNSCC is a heterogeneous cancer, one of the aims of this thesis was to evaluate the prognostic role of TSPO in HNSCC in a large patient cohort, consisting of HNSCCs originating from different sites. Surprisingly, the results in study **I** were opposite to those of a previous study by Nagler *et al*. (2010), which showed an association between low TSPO tumour expression and poor survival in patients with HNSCC. This association was most prominent in patients with p16-positive oropharyngeal carcinoma. This is an important finding, as p16-positive tumours are often poorly differentiated (Fakhry et al., 2008), although they respond better to radiotherapy (Tawk et al., 2021). Furthermore, our *in silico* analyses revealed that genes co-expressed with *TSPO* participate in OXPHOS and DNA damage response, indicating a possible connection between TSPO, p16, and radiotherapy response in HNSCC.

Several studies have proposed different mechanisms by which p16 regulates radiotherapy response (Wang et al., 2020). One suggested mechanism is that p16 increases the number of DNA DSBs in irradiated cells by impairing the homologous recombination repair pathway (Rieckmann et al., 2013) by reducing the expression of cyclin D1 expression, thereby decreasing the recruitment of RAD51 to the DNA damage site (Dok et al., 2014). Studies have also reported that p16-positive cells cannot proceed from the G2/M phase of the cell cycle after irradiation, leading to dysregulated cell cycle and increased apoptosis (Rieckmann et al., 2013; Arenz et al., 2014). *In silico* analyses in study **I** indicated that some genes (i.e. *CDC27* and *CCNT1*) co-expressed negatively with *TSPO* regulate the G2/M checkpoint. CDC27 is part of the anaphase-promoting complex/cyclosome (APC/C) and regulates G2/M transition by interacting with CDC20 and promoting protein proteolysis, which leads

to chromosome segregation. It has been hypothesised to have either a tumour suppressive or an oncogenic role in different cancers (Kazemi-Sefat et al., 2021). CCNT1 is part of protein kinase complex P-TEFb that phosphorylates RNA polymerase II and promotes transcriptional elongation (Ramakrishnan et al., 2012). P-TEFb has been suggested to mediate DNA damage response after radiotherapy by regulating DNA repair and cell cycle in glioma; hence, inhibition of this complex would lead to a better radiotherapy response (Walker et al., 2024). Thus, a possible hypothesis is that decreased TSPO expression increases the expression of these G2 checkpoint regulating-proteins, which help cancer cells proceed to the M phase of the cell cycle, leading to increased cell proliferation and tumour expansion. However, it is not known how p16 regulates the G2 phase after irradiation and how TSPO is connected in this process; thus, more studies are needed. In contrast, another negatively co-expressed gene (*ATRX*) may inhibit G2/M checkpoint progression. ATRX belongs to the switch/sucrose non-fermentable (SWI/SNF) family, also known as BRG1/BRM-associated factor (BAF) complexes, which remodel chromatin and regulate gene expression. Several studies have proposed a tumour suppressor role for ATRX, which is often mutated in cancers (reviewed in Pang et al., 2023). ATRX has also been suggested to regulate Chk1, which inhibits transition from the G2/M checkpoint (Qin et al., 2022). Thus, no clear conclusions between TSPO and cell cycle regulation can be reached based on *in silico* data alone.

The results of the TMA and TCGA patient cohorts showed that the association between TSPO expression and survival differed between tumour sites. In addition to p16-positive oropharyngeal cancer, poor DSS was significantly associated with low TSPO tumour protein expression in patients with oral cancer. However, this association was not found in the TCGA cohort. However, a trend towards an association between low TSPO tumour expression and 5-year and 3-year DSS was observed in laryngeal cancer in the TMA and TCGA cohort, respectively. Furthermore, a trend towards an association between low TSPO expression and DFS in the TMA cohort and a significant association between 3-year PFS survival and low TSPO tumour expression in the TCGA cohort in laryngeal cancer was seen. These findings indicate a distinctive role for TSPO in laryngeal cancer, especially in terms of treatment response. This is important because the treatment approach for laryngeal cancer requires extreme consideration to preserve the larynx, while achieving the best chance of cure (Obid et al., 2019). No differences were found at other tumour sites (hypopharynx, nasopharynx, sinonasal, and unknown) that were combined in the TMA cohort. These results indicate that the association between TSPO expression and survival differs among HNSCC subsites. In particular, the possible association between TSPO expression and DFS and PFS survival in patients with laryngeal cancer indicates a distinct role for TSPO that is observed in other HNSCC sites. However, studies with patients and cell lines originating from

different tumour sites, especially the larynx, are needed to draw conclusions from these findings.

Our *in silico* gene overlap analyses in study **I** using three different databases indicated a clear role for TSPO in OXPHOS in HNSCC. The role of TSPO in oxidative metabolism has been widely studied, with reports of an association between increased oxidative metabolism and higher TSPO expression in cells and mice (Liu et al., 2017; Milenkovic et al., 2019; Fu et al., 2020). However, contradicting results regarding the association between OXPHOS and survival in HNSCC have been reported. Frederick *et al*. (2020) found that higher expression of genes involved in OXPHOS was associated with better survival in HNSCC. Another study showed that mediator complex subunit 30 (MED30)-mediated OXPHOS was associated with poor survival in HNSCC (Noh et al., 2023). In addition, HPV-negative tumours rely more on glycolysis as their metabolic pathway than HPV-positive tumours (Fleming et al., 2019), which rely more on OXPHOS (Jung et al., 2017; Cruz-Gregorio et al., 2019; Prusinkiewicz et al., 2020). However, HPV-positive HNSCC is also reported to have low levels of OXPHOS (Li et al., 2023). In addition, recurrent HPV-positive HNSCC with enriched nuclear factor erythroid 2-related factor 2 (NRF2) and OXPHOS signalling were associated with worse survival (Vyas et al., 2021). Furthermore, NRF2 overexpressing HPV-positive cells were sensitive to OXPHOS inhibitors. NRF2 participates in many cellular functions, such as metabolic reprogramming (He et al., 2020). These results indicate that higher OXPHOS is associated with worse survival in recurrent HPV-positive HNSCC. Nevertheless, a higher glycolytic metabolism may explain the increased radioresistance of HPV-negative HNSCC (Mims et al., 2015). Our findings indirectly support this, as low TSPO tumour expression is associated with worse DSS in patients with p16-positive oropharyngeal cancer. Lower TSPO levels may reduce oxidative metabolism in these tumours; thus, making them rely more on glycolysis, which in turn leads to worse radiotherapy response.

6.2 Role of TSPO in DNA damage response

The functional role of TSPO has been studied for decades, but its role in cells remains unclear (Bonsack & Sukumari-Ramesh, 2018; Betlazar et al., 2020). Moreover, no previous studies have investigated whether there is an association between TSPO and DNA damage response. In study **II**, we found an association between worse DSS and low TSPO tumour expression in patients treated with radiotherapy alone. Such a difference in survival was not observed in patients treated with chemoradiotherapy, surgery, or combination treatment. These findings indicate that TSPO is related to radiotherapy response in HNSCC. Despite only a trend towards lower DNA damage levels quantified by γH2Ax expression was observed in TSPO-silenced FaDu cells

compared with control-silenced cells after irradiation in our *in vitro* studies (study **II**), these results suggest that TSPO may indeed have a role in the DNA damage response. This response was not related to changes in TSPO expression after irradiation, as we found a decrease in TSPO expression 1 h after irradiation in NT siRNA cells and an increase 16 h after irradiation. Thus, irradiation may cause functional changes in TSPO, which promote DNA damage response in a proapoptotic manner.

In contrast, no differences were found in γH2Ax expression after irradiation between NT and TSPO siRNA-transfected Cal33 cells, indicating different DNA response pathways between Cal33 and FaDu cells. Differences in the DNA damage response after irradiation between these cell lines were also indicated in a previous study by Kurth *et al*. (2015) in which they established radioresistant subclones from these cell lines. Only a slightly more radioresistant subclone was generated from FaDu cells with a higher surviving fraction after 2-Gy dose irradiation, whereas a significantly more radioresistant subclone was generated from Cal33 cells.

One possible reason for not reaching statistical significance in DNA damage response between NT and TSPO siRNA FaDu cells could be the selected time points (1 and 16 h after irradiation) when the DNA damage response was measured. The first responses to DNA damage occur within seconds after irradiation, and depending on the DNA damage repair pathway, the damage can be repaired within a couple of hours or several hours (Penninckx et al., 2021). There is a chance that we could have observed a more prominent difference in the DNA damage response between control and TSPO-silenced cells if we had done measurements between the selected time points. Nevertheless, our *in vitro* findings support our TMA patient cohort results in which low TSPO tumour expression is associated with worse survival. Thus, further studies should be conducted to find the exact regulatory role of TSPO in DNA damage response.

We did not observe any differences in DNA damage response between TSPO-silenced and control FaDu and Cal33 cells after cisplatin treatment, indicating that TSPO does not participate in DNA damage response caused by chemotherapy. This was also supported by our findings in the TMA data in study **II**, which showed no association between TSPO tumour expression and survival in patients treated with chemoradiotherapy.

In study **I**, we found negative co-expression between *TSPO* and *ATRX*, and between *TSPO* and *TP53BP1*. ATRX and TP53BP1 participate in DNA damage response. In section 6.1, the role of ARTX in cell cycle regulation was discussed. In addition, ATRX may participate in DNA repair. Although ATRX has been reported to play a tumour suppressor role in cancer, patients with ATRX-negative nasopharyngeal cancer have better OS than those with ATRX-positive cancer (Xie et al., 2021). TP53BP1, also known as 53BP1, is a tumour suppressor gene that

participates in DNA DSB repair and promotes non-homologous end-joining during the G1 phase of the cell cycle (Panier & Boulton, 2014). It is mutated in many cancers (Zhang et al., 2021), but it also plays a protective role after radiotherapy in glioblastoma (Squatrito et al., 2012) and after fractionated radiotherapy in different types of cancer cells (Eke et al., 2020). Based on these studies and their co-expression with *TSPO*, low TSPO expression in HNSCC could lead to increased ATRX and TP53BP1 expression, leading to increased DNA repair after radiotherapy, thereby promoting cancer cell survival.

Our results in study **II** did not indicate that TSPO regulates apoptosis (cPARP1) and proliferation (pAkt) after irradiation. However, previous *in vitro* studies have suggested that TSPO may play either an antiapoptotic or proapoptotic role, depending on the cancer. A proapoptotic role of TSPO has been reported in colorectal cancer (Shoukrun et al., 2008), Leydig tumour cells (Weisinger et al., 2004), glioma (Levin et al., 2005; Fu et al., 2020), and glioblastoma (Bode et al., 2012), whereas an antiapoptotic role has been proposed for breast cancer (Li et al., 2007), glioma (Rechichi et al., 2008; Bader et al., 2019), and T cell leukaemia (Liu et al., 2017). Furthermore, a study by Lin *et al*. (2014) found that TSPO inhibition reduced apoptosis in Drosophila induced by γ-radiation. However, Stoebner *et al*. (2001) reported increased survival in TSPO-transfected Jurkat cells after UVB exposure. As we studied apoptosis and proliferation in a relatively short time (1 and 16 h) after irradiation, it is possible that we were not able to detect long-term changes in apoptosis and proliferation caused by TSPO deficiency. In addition, we irradiated the cells with one dose (5 Gy) only, which may also explain why we did not observe any differences in apoptosis and proliferation.

6.3 Role of TSPO in OXPHOS

As discussed in the previous section (section 6.1), the results in study **I** indicate that TSPO participates in OXPHOS. In study **II**, we measured OCRs after irradiation and acute PK11195 injection and found significantly lower respiration levels in TSPO-silenced FaDu cells, indicating a regulatory role of TSPO in mitochondrial respiration. Our results are in line with several other studies showing a positive regulatory role for TSPO in oxidative metabolism (Banati et al., 2014; Zhao et al., 2016; Liu et al., 2017; Bader et al., 2019; Milenkovic et al., 2019; Fairley et al., 2023). Typically, OXPHOS is reduced in cancer cells, leading to reduced ROS levels and increased cancer cell survival (Arfin et al., 2021). Furthermore, several studies have suggested that TSPO regulates ROS homeostasis, although both positive and negative regulatory mechanisms have been proposed (Carayon et al., 1996; Stoebner et al., 2001; Jayakumar et al., 2002; Zeno et al., 2009; Choi et al., 2011; Joo et al., 2012; Gatliff et al., 2014; Joo et al., 2015; Tu et al., 2016; Fu et al., 2020). ROS

production is induced after radiotherapy, causing oxidative stress and leading to excessive ROS levels, which promotes apoptosis (Kim et al., 2019). Based on our *in silico* findings, indicating a positive regulatory role for TSPO in OXPHOS, lower TSPO expression may induce ROS production due to reduced OXPHOS levels. This may lead to worse radiotherapy response and induced cancer cell proliferation, as a slight increase in ROS levels promotes immunosuppression in the tumour microenvironment (Greene et al., 2022).

In Cal33 cells, we did not observe such a decrease in oxidative metabolism. This was most likely due to siRNA transfection, which can be stressful for the cells. Cal33 cells are likely more sensitive to such methods because we observed decreased respiration levels also in control-silenced cells.

We also studied the effects of acute PK11195 addition on mitochondrial respiration. PK11195 treatment alone did not affect mitochondrial respiration in FaDu cells. However, after radiotherapy, increased basal respiration and ATP production and decreased maximal respiration were observed in PK11195-treated cells. In Cal33 cells, PK11195 treatment decreased basal respiration and ATP production. These results indicate differences in the responses of these cell lines. PK11195 inhibits ATPase and decrease ATP synthesis (Cleary et al., 2007). These findings agree with our results in Cal33 cells, further supporting a regulatory role of TSPO in oxidative metabolism.

In addition to measuring mitochondrial respiration, we studied changes in the expression of specific subunits of OXPHOS complexes. Our results did not show that TSPO regulates OXPHOS, as no significant changes were observed. However, because these experiments were performed by quantifying the expression of specific subunits in the OXPHOS complexes, we do not know whether there were changes in the expression of other subunits.

6.4 [^{18}F]F-DPA uptake by HNSCC xenografts and cells

The use of TSPO-PET tracers in peripheral cancers has not been widely studied, but few preclinical studies have focused on TSPO imaging in cancers other than gliomas (Zheng et al., 2011; Vasdev et al., 2013; Zheng et al., 2016; Berroterán-Infante et al., 2018; Lanfranca et al., 2018; Tantawy et al., 2018; Zhang et al., 2019; de Souza et al., 2022). However, none of these studies were performed in HNSCC. Thus, our study was the first to evaluate the feasibility of TSPO-PET imaging in HNSCC. We demonstrated that [^{18}F]F-DPA specifically binds to TSPO in HNSCC and that irradiation increases tracer uptake. Importantly, these results show that TSPO-PET imaging is feasible for HNSCC. Two weeks after irradiation, we still detected increased [^{18}F]F-DPA uptake into tumours. In contrast, we observed no difference

in [^{18}F]FDG uptake 1 week after irradiation. However, a significant decrease in tracer uptake was observed 2 weeks after irradiation. [^{18}F]FDG imaging is used in HNSCC, reflecting glucose metabolism in tumours. Therefore, the reduction in tracer uptake 2 weeks after radiotherapy in our study indicates decreased glucose metabolism in tumours due to decrease in tumour size. In contrast, increased [^{18}F]F-DPA uptake two weeks after radiotherapy indicates a radiation-induced functional role of TSPO. The regulatory mechanism behind this increased uptake remains to be solved. Consequently, the usability of TSPO-PET imaging for estimating radiotherapy response should be further investigated.

Surprisingly, we did not find a significant correlation between [^{18}F]F-DPA uptake and TSPO expression in HNSCC cells. An increase in TSPO protein expression was observed in the xenografts after radiotherapy, whereas only a slight non-significant increase was observed in the cells. Thus, higher TSPO expression in the xenografts may indicate that an interaction between tumour cells and tumour microenvironment is required for TSPO activation. Non-tumorigenic cells present in the tumour microenvironment might also upregulate TSPO expression; thus, contributing to increased tracer uptake. Indeed, Zheng *et al*. (2011) reported that TSPO expression in breast cancer xenografts is affected by several cell types. Increased [^{18}F]F-DPA uptake in cells may also be affected by other changes in TSPO function. One possible hypothesis is the tracer binding capability of TSPO. TSPO functions as a monomer, dimer, and polymer (Hinsen et al., 2015; Jaipuria et al., 2017). Perhaps different forms of TSPO affect the binding potential of the tracer. It has been reported that photoirradiation increases the binding potential of PK11195 in Leydig cells (Delavoie et al., 2003); therefore, irradiation (X-ray) may change the tracer's binding potential. However, it should be noted that different TSPO-PET tracers bind to different parts of the protein, and do not necessarily work in the same manner. Thus, using different TSPO-PET tracers may not yield results similar to those we reported for [^{18}F]F-DPA.

Pre-treatment with PK11195 inhibited the [^{18}F]F-DPA T/B uptake ratio into tumours up to 73%, proving the tracer to be specific for TSPO. After PK11195 treatment, a significant increase in tracer uptake was found in the blood due to the successful blockade of TSPO in tissues. Successful blocking of [^{18}F]F-DPA uptake was also detected by *ex vivo* and *in vitro* autoradiography from tumour sections after PK11195 pre-treatment.

Moreover, only a small percentage (~5%) of radioactive metabolites was detected in non-irradiated and irradiated tumours, demonstrating that the uptake was mostly unchanged [^{18}F]F-DPA. The percentage of radioactive metabolites in the tumour increased (~30%) after PK11195 pre-treatment, being in line with the higher amount detected in plasma (~50%).

Whole-body biodistribution in study **III** showed surprising increases after local tumour irradiation in the adrenal glands and kidneys of mice with FaDu tumours. In addition to the possible immunological response discussed in original publication **III**, a long-range non-targeted response could explain the increase in tracer uptake. Radiation-induced non-targeted effects are a relatively new finding, indicating the effect of radiation on surrounding tissues and cells outside the irradiated area. This can mean either an effect on surrounding cells within a few millimetres range (bystander effect) or an effect on farther tissues and organs (abscopal effect). In addition, the heterogeneous effect between cells in the irradiated area might lead to a cohort effect, in which cells that received high-dose irradiation affect low-dose irradiated cells and vice versa (Daguenet et al., 2020). The bystander effect has been studied more extensively than the abscopal and cohort effects. One study showed that irradiation of the thyroid affected other organs as well (Langen et al., 2016). We speculate that tumour irradiation in our study had such abscopal effects on the kidneys and adrenal glands, leading to higher tracer uptake. However, as the cancer cells were inoculated into the hind limbs, the tumours formed relatively close to these organs. Thus, it is possible that some of the irradiation was also directed to these organs.

6.5 Study limitations

6.5.1 HNSCC patient cohort and *in silico* analysis

The main study limitations, such as the retrospective study design and the low number of patients in some of the subgroups, in study **I** have been discussed in the original publication. Additionally, in study **II**, due to the low number of patients treated with radiotherapy alone, we could not investigate the prognostic role of TSPO at each site separately. As it was also stated in study **I**, there was only one publicly available patient cohort (TCGA) that could be used to study the functional and prognostic role of TSPO in HNSCC. These public patient cohort data are based on mRNA expression rather than protein expression. Protein expression should be associated with the functional role of TSPO because mRNA can be degraded before protein synthesis and does not reflect tumour protein levels. However, having both mRNA and protein data can provide information from several aspects and can be considered an advantage instead of a limitation.

6.5.2 TSPO function in HNSCC

One limitation of the functional studies was the cell lines that were used. As discussed in section 6.2, the FaDu and Cal33 cell lines may exhibit different DNA damage responses. However, we selected these cell lines as radioresistant subclones

have been generated (see section 6.2), and our future studies will focus on identifying the connection between radioresistance and TSPO.

Another limitation was the use of siRNAs for TSPO silencing (also discussed in section 6.3). Silencing itself is a relatively stressful method for cells because siRNA can be toxic to cells and the medium used for transfection usually lacks FBS and other nutrients that are conventionally added to culture medium for cell survival and growth.

Additionally, when studying DNA damage response, we focused on only two DNA damage markers. Because DNA damage response is regulated via different signalling pathways, there is a chance of overlooking important findings when only selected DNA damage markers are analysed. However, studying every possible DNA damage marker is laborious and costly. As no previous studies have investigated the role of TSPO in DNA damage response, there were no indications on which DNA damage markers should be studied.

TSPO has been suggested to participate in cell cycle regulation and apoptosis in cancer cells. Again, we only studied changes in cPARP1 and activated Akt expression. Perhaps investigating other proteins involved in cell cycle regulation and apoptosis would have provided clearer results. In addition, studying apoptosis and proliferation at several time points and for a longer duration may also have shown changes that we were not able to detect with the selected time points. Nevertheless, there is evidence that TSPO regulates the DNA damage response after radiotherapy, although the exact mechanism remains unclear.

We studied the role of TSPO in OXPHOS regulation by measuring the protein levels of the subunits of the five OXPHOS complexes using Western blotting. This method is relatively limited, as Complex I consists of 45, Complex II of 4, Complex III of 10, Complex IV of 13 or 14, and Complex V of 17 subunits (Signes & Fernandez-Vizarra, 2018). Thus, measuring the protein levels of one subunit in each complex does not necessarily reflect functional changes in the complexes. However, this method was relatively straightforward, cost-efficient, and fast, providing reliable preliminary results that could be further studied. Another limitation was the use of cell lines instead of e.g. animal models. Our co-expression studies were conducted using publicly available patient cohort data. Moreover, cells are lacking the surrounding tissues and vascularisation, which affect the tumour. Thus, it is important to further study OXPHOS using other methods that include the effect of surrounding tissues.

6.5.3 [^{18}F]F-DPA uptake studies

A limitation of our PET studies was the use of immunodeficient mice because they have an abnormal immune system that does not reflect the immune system of

humans. As discussed in the original publication **III**, another limitation was the low number of mice used in some experimental setups. Although we found interesting changes in tracer uptake in the adrenal glands and kidneys after radiotherapy, we cannot draw any conclusions based on these findings due to the low sample (n = 3) number. On a final note, we could not study the role of p16 in these mouse models and cell lines. However, our findings in study **I** indicate an association between TSPO and p16 in patients with oropharyngeal cancer, which is an interesting finding that should be further studied.

6.6 Future aspects

6.6.1 Studying radioresistance in patients with HNSCC

As our results indicated that [^{18}F]F-DPA bound specifically to TSPO in HNSCC cells and xenografts, it is feasible to further evaluate the use of a TSPO-PET tracer in patients with HNSCC. It should be noted that experimental studies using mouse models, and especially cells, do not necessarily reflect a clinical setting. Thus, findings from preclinical studies cannot always be repeated in patient studies. Furthermore, in this thesis, I used HNSCC cell lines originating from the hypopharynx and tongue. It is important to evaluate tracer uptake at other HNSCC sites, particularly in laryngeal and p16-positive oropharyngeal cancers, in which we saw the clearest association between TSPO tumour expression and survival.

PET imaging can be performed before and several weeks after starting treatment. Novel information about TSPO expression in HNSCC is obtained with PET imaging at baseline and after starting radiotherapy. This information may help in estimating radiotherapy outcome and therefore would be of clinical significance.

6.6.2 Studying the biological functions of TSPO in HNSCC

Cells, animal models, or tumour tissues can be used to further study the functional role of TSPO in HNSCC. In general, using cell lines is the easiest and most-affordable way to continue studying the role of TSPO in HNSCC. Moreover, studying the oxidative metabolism of the tumour tissues could be performed using Agilent Seahorse, for example, by modifying the methods used in other studies (Underwood et al., 2020; Kluza et al., 2021; Wang et al., 2024).

We investigated the role of TSPO in OXPHOS and DNA damage response using TSPO-silenced cells transfected with siRNAs. This silencing method is often stressful for cells and can affect their function. Thus, using other methods to produce TSPO-deficient cells could reduce the risk of transfection itself affecting cell function. One option would be to develop TSPO-knockout cell lines with e.g.

CRISPR-Cas9 method. Furthermore, because knockout cell lines are stably transfected, longer-duration experiments, such as *in vivo* experiments, would be easier to perform. Another option would be to use cell lines that have naturally high or low TSPO expression. However, in HNSCC, it should be noted that the tumour sites can differ according to their properties. Therefore, cells with naturally different TSPO levels should be from the same site.

As another consideration, because TSPO is mainly located in mitochondria, studying only the mitochondrial fraction may be useful. Using this approach, the possible effects of other parts of the cell on, for example, protein quantification by Western blotting, are avoided, which could otherwise hide or mask the changes in mitochondria. However, studying only the mitochondrial fraction may also yield misleading results, as changes in the mitochondrial fraction do not necessarily reflect changes in the entire cell.

Our research group is currently performing spatial transcriptomics on selected samples from the TMA cohort. We selected samples that originated from the larynx or oropharynx and have different p16 status and TSPO tumour expression. Furthermore, we will combine these analyses with proteomics data to measure the immune markers in these sections. As a result, we will gain information about the role of TSPO in our TMA cohort and possibly identify important genes that are co-expressed with TSPO. In addition, we will determine whether there are immune markers that show differences between low- and high TSPO-expressing tumours. These results could provide more insight into the results found in study **I**.

6.6.3 Studying the molecular background in [^{18}F]F-DPA uptake

We will further evaluate the association between [^{18}F]F-DPA uptake and radioresistance using FaDu and Cal33 cell lines, which have different levels of radioresistance compared with parental cells (Kurth et al., 2015).

The possibility of increased tracer uptake due to TSPO multimerisation could be studied by blue native electrophoresis (Schägger & von Jagow, 1991) combined with Western blot. This method is based on the use of mild detergents and Coomassie blue to separate proteins without denaturing them, as in SDS-PAGE. Thus, this method can easily provide information about the multimerisation in HNSCC cells. Mass spectrometry (Richards et al., 2021) or mass photometry (Liebthal et al., 2021) can also be used to study protein–protein interactions.

7 Conclusions

In summary:

I. TSPO is a potential independent prognostic factor of HNSCC. Low TSPO tumour expression is associated with poor survival, particularly in patients with p16-positive oropharyngeal cancer. Lower TSPO expression associated with advanced disease. Genes co-expressed with TSPO indicate a possible role of TSPO in OXPHOS regulation and DNA damage response.

II. TSPO modulates DNA damage response after radiotherapy. Low TSPO tumour expression is associated with poor survival in patients treated with radiotherapy. DNA damage response is lower after irradiation in TSPO-silenced HNSCC cells. This difference was not observed after cisplatin treatment. Mitochondrial respiration is lower in TSPO-silenced HNSCC cells.

III. TSPO-PET imaging with [^{18}F]F-DPA is suitable for TSPO imaging in HNSCC. Tracer uptake increased after radiotherapy, which was not caused by inflammatory responses.

In conclusion, these findings indicate that low TSPO tumour expression leads to worse survival in HNSCC, which may be due to changes in the DNA damage response after radiotherapy. After irradiation, HNSCC cells with normal or high TSPO expression produce excessive ROS, resulting to increased DNA damage and cell death (**Figure 14**). When TSPO is absent in cancer cells, OXPHOS levels are lower. This, in turn, leads to reduced ROS levels and DNA damage after radiotherapy, resulting in increased cancer cell survival. Therefore, TSPO-PET imaging could be used as an indicator of TSPO activation; low TSPO tracer uptake would indicate patients without active TSPO in the tumour and a reduced response to radiotherapy.

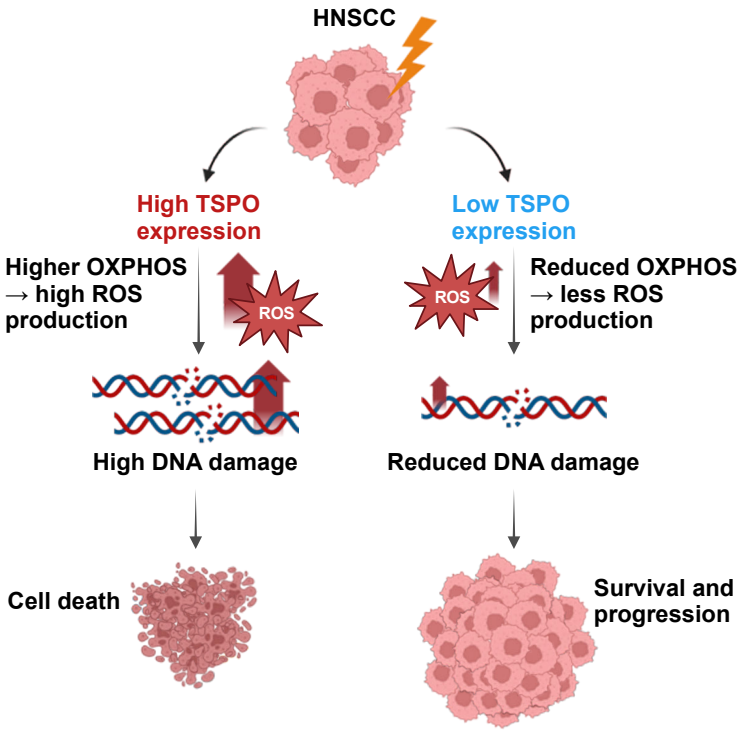

Figure 14. Hypothesised role of TSPO in HNSCC. Created with BioRender.com.

Acknowledgements

This thesis work was carried out at the Faculty of Medicine, Department of Clinical Medicine, Clinical Physiology and Nuclear Medicine, Turku PET Centre, MediCity Research Laboratory, and FICAN West, University of Turku. I would sincerely like to thank Professor Juhani Knuuti, the director of Turku PET Centre, Professor Sirpa Jalkanen and Professor Marko Salmi, the previous and current directors of MediCity Research Laboratory, Professor Panu Jaakkola, the scientific director of FICAN West, and Adjunct Professor Pia Vihinen, the director of FICAN West, for providing excellent facilities for conducting research. I would also like to thank Professor Jukka Kemppainen, the head of Department of Clinical Physiology and Nuclear Medicine, for accepting me as a doctoral candidate at the Department of Clinical Medicine, Clinical Physiology and Nuclear Medicine.

I want to express my deepest gratitude to my thesis supervisors, Adjunct Professor Tove Grönroos and Adjunct Professor Maria Sundvall, for all the support during these years. You have truly helped me to grow as a scientist. I would also like to express my gratitude to Adjunct Professor Johanna Tuomela who supervised my master's thesis and was my supervisor during the first years of my PhD studies. Johanna was one of the most empathetic and inspirational people I have met.

I would like to thank my thesis pre-examiners Associate Professor Ahmed Al-Samadi and Professor Linda Marklund for evaluating my work. I want to express my gratitude to Professor Esther Troost for accepting to be my opponent. I look forward to the discussion about my thesis.

I sincerely thank my thesis follow-up committee members, Professor Tuula Salo and Professor Olof Solin, for all the great ideas for my PhD projects. I would also like to acknowledge the Turku Doctoral Programme of Molecular Medicine for all the valuable support it provides to the doctoral students. I would also like to thank Professor Noora Kotaja, the director of TuDMM, and Eeva Valve and Verna Louhivuori, the previous and current coordinators of TuDMM. I sincerely thank Outi Irjala and Anni Halonen, the previous and current chief academic officers of the Faculty of Medicine, for all the support related to the thesis submission process.

I would like to express my gratitude to all the co-authors for their valuable contribution to the PhD projects. I especially want to thank Linda Nissi, Sami

Ventelä, Heikki Irjala, and Heikki Minn for their clinical expertise. I sincerely thank Jouko Sandholm for his expertise in image analysis. I also want to thank Francisco López-Picón for his expertise in PET studies, Eliisa Löyttyniemi for all the support on the statistical analyses, and Antti Kukkula for the assistance with the *in silico* analyses. I would like to express my gratitude to Sami Suilamo for making it possible for me to continue my experiments after the irradiator broke.

I would like to thank all the lab members of the past Tuomela lab. Thank you Afshan, Alejandra, Arafat, Nataliia, Soili, and Tami for all the help and support during my thesis work. I will always remember the great times we had at our summer days. I am especially grateful for the friendship I have formed with Alejandra. Alejandra, you truly are a great friend. I will always remember our Zoom coffee breaks during the COVID-19 lockdown. I would also like to thank all the current and previous personnel at the Turku PET Centre Preclinical Imaging Laboratory. I especially want to thank Aake Honkaniemi, Marko Vehmanen, Mira Eisala, and Päivi Kotitalo for their assistance with the PET studies. I also want to express my thanks to the radiochemists at Turku PET Centre, especially Thomas Keller. I would like to express my deepest gratitude to Petra Ketonen and Kaneli Auer for all the help with the Western blots.

I would like to thank all my friends who have supported me through my PhD studies. Thank you, Aino, Anni, Lissu, and Tiina! I would especially like to thank Anni for all the emotional support. I would also like to sincerely thank Jasmin. Jasmin, you are like a sister to me to whom I can talk about all my worries. I wish we could have shared an office during our PhD studies as we did during our master's studies. I need to listen to all the great songs we found during those times!

I would like to express my deepest gratitude to my mother Eeva and my brothers Atte and Iiro. Thank you for all the support during my PhD studies. You are the greatest family I could ever have. Äiti, kiitos että olet jaksanut kuunnella valitustani milloin mistäkin asiasta ja ollut aina tukenani. Täsä tää ny o. Finally, I would like to thank my father Jari. He was a great father who always supported and encouraged me throughout my school years and the first years of my university studies.

This work has been financially supported by the Swedish Cultural Foundation in Finland, Finnish Cultural Foundation Satakunta Regional Fund, Maud Kuistila Memorial Foundation, Cancer Foundation Finland sr., and Turku Doctoral Programme of Molecular Medicine.

Turku, April 2025
Sanni Tuominen

References

Albert, N. L., Unterrainer, M., Fleischmann, D. F., Lindner, S., Vettermann, F., Brunegraf, A., Vomacka, L., Brendel, M., Wenter, V., Wetzel, C., Rupprecht, R., Tonn, J.-C., Belka, C., Bartenstein, P., & Niyazi, M. (2017). TSPO PET for glioma imaging using the novel ligand (18)F-GE-180: First results in patients with glioblastoma. *European Journal of Nuclear Medicine and Molecular Imaging*, *44*(13), 2230–2238. https://doi.org/10.1007/s00259-017-3799-9

Albert, N. L., Nelwan, D. V., Fleischmann, D. F., Quach, S., von Rohr, K., Kaiser, L., Teske, N., Unterrainer, L. M., Bartos, L. M., Ruf, V. C., Brendel, M., Riemenschneider, M. J., Wetzel, C., Herms, J., Rupprecht, R., Thon, N., Tonn, J.-C., Belka, C., Bartenstein, P., … Holzgreve, A. (2023). Prognostic Value of TSPO PET Before Radiotherapy in Newly Diagnosed IDH-Wild-Type Glioblastoma. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *64*(10), 1519–1525. https://doi.org/10.2967/jnumed.122.265247

Alho, H., Ferrarese, C., Vicini, S., & Vaccarino, F. (1988). Subsets of GABAergic neurons in dissociated cell cultures of neonatal rat cerebral cortex show co-localization with specific modulator peptides. *Brain Research*, *467*(2), 193–204. https://doi.org/10.1016/0165-3806(88)90023-5

Alsahafi, E., Begg, K., Amelio, I., Raulf, N., Lucarelli, P., Sauter, T., & Tavassoli, M. (2019). Clinical update on head and neck cancer: Molecular biology and ongoing challenges. *Cell Death and Disease*, *10*(8). https://doi.org/10.1038/s41419-019-1769-9

Alsner, J., Sørensen, S. B., & Overgaard, J. (2001). TP53 mutation is related to poor prognosis after radiotherapy, but not surgery, in squamous cell carcinoma of the head and neck. *Radiotherapy and Oncology : Journal of the European Society for Therapeutic Radiology and Oncology*, *59*(2), 179–185. https://doi.org/10.1016/s0167-8140(01)00301-2

Amin, M. B., Greene, F. L., Edge, S. B., Compton, C. C., Gershenwald, J. E., Brookland, R. K., Meyer, L., Gress, D. M., Byrd, D. R., & Winchester, D. P. (2017). The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: A Cancer Journal for Clinicians*, *67*(2), 93–99. https://doi.org/10.3322/caac.21388

Ammer, L. M., Vollmann-Zwerenz, A., Ruf, V., Wetzel, C. H., Riemenschneider, M. J., Albert, N. L., Beckhove, P., & Hau, P. (2020). The role of translocator protein TSPO in hallmarks of glioblastoma. *Cancers*, *12*(10), 1–26. https://doi.org/10.3390/cancers12102973

Anholt, R. R., De Souza, E. B., Kuhar, M. J., & Snyder, S. H. (1985). Depletion of peripheral-type benzodiazepine receptors after hypophysectomy in rat adrenal gland and testis. *European Journal of Pharmacology*, *110*(1), 41–46. https://doi.org/10.1016/0014-2999(85)90026-3

Anholt, R. R. H. (1986). Mitochondrial benzodiazepine receptors as potential modulators of intermediary metabolism. *Trends in Pharmacological Sciences*, *7*(C), 506–511. https://doi.org/10.1016/0165-6147(86)90438-4

Antkiewicz-Michaluk, L., Guidotti, A., & Krueger, K. E. (1988). Molecular characterization and mitochondrial density of a recognition site for peripheral-type benzodiazepine ligands. *Molecular Pharmacology*, *34*(3), 272–278.

Arenz, A., Ziemann, F., Mayer, C., Wittig, A., Dreffke, K., Preising, S., Wagner, S., Klussmann, J.-P., Engenhart-Cabillic, R., & Wittekindt, C. (2014). Increased radiosensitivity of HPV-positive head and neck cancer cell lines due to cell cycle dysregulation and induction of apoptosis.

Strahlentherapie Und Onkologie : Organ Der Deutschen Rontgengesellschaft ... [et Al], *190*(9), 839–846. https://doi.org/10.1007/s00066-014-0605-5

Arfin, S., Jha, N. K., Jha, S. K., Kesari, K. K., Ruokolainen, J., Roychoudhury, S., Rathi, B., & Kumar, D. (2021). Oxidative Stress in Cancer Cell Metabolism. *Antioxidants (Basel, Switzerland)*, *10*(5). https://doi.org/10.3390/antiox10050642

Attner, P., Du, J., Näsman, A., Hammarstedt, L., Ramqvist, T., Lindholm, J., Marklund, L., Dalianis, T., & Munck-Wikland, E. (2011). Human papillomavirus and survival in patients with base of tongue cancer. *International journal of cancer*, *128*(12), 2892–2897. https://doi.org/10.1002/ijc.25625

Bader, S., Wolf, L., Milenkovic, V. M., Gruber, M., Nothdurfter, C., Rupprecht, R., & Wetzel, C. H. (2019). Differential effects of TSPO ligands on mitochondrial function in mouse microglia cells. *Psychoneuroendocrinology*, *106*, 65–76. https://doi.org/10.1016/j.psyneuen.2019.03.029

Bae, K.-R., Shim, H.-J., Balu, D., Kim, S. R., & Yu, S.-W. (2014). Translocator protein 18 kDa negatively regulates inflammation in microglia. *Journal of Neuroimmune Pharmacology: The Official Journal of the Society on NeuroImmune Pharmacology*, *9*(3), 424–437. https://doi.org/10.1007/s11481-014-9540-6

Baehring, E., & McCorkle, R. (2012). Postoperative complications in head and neck cancer. *Clinical Journal of Oncology Nursing*, *16*(6), E203-9. https://doi.org/10.1188/12.CJON.E203-E209

Bagan, J., Sarrion, G., & Jimenez, Y. (2010). Oral cancer: clinical features. *Oral oncology*, *46*(6), 414–417. https://doi.org/10.1016/j.oraloncology.2010.03.009

Baines, C. P., Kaiser, R. A., Sheiko, T., Craigen, W. J., & Molkentin, J. D. (2007). Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nature Cell Biology*, *9*(5), 550–555. https://doi.org/10.1038/ncb1575

Banati, R. B., Middleton, R. J., Chan, R., Hatty, C. R., Wai-Ying Kam, W., Quin, C., Graeber, M. B., Parmar, A., Zahra, D., Callaghan, P., Fok, S., Howell, N. R., Gregoire, M., Szabo, A., Pham, T., Davis, E., & Liu, G. J. (2014). Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. *Nature Communications*, *5*, 1–12. https://doi.org/10.1038/ncomms6452

Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P. D., McQuaid, S., Gray, R. T., Murray, L. J., Coleman, H. G., James, J. A., Salto-Tellez, M., & Hamilton, P. W. (2017). QuPath: Open source software for digital pathology image analysis. *Scientific Reports*, *7*(1), 16878. https://doi.org/10.1038/s41598-017-17204-5

Barron, A. M., Higuchi, M., Hattori, S., Kito, S., Suhara, T., & Ji, B. (2021). Regulation of Anxiety and Depression by Mitochondrial Translocator Protein-Mediated Steroidogenesis: The Role of Neurons. *Molecular Neurobiology*, *58*(2), 550–563. https://doi.org/10.1007/s12035-020-02136-5

Barsouk, A., Aluru, J. S., Rawla, P., Saginala, K., & Barsouk, A. (2023). Epidemiology, Risk Factors, and Prevention of Head and Neck Squamous Cell Carcinoma. *Medical Sciences (Basel, Switzerland)*, *11*(2). https://doi.org/10.3390/medsci11020042

Basu, S., Hess, S., Nielsen Braad, P.-E., Olsen, B. B., Inglev, S., & Høilund-Carlsen, P. F. (2014). The Basic Principles of FDG-PET/CT Imaging. *PET Clinics*, *9*(4), 355–370, v. https://doi.org/10.1016/j.cpet.2014.07.006

Batra, S., & Alenfall, J. (1994). Characterization of peripheral benzodiazepine receptors in rat prostatic adenocarcinoma. *The Prostate*, *24*(5), 269–278. https://doi.org/10.1002/pros.2990240509

Beinlich, A., Strohmeier, R., Kaufmann, M., & Kuhl, H. (2000). Relation of cell proliferation to expression of peripheral benzodiazepine receptors in human breast cancer cell lines. *Biochemical Pharmacology*, *60*(3), 397–402. https://doi.org/10.1016/s0006-2952(00)00325-7

Benavides, J., Dubois, A., Gotti, B., Bourdiol, F., & Scatton, B. (1990). Cellular distribution of omega 3 (peripheral type benzodiazepine) binding sites in the normal and ischaemic rat brain: An autoradiographic study with the photoaffinity ligand [^{3}H]PK 14105. *Neuroscience Letters*, *114*(1), 32–38. https://doi.org/10.1016/0304-3940(90)90424-8

Berkovich, A., McPhie, P., Campagnone, M., Guidotti, A., & Hensley, P. (1990). A natural processing product of rat diazepam binding inhibitor, triakontatetraneuropeptide (diazepam binding inhibitor 17-50) contains an alpha-helix, which allows discrimination between benzodiazepine binding site subtypes. *Molecular Pharmacology*, *37*(2), 164–172.

Bernassau, J. M., Reversat, J. L., Ferrara, P., Caput, D., & Lefur, G. (1993). A 3D model of the peripheral benzodiazepine receptor and its implication in intra mitochondrial cholesterol transport. *Journal of Molecular Graphics*, *11*(4), 235,236-244. https://doi.org/10.1016/0263-7855(93)80003-a

Berroterán-Infante, N., Balber, T., Fürlinger, P., Bergmann, M., Lanzenberger, R., Hacker, M., Mitterhauser, M., & Wadsak, W. (2018). [(18)F]FEPPA: Improved Automated Radiosynthesis, Binding Affinity, and Preliminary in Vitro Evaluation in Colorectal Cancer. *ACS Medicinal Chemistry Letters*, *9*(3), 177–181. https://doi.org/10.1021/acsmedchemlett.7b00367

Besman, M. J., Yanagibashi, K., Lee, T. D., Kawamura, M., Hall, P. F., & Shively, J. E. (1989). Identification of des-(Gly-Ile)-endozepine as an effector of corticotropin-dependent adrenal steroidogenesis: Stimulation of cholesterol delivery is mediated by the peripheral benzodiazepine receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(13), 4897–4901. https://doi.org/10.1073/pnas.86.13.4897

Betlazar, C., Middleton, R. J., Banati, R., & Liu, G. J. (2020). The Translocator Protein (TSPO) in Mitochondrial Bioenergetics and Immune Processes. *Cells*, *9*(2), 1–18. https://doi.org/10.3390/cells9020512

Bhoola, N. H., Mbita, Z., Hull, R., & Dlamini, Z. (2018). Translocator protein (TSPO) as a potential biomarker in human cancers. *International Journal of Molecular Sciences*, *19*(8). https://doi.org/10.3390/ijms19082176

Blahos, J. 2nd, Whalin, M. E., & Krueger, K. E. (1995). Identification and purification of a 10-kilodalton protein associated with mitochondrial benzodiazepine receptors. *The Journal of Biological Chemistry*, *270*(35), 20285–20291. https://doi.org/10.1074/jbc.270.35.20285

Blot, W. J., McLaughlin, J. K., Winn, D. M., Austin, D. F., Greenberg, R. S., Preston-Martin, S., Bernstein, L., Schoenberg, J. B., Stemhagen, A., & Fraumeni, J. F. J. (1988). Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Research*, *48*(11), 3282–3287.

Bode, J., Veenman, L., Caballero, B., Lakomek, M., Kugler, W., & Gavish, M. (2012). The 18 kDa translocator protein influences angiogenesis, as well as aggressiveness, adhesion, migration, and proliferation of glioblastoma cells. *Pharmacogenetics and Genomics*, *22*(7), 538–550. https://doi.org/10.1097/FPC.0b013e3283539cdc

Bogan, R. L., Davis, T. L., & Niswender, G. D. (2007). Peripheral-type benzodiazepine receptor (PBR) aggregation and absence of steroidogenic acute regulatory protein (StAR)/PBR association in the mitochondrial membrane as determined by bioluminescence resonance energy transfer (BRET). *The Journal of Steroid Biochemistry and Molecular Biology*, *104*(1–2), 61–67. https://doi.org/10.1016/j.jsbmb.2006.10.007

Bono, F., Lamarche, I., Prabonnaud, V., Le Fur, G., & Herbert, J. M. (1999). Peripheral benzodiazepine receptor agonists exhibit potent antiapoptotic activities. *Biochemical and Biophysical Research Communications*, *265*(2), 457–461. https://doi.org/10.1006/bbrc.1999.1683

Bonsack, F., & Sukumari-Ramesh, S. (2018). TSPO: An evolutionarily conserved protein with elusive functions. *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061694

Boujrad, N., Hudson, J. R. J., & Papadopoulos, V. (1993). Inhibition of hormone-stimulated steroidogenesis in cultured Leydig tumor cells by a cholesterol-linked phosphorothioate oligodeoxynucleotide antisense to diazepam-binding inhibitor. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(12), 5728–5731. https://doi.org/10.1073/pnas.90.12.5728

Boujrad, N., Vidic, B., & Papadopoulos, V. (1996). Acute action of choriogonadotropin on Leydig tumor cells: Changes in the topography of the mitochondrial peripheral-type benzodiazepine receptor. *Endocrinology*, *137*(12), 5727–5730. https://doi.org/10.1210/endo.137.12.8940407

Bovolin, P., Schlichting, J., Miyata, M., Ferrarese, C., Guidotti, A., & Alho, H. (1990). Distribution and characterization of diazepam binding inhibitor (DBI) in peripheral tissues of rat. *Regulatory Peptides*, *29*(2–3), 267–281. https://doi.org/10.1016/0167-0115(90)90089-f

Bradley, P. J. (2019). Epidemiology of Hypopharyngeal Cancer. *Advances in Oto-Rhino-Laryngology*, *83*, 1–14. https://doi.org/10.1159/000492299

Braestrup, C., & Squires, R. F. (1977). Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H]diazepam binding. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(9), 3805–3809. https://doi.org/10.1073/pnas.74.9.3805

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I., & Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, 74(3), 229–263. https://doi.org/10.3322/caac.21834

Bribes, E., Carrière, D., Goubet, C., Galiègue, S., Casellas, P., & Simony-Lafontaine, J. (2004). Immunohistochemical assessment of the peripheral benzodiazepine receptor in human tissues. *The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society*, *52*(1), 19–28. https://doi.org/10.1177/002215540405200103

Brook, I. (2020). Late side effects of radiation treatment for head and neck cancer. *Radiation Oncology Journal*, *38*(2), 84–92. https://doi.org/10.3857/roj.2020.00213

Bruce, J. P., Yip, K., Bratman, S. V., Ito, E., & Liu, F.-F. (2015). Nasopharyngeal Cancer: Molecular Landscape. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology*, *33*(29), 3346–3355. https://doi.org/10.1200/JCO.2015.60.7846

Budach, V., & Tinhofer, I. (2019). Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: A systematic review. *The Lancet. Oncology*, *20*(6), e313–e326. https://doi.org/10.1016/S1470-2045(19)30177-9

Butlen, D. (1984). Benzodiazepine receptors along the nephron: [^{3}H]PK 11195 binding in rat tubules. *FEBS Letters*, *169*(2), 138–142. https://doi.org/10.1016/0014-5793(84)80305-1

Cacicedo, J., Navarro, A., Del Hoyo, O., Gomez-Iturriaga, A., Alongi, F., Medina, J. A., Elicin, O., Skanjeti, A., Giammarile, F., Bilbao, P., Casquero, F., de Bari, B., & Dal Pra, A. (2016). Role of fluorine-18 fluorodeoxyglucose PET/CT in head and neck oncology: The point of view of the radiation oncologist. *The British Journal of Radiology*, *89*(1067), 20160217. https://doi.org/10.1259/bjr.20160217

Canat, X., Carayon, P., Bouaboula, M., Cahard, D., Shire, D., Roque, C., Le Fur, G., & Casellas, P. (1993). Distribution profile and properties of peripheral-type benzodiazepine receptors on human hemopoietic cells. *Life Sciences*, *52*(1), 107–118. https://doi.org/10.1016/0024-3205(93)90293-c

Carayon, P., Portier, M., Dussossoy, D., Bord, A., Petitprêtre, G., Canat, X., Le Fur, G., & Casellas, P. (1996). Involvement of peripheral benzodiazepine receptors in the protection of hematopoietic cells against oxygen radical damage. *Blood*, *87*(8), 3170–3178.

Carmel, I., Fares, F. A., Leschiner, S., Scherübl, H., Weisinger, G., & Gavish, M. (1999). Peripheral-type benzodiazepine receptors in the regulation of proliferation of MCF-7 human breast carcinoma cell line. *Biochemical Pharmacology*, *58*(2), 273–278. https://doi.org/10.1016/s0006-2952(99)00093-3

Cavallaro, S., Pani, L., Guidotti, A., & Costa, E. (1993). ACTH-induced mitochondrial DBI receptor (MDR) and diazepam binding inhibitor (DBI) expression in adrenals of hypophysectomized rats is not cause-effect related to its immediate steroidogenic action. *Life Sciences*, *53*(14), 1137–1147. https://doi.org/10.1016/0024-3205(93)90550-m

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., & Sumer, O. S. (2012). The CBio Cancer Genomics. *Cancer Discov.*, *2*(5), 1–7. https://doi.org/10.1158/2159-8290.CD-12-0095.

Chamoli, A., Gosavi, A. S., Shirwadkar, U. P., Wangdale, K. V., Behera, S. K., Kurrey, N. K., Kalia, K., & Mandoli, A. (2021). Overview of oral cavity squamous cell carcinoma: Risk factors, mechanisms, and diagnostics. *Oral oncology*, 121, 105451. https://doi.org/10.1016/j.oraloncology.2021.105451

Chang, Y. J., McCabe, R. T., Rennert, H., Budarf, M. L., Sayegh, R., Emanuel, B. S., Skolnick, P., & Strauss, J. F. 3rd. (1992). The human "peripheral-type" benzodiazepine receptor: Regional mapping of the gene and characterization of the receptor expressed from cDNA. *DNA and Cell Biology*, *11*(6), 471–480. https://doi.org/10.1089/dna.1992.11.471

Charbonneau, P., Syrota, A., Crouzel, C., Valois, J. M., Prenant, C., & Crouzel, M. (1986). Peripheral-type benzodiazepine receptors in the living heart characterized by positron emission tomography. *Circulation*, *73*(3), 476–483. https://doi.org/10.1161/01.cir.73.3.476

Charoentong, P., Finotello, F., Angelova, M., Mayer, C., Efremova, M., Rieder, D., Hackl, H., & Trajanoski, Z. (2017). Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Reports*, *18*(1), 248–262. https://doi.org/10.1016/j.celrep.2016.12.019

Chelli, B., Falleni, A., Salvetti, F., Gremigni, V., Lucacchini, A., & Martini, C. (2001). Peripheral-type benzodiazepine receptor ligands: Mitochondrial permeability transition induction in rat cardiac tissue. *Biochemical Pharmacology*, *61*(6), 695–705. https://doi.org/10.1016/s0006-2952(00)00588-8

Chen, A. Y., & Myers, J. N. (2001). Cancer of the oral cavity. *Disease-a-Month: DM*, *47*(7), 275–361. https://doi.org/10.1067/mcd.2001.109374

Chen, S. M. Y., Krinsky, A. L., Woolaver, R. A., Wang, X., Chen, Z., & Wang, J. H. (2020). Tumor immune microenvironment in head and neck cancers. *Molecular Carcinogenesis*, *59*(7), 766–774. https://doi.org/10.1002/mc.23162

Chen, Y. F., Xie, J. D., Jiang, Y. C., Chen, D. T., Pan, J. H., Chen, Y. H., Yuan, Y. F., Wen, Z. S., & Zeng, W. A. (2017). The prognostic value of peripheral benzodiazepine receptor in patients with esophageal squamous cell carcinoma. *Journal of Cancer*, *8*(16). https://doi.org/10.7150/jca.20739

Chen, Y.-P., Chan, A. T. C., Le, Q.-T., Blanchard, P., Sun, Y., & Ma, J. (2019). Nasopharyngeal carcinoma. *Lancet (London, England)*, *394*(10192), 64–80. https://doi.org/10.1016/S0140-6736(19)30956-0

Chen, Z., Han, F., Du, Y., Shi, H., & Zhou, W. (2023). Hypoxic microenvironment in cancer: Molecular mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy*, *8*(1), 70. https://doi.org/10.1038/s41392-023-01332-8

Cheraghlou, S., Yu, P. K., Otremba, M. D., Park, H. S., Bhatia, A., Zogg, C. K., Mehra, S., Yarbrough, W. G., & Judson, B. L. (2018). Treatment deintensification in human papillomavirus-positive oropharynx cancer: Outcomes from the National Cancer Data Base. *Cancer*, *124*(4), 717–726. https://doi.org/10.1002/cncr.31104

Cherry, S. R., & Dahlbom, M. (2006). PET: Physics, Instrumentation, and Scanners. In M. E. Phelps (Ed.), *PET: Physics, Instrumentation, and Scanners* (pp. 1–117). Springer New York. https://doi.org/10.1007/0-387-34946-4_1

Choi, H. B., Khoo, C., Ryu, J. K., van Breemen, E., Kim, S. U., & McLarnon, J. G. (2002). Inhibition of lipopolysaccharide-induced cyclooxygenase-2, tumor necrosis factor-alpha and $[Ca^{2+}]i$ responses in human microglia by the peripheral benzodiazepine receptor ligand PK11195. *Journal of Neurochemistry*, *83*(3), 546–555. https://doi.org/10.1046/j.1471-4159.2002.01122.x

Choi, J., Ifuku, M., Noda, M., & Guilarte, T. R. (2011). Translocator protein (18 kDa)/peripheral benzodiazepine receptor specific ligands induce microglia functions consistent with an activated state. *Glia*, *59*(2), 219–230. https://doi.org/10.1002/glia.21091

Chow, L. Q. M. (2020). Head and Neck Cancer. *New England Journal of Medicine*, *382*(1), 60–72. https://doi.org/10.1056/NEJMra1715715

Chung, J.-Y., Chen, H., Papadopoulos, V., & Zirkin, B. (2020). Cholesterol accumulation, lipid droplet formation, and steroid production in Leydig cells: Role of translocator protein (18-kDa). *Andrology*, *8*(3), 719–730. https://doi.org/10.1111/andr.12733

Cleary, J., Johnson, K. M., Opipari, A. W. J., & Glick, G. D. (2007). Inhibition of the mitochondrial F_1F_0-ATPase by ligands of the peripheral benzodiazepine receptor. *Bioorganic & Medicinal Chemistry Letters*, *17*(6), 1667–1670. https://doi.org/10.1016/j.bmcl.2006.12.102

Cohen, A. S., Li, J., Hight, M. R., McKinley, E., Fu, A., Payne, A., Liu, Y., Zhang, D., Xie, Q., Bai, M., Ayers, G. D., Tantawy, M. N., Smith, J. A., Revetta, F., Washington, M. K., Shi, C., Merchant, N., & Manning, H. C. (2020). TSPO-targeted PET and Optical Probes for the Detection and Localization of Premalignant and Malignant Pancreatic Lesions. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *26*(22), 5914–5925. https://doi.org/10.1158/1078-0432.CCR-20-1214

Comprehensive genomic characterization of head and neck squamous cell carcinomas. (2015). *Nature*, *517*(7536), 576–582. https://doi.org/10.1038/nature14129

Couturier, O., Luxen, A., Chatal, J.-F., Vuillez, J.-P., Rigo, P., & Hustinx, R. (2004). Fluorinated tracers for imaging cancer with positron emission tomography. *European Journal of Nuclear Medicine and Molecular Imaging*, *31*(8), 1182–1206. https://doi.org/10.1007/s00259-004-1607-9

Craig, S. G., Anderson, L. A., Schache, A. G., Moran, M., Graham, L., Currie, K., Rooney, K., Robinson, M., Upile, N. S., Brooker, R., Mesri, M., Bingham, V., McQuaid, S., Jones, T., McCance, D. J., Salto-Tellez, M., McDade, S. S., & James, J. A. (2019). Recommendations for

determining HPV status in patients with oropharyngeal cancers under TNM8 guidelines: A two-tier approach. *British Journal of Cancer*, *120*(8), 827–833. https://doi.org/10.1038/s41416-019-0414-9

Cramer, J. D., Burtness, B., Le, Q. T., & Ferris, R. L. (2019). The changing therapeutic landscape of head and neck cancer. *Nature Reviews. Clinical Oncology*, *16*(11), 669–683. https://doi.org/10.1038/s41571-019-0227-z

Cruz-Gregorio, A., Martínez-Ramírez, I., Pedraza-Chaverri, J., & Lizano, M. (2019). Reprogramming of energy metabolism in response to radiotherapy in head and neck squamous cell carcinoma. *Cancers*, *11*(2), 1–17. https://doi.org/10.3390/cancers11020182

Daguenet, E., Louati, S., Wozny, A.-S., Vial, N., Gras, M., Guy, J.-B., Vallard, A., Rodriguez-Lafrasse, C., & Magné, N. (2020). Radiation-induced bystander and abscopal effects: Important lessons from preclinical models. *British Journal of Cancer*, *123*(3), 339–348. https://doi.org/10.1038/s41416-020-0942-3

Dahiya, K., & Dhankhar, R. (2016). Updated overview of current biomarkers in head and neck carcinoma. *World Journal of Methodology*, *6*(1), 77–86. https://doi.org/10.5662/wjm.v6.i1.77

de Souza, A. M., Real, C. C., Junqueira, M. de S., Estessi de Souza, L., Navarro Marques, F. L., Buchpiguel, C. A., Chammas, R., Sapienza, M. T., & de Paula Faria, D. (2022). Potential of [(11)C](R)-PK11195 PET Imaging for Evaluating Tumor Inflammation: A Murine Mammary Tumor Model. *Pharmaceutics*, *14*(12). https://doi.org/10.3390/pharmaceutics14122715

De Souza, E. B., Anholt, R. R., Murphy, K. M., Snyder, S. H., & Kuhar, M. J. (1985). Peripheral-type benzodiazepine receptors in endocrine organs: Autoradiographic localization in rat pituitary, adrenal, and testis. *Endocrinology*, *116*(2), 567–573. https://doi.org/10.1210/endo-116-2-567

Delavoie, F., Li, H., Hardwick, M., Robert, J.-C., Giatzakis, C., Péranzi, G., Yao, Z.-X., Maccario, J., Lacapère, J.-J., & Papadopoulos, V. (2003). In vivo and in vitro peripheral-type benzodiazepine receptor polymerization: Functional significance in drug ligand and cholesterol binding. *Biochemistry*, *42*(15), 4506–4519. https://doi.org/10.1021/bi0267487

Denissoff, A., Huusko, T., Ventelä, S., Niemelä, S., & Routila, J. (2022). Exposure to alcohol and overall survival in head and neck cancer: A regional cohort study. *Head and Neck*, *44*(10), 2109–2117. https://doi.org/10.1002/hed.27125

Dickens, A. M., Vainio, S., Marjamäki, P., Johansson, J., Lehtiniemi, P., Rokka, J., Rinne, J., Solin, O., Haaparanta-Solin, M., Jones, P. A., Trigg, W., Anthony, D. C., & Airas, L. (2014). Detection of microglial activation in an acute model of neuroinflammation using PET and radiotracers ^{11}C-(R)-PK11195 and ^{18}F-GE-180. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *55*(3), 466–472. https://doi.org/10.2967/jnumed.113.125625

DiMaio, D., & Mattoon, D. (2001). Mechanisms of cell transformation by papillomavirus E5 proteins. *Oncogene*, *20*(54), 7866–7873. https://doi.org/10.1038/sj.onc.1204915

Dimitrova-Shumkovska, J., Krstanoski, L., & Veenman, L. (2020). Diagnostic and Therapeutic Potential of TSPO Studies Regarding Neurodegenerative Diseases, Psychiatric Disorders, Alcohol Use Disorders, Traumatic Brain Injury, and Stroke: An Update. *Cells*, *9*(4). https://doi.org/10.3390/cells9040870

Dittmann, K., Mayer, C., Fehrenbacher, B., Schaller, M., Raju, U., Milas, L., Chen, D. J., Kehlbach, R., & Rodemann, H. P. (2005). Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *The Journal of Biological Chemistry*, *280*(35), 31182–31189. https://doi.org/10.1074/jbc.M506591200

Doble, A., Malgouris, C., Daniel, M., Daniel, N., Imbault, F., Basbaum, A., Uzan, A., Guérémy, C., & Le Fur, G. (1987). Labelling of peripheral-type benzodiazepine binding sites in human brain with [^{3}H]PK 11195: Anatomical and subcellular distribution. *Brain Research Bulletin*, *18*(1), 49–61. https://doi.org/10.1016/0361-9230(87)90033-5

Dok, R., Kalev, P., Van Limbergen, E. J., Asbagh, L. A., Vázquez, I., Hauben, E., Sablina, A., & Nuyts, S. (2014). p16INK4a impairs homologous recombination-mediated DNA repair in human papillomavirus-positive head and neck tumors. *Cancer Research*, *74*(6), 1739–1751. https://doi.org/10.1158/0008-5472.CAN-13-2479

Dolder, M., Wendt, S., & Wallimann, T. (2001). Mitochondrial creatine kinase in contact sites: Interaction with porin and adenine nucleotide translocase, role in permeability transition and

sensitivity to oxidative damage. *Biological Signals and Receptors*, *10*(1–2), 93–111. https://doi.org/10.1159/000046878

Eberly, H. W., Sciscent, B. Y., Lorenz, F. J., Rettig, E. M., & Goyal, N. (2024). Current and Emerging Diagnostic, Prognostic, and Predictive Biomarkers in Head and Neck Cancer. *Biomedicines*, *12*(2). https://doi.org/10.3390/biomedicines12020415

Economopoulou, P., de Bree, R., Kotsantis, I., & Psyrri, A. (2019). Diagnostic Tumor Markers in Head and Neck Squamous Cell Carcinoma (HNSCC) in the Clinical Setting. *Frontiers in Oncology*, *9*, 827. https://doi.org/10.3389/fonc.2019.00827

Eke, I., Zong, D., Aryankalayil, M. J., Sandfort, V., Bylicky, M. A., Rath, B. H., Graves, E. E., Nussenzweig, A., & Coleman, C. N. (2020). 53BP1/RIF1 signaling promotes cell survival after multifractionated radiotherapy. *Nucleic Acids Research*, *48*(3), 1314–1326. https://doi.org/10.1093/nar/gkz1139

Fafalios, A., Akhavan, A., Parwani, A. V., Bies, R. R., McHugh, K. J., & Pflug, B. R. (2009). Translocator protein blockade reduces prostate tumor growth. *Clinical Cancer Research*, *15*(19), 6177–6184. https://doi.org/10.1158/1078-0432.CCR-09-0844

Fairley, L. H., Lai, K. O., Wong, J. H., Chong, W. J., Vincent, A. S., D'Agostino, G., Wu, X., Naik, R. R., Jayaraman, A., Langley, S. R., Ruedl, C., & Barron, A. M. (2023). Mitochondrial control of microglial phagocytosis by the translocator protein and hexokinase 2 in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(8), e2209177120. https://doi.org/10.1073/pnas.2209177120

Fakhry, C., Westra, W. H., Li, S., Cmelak, A., Ridge, J. A., Pinto, H., Forastiere, A., & Gillison, M. L. (2008). Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *Journal of the National Cancer Institute*, *100*(4), 261–269. https://doi.org/10.1093/jnci/djn011

Fan, J., Campioli, E., Midzak, A., Culty, M., & Papadopoulos, V. (2015). Conditional steroidogenic cell-targeted deletion of TSPO unveils a crucial role in viability and hormone-dependent steroid formation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(23), 7261–7266. https://doi.org/10.1073/pnas.1502670112

Fan, J., & Papadopoulos, V. (2020). Mitochondrial TSPO Deficiency Triggers Retrograde Signaling in MA-10 Mouse Tumor Leydig Cells. *International Journal of Molecular Sciences*, *22*(1). https://doi.org/10.3390/ijms22010252

Fan, Z., Calsolaro, V., Atkinson, R. A., Femminella, G. D., Waldman, A., Buckley, C., Trigg, W., Brooks, D. J., Hinz, R., & Edison, P. (2016). Flutriciclamide (^{18}F-GE180) PET: First-in-Human PET Study of Novel Third-Generation In Vivo Marker of Human Translocator Protein. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *57*(11), 1753–1759. https://doi.org/10.2967/jnumed.115.169078

Fan, Z., Li, M., Chen, X., Wang, J., Liang, X., Wang, H., Wang, Z., Cheng, B., & Xia, J. (2017). Prognostic Value of Cancer Stem Cell Markers in Head and Neck Squamous Cell Carcinoma: A Meta-analysis. *Scientific Reports*, *7*, 43008. https://doi.org/10.1038/srep43008

Farhan, F., Almarhoun, M., Wong, A., Findlay, A. S., Bartholomew, C., Williams, M. T. S., Hurd, T. W., & Shu, X. (2021). Deletion of TSPO Causes Dysregulation of Cholesterol Metabolism in Mouse Retina. *Cells*, *10*(11). https://doi.org/10.3390/cells10113066

Feeney, C., Scott, G., Raffel, J., Roberts, S., Coello, C., Jolly, A., Searle, G., Goldstone, A. P., Brooks, D. J., Nicholas, R. S., Trigg, W., Gunn, R. N., & Sharp, D. J. (2016). Kinetic analysis of the translocator protein positron emission tomography ligand [(18)F]GE-180 in the human brain. *European Journal of Nuclear Medicine and Molecular Imaging*, *43*(12), 2201–2210. https://doi.org/10.1007/s00259-016-3444-z

Ferlay J, Laversanne M, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F (2024). Global Cancer Observatory: Cancer Tomorrow (version 1.1). Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.who.int/tomorrow, accessed [28 June 2024].

Ferrero, P., Santi, M. R., Conti-Tronconi, B., Costa, E., & Guidotti, A. (1986). Study of an octadecaneuropeptide derived from diazepam binding inhibitor (DBI): Biological activity and

presence in rat brain. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(3), 827–831. https://doi.org/10.1073/pnas.83.3.827

Ferris, R. L. (2015). Immunology and Immunotherapy of Head and Neck Cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *33*(29), 3293–3304. https://doi.org/10.1200/JCO.2015.61.1509

Filippi, L., Frantellizzi, V., Vincentis, G. D., Schillaci, O., & Evangelista, L. (2023). Clinical Applications of TSPO PET for Glioma Imaging: Current Evidence and Future Perspective-A Systematic Review. *Diagnostics (Basel, Switzerland)*, *13*(10). https://doi.org/10.3390/diagnostics13101813

Fischer, R., Schmitt, M., Bode, J. G., & Häussinger, D. (2001). Expression of the peripheral-type benzodiazepine receptor and apoptosis induction in hepatic stellate cells. *Gastroenterology*, *120*(5), 1212–1226. https://doi.org/10.1053/gast.2001.23260

Fleming, J. C., Woo, J., Moutasim, K., Mellone, M., Frampton, S. J., Mead, A., Ahmed, W., Wood, O., Robinson, H., Ward, M., Woelk, C. H., Ottensmeier, C. H., King, E., Kim, D., Blaydes, J. P., & Thomas, G. J. (2019). HPV, tumour metabolism and novel target identification in head and neck squamous cell carcinoma. *British Journal of Cancer*, *120*(3), 356–367. https://doi.org/10.1038/s41416-018-0364-7

Frederick, M., Skinner, H. D., Kazi, S. A., Sikora, A. G., & Sandulache, V. C. (2020). High expression of oxidative phosphorylation genes predicts improved survival in squamous cell carcinomas of the head and neck and lung. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-63448-z

Fu, Y., Wang, D., Wang, H., Cai, M., Li, C., Zhang, X., Chen, H., Hu, Y., Zhang, X., Ying, M., He, W., & Zhang, J. (2020). TSPO deficiency induces mitochondrial dysfunction, leading to hypoxia, angiogenesis, and a growth-promoting metabolic shift toward glycolysis in glioblastoma. *Neuro-Oncology*, *22*(2), 240–252. https://doi.org/10.1093/neuonc/noz183

Galiègue, S., Jbilo, O., Combes, T., Bribes, E., Carayon, P., Le Fur, G., & Casellas, P. (1999). Cloning and characterization of PRAX-1. A new protein that specifically interacts with the peripheral benzodiazepine receptor. *The Journal of Biological Chemistry*, *274*(5), 2938–2952. https://doi.org/10.1074/jbc.274.5.2938

Galiègue, S., Casellas, P., Kramar, A., Tinel, N., & Simony-Lafontaine, J. (2004). Immunohistochemical assessment of the peripheral benzodiazepine receptor in breast cancer and its relationship with survival. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *10*(6), 2058–2064. https://doi.org/10.1158/1078-0432.ccr-03-0988

Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C., & Schultz, N. (2013). Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal Complementary Data Sources and Analysis Options. *Science Signaling*, *6*(269), 1–20. https://doi.org/10.1126/scisignal.2004088.Integrative

Garneau, J. C., Bakst, R. L., & Miles, B. A. (2018). Hypopharyngeal cancer: A state of the art review. *Oral Oncology*, *86*, 244–250. https://doi.org/10.1016/j.oraloncology.2018.09.025

Garza, S., Chen, L., Galano, M., Cheung, G., Sottas, C., Li, L., Li, Y., Zirkin, B. R., & Papadopoulos, V. (2022). Mitochondrial dynamics, Leydig cell function, and age-related testosterone deficiency. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *36*(12), e22637. https://doi.org/10.1096/fj.202201026R

Gatliff, J., East, D., Crosby, J., Abeti, R., Harvey, R., Craigen, W., Parker, P., & Campanella, M. (2014). TSPO interacts with VDAC1 and triggers a ROS-mediated inhibition of mitochondrial quality control. *Autophagy*, *10*(12), 2279–2296. https://doi.org/10.4161/15548627.2014.991665

Gauss, C., Stone, L. D., Ghafouri, M., Quan, D., Johnson, J., Fribley, A. M., & Amm, H. M. (2024). Overcoming Resistance to Standard-of-Care Therapies for Head and Neck Squamous Cell Carcinomas. *Cells*, *13*(12). https://doi.org/10.3390/cells13121018

Gavish, A., Krayzler, E., & Nagler, R. (2017). Two populations of TSPO binding sites in oral cancer SCC-15 cells. *Experimental Cell Research*, *350*(1), 279–283. https://doi.org/10.1016/j.yexcr.2016.12.005

Gavish, M., Bachman, I., Shoukrun, R., Katz, Y., Veenman, L., Weisinger, G., & Weizman, A. (1999). Enigma of the peripheral benzodiazepine receptor. *Pharmacological Reviews*, *51*(4), 629–650.

Gehlert, D. R., Yamamura, H. I., & Wamsley, J. K. (1985). Autoradiographic localization of "peripheral-type" benzodiazepine binding sites in the rat brain, heart and kidney. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *328*(4), 454–460. https://doi.org/10.1007/BF00692915

Giesel, F. L., Kratochwil, C., Lindner, T., Marschalek, M. M., Loktev, A., Lehnert, W., Debus, J., Jäger, D., Flechsig, P., Altmann, A., Mier, W., & Haberkorn, U. (2019). ^{68}Ga-FAPI PET/CT: Biodistribution and Preliminary Dosimetry Estimate of 2 DOTA-Containing FAP-Targeting Agents in Patients with Various Cancers. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*, 60(3), 386–392. https://doi.org/10.2967/jnumed.118.215913

Gillison, M. L., Koch, W. M., & Shah, K. V. (1999). Human papillomavirus in head and neck squamous cell carcinoma: Are some head and neck cancers a sexually transmitted disease? *Current Opinion in Oncology*, *11*(3), 191–199. https://doi.org/10.1097/00001622-199905000-00010

Gillison, M. L., Chaturvedi, A. K., Anderson, W. F., & Fakhry, C. (2015). Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *33*(29), 3235–3242. https://doi.org/10.1200/JCO.2015.61.6995

Giorgio, V., von Stockum, S., Antoniel, M., Fabbro, A., Fogolari, F., Forte, M., Glick, G. D., Petronilli, V., Zoratti, M., Szabó, I., Lippe, G., & Bernardi, P. (2013). Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(15), 5887–5892. https://doi.org/10.1073/pnas.1217823110

Goldman, M. J., Craft, B., Hastie, M., Repecka, K., McDade, F., Kamath, A., Banerjee, A., Luo, Y. H., Rogers, D., Brooks, A. N., Zhu, J. C., & Haussler, D. (2020). Visualizing and interpreting cancer genomics data via the Xena platform. *Nature Biotechnology*, *38*(6), 675–678. https://doi.org/10.1038/s41587-020-0546-8

Gormley, M., Gray, E., Richards, C., Gormley, A., Richmond, R. C., Vincent, E. E., Dudding, T., Ness, A. R., & Thomas, S. J. (2022). An update on oral cavity cancer: epidemiological trends, prevention strategies and novel approaches in diagnosis and prognosis. *Community dental health*, *39*(3), 197–205. https://doi.org/10.1922/CDH_00032Gormley09

Graeber, E., & Korkhov, V. M. (2019). Characterisation of the ligand binding sites in the translocator protein TSPO using the chimeric bacterial-mammalian constructs. *Protein Expression and Purification*, *164*, 105456. https://doi.org/10.1016/j.pep.2019.105456

Granov, A., Tiutin, L., & Schwarz, T. (2013). The Physical Basis of Positron Emission Tomography. In A. Granov, L. Tiutin, & T. Schwarz (Eds.), *Positron Emission Tomography* (pp. 3–24). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-21120-1_1

Gray, L. H., Conger, A. D., Ebert, M., Horsney, S., & Scott, O. C. (1953). The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *The British Journal of Radiology*, *26*(312), 638–648. https://doi.org/10.1259/0007-1285-26-312-638

Greene, J., Segaran, A., & Lord, S. (2022). Targeting OXPHOS and the electron transport chain in cancer; Molecular and therapeutic implications. *Seminars in Cancer Biology*, *86*(Pt 2), 851–859. https://doi.org/10.1016/j.semcancer.2022.02.002

Grimm, A., Lejri, I., Hallé, F., Schmitt, M., Götz, J., Bihel, F., & Eckert, A. (2020). Mitochondria modulatory effects of new TSPO ligands in a cellular model of tauopathies. *Journal of Neuroendocrinology*, *32*(1), e12796. https://doi.org/10.1111/jne.12796

Grønhøj Larsen, C., Gyldenløve, M., Jensen, D. H., Therkildsen, M. H., Kiss, K., Norrild, B., Konge, L., & von Buchwald, C. (2014). Correlation between human papillomavirus and p16 overexpression in oropharyngeal tumours: A systematic review. *British Journal of Cancer*, *110*(6), 1587–1594. https://doi.org/10.1038/bjc.2014.42

Guilarte, T. R., Kuhlmann, A. C., O'Callaghan, J. P., & Miceli, R. C. (1995). Enhanced expression of peripheral benzodiazepine receptors in trimethyltin-exposed rat brain: A biomarker of neurotoxicity. *Neurotoxicology*, *16*(3), 441–450.

Guo, Y., Kalathur, R. C., Liu, Q., Kloss, B., Bruni, R., Ginter, C., Kloppmann, E., Rost, B., & Hendrickson, W. A. (2015). Protein structure. Structure and activity of tryptophan-rich TSPO proteins. *Science (New York, N.Y.)*, *347*(6221), 551–555. https://doi.org/10.1126/science.aaa1534

Gut, P. (2015). Targeting mitochondrial energy metabolism with TSPO ligands. *Biochemical Society Transactions*, *43*, 537–542. https://doi.org/10.1042/BST20150019

Hall, S. F., Groome, P. A., Irish, J., & O'Sullivan, B. (2008). The natural history of patients with squamous cell carcinoma of the hypopharynx. *The Laryngoscope*, *118*(8), 1362–1371. https://doi.org/10.1097/MLG.0b013e318173dc4a

Hamacher, K., Coenen, H. H., & Stöcklin, G. (1986). Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *27*(2), 235–238.

Han, Y., Liu, D., & Li, L. (2020). PD-1/PD-L1 pathway: Current researches in cancer. *American Journal of Cancer Research*, *10*(3), 727–742.

Han, Z. Q., Slack, R. S., Li, W. P., & Papadopoulos, V. (2003). Expression of peripheral benzodiazepine receptor (PBR) in human tumors: Relationship to breast, colorectal, and prostate tumor progression. *Journal of Receptors and Signal Transduction*, *23*(2–3), 225–238. https://doi.org/10.1081/RRS-120025210

Hardwick, M., Fertikh, D., Culty, M., Li, H., Vidic, B., & Papadopoulos, V. (1999). Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: Correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol. *Cancer Research*, *59*(4), 831–842.

Hardwick, M., Cavalli, L. R., Barlow, K. D., Haddad, B. R., & Papadopoulos, V. (2002). Peripheral-type benzodiazepine receptor (PBR) gene amplification in MDA-MB-231 aggressive breast cancer cells. *Cancer Genetics and Cytogenetics*, *139*(1), 48–51. https://doi.org/10.1016/s0165-4608(02)00604-0

Hashibe, M., Brennan, P., Benhamou, S., Castellsague, X., Chen, C., Curado, M. P., Dal Maso, L., Daudt, A. W., Fabianova, E., Fernandez, L., Wünsch-Filho, V., Franceschi, S., Hayes, R. B., Herrero, R., Koifman, S., La Vecchia, C., Lazarus, P., Levi, F., Mates, D., … Boffetta, P. (2007). Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: Pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Journal of the National Cancer Institute*, *99*(10), 777–789. https://doi.org/10.1093/jnci/djk179

Hauet, T., Yao, Z.-X., Bose, H. S., Wall, C. T., Han, Z., Li, W., Hales, D. B., Miller, W. L., Culty, M., & Papadopoulos, V. (2005). Peripheral-type benzodiazepine receptor-mediated action of steroidogenic acute regulatory protein on cholesterol entry into leydig cell mitochondria. *Molecular Endocrinology (Baltimore, Md.)*, *19*(2), 540–554. https://doi.org/10.1210/me.2004-0307

He, F., Ru, X., & Wen, T. (2020). NRF2, a Transcription Factor for Stress Response and Beyond. *International Journal of Molecular Sciences*, *21*(13). https://doi.org/10.3390/ijms21134777

He, J., Mao, C.-C., Reyes, A., Sembongi, H., Di Re, M., Granycome, C., Clippingdale, A. B., Fearnley, I. M., Harbour, M., Robinson, A. J., Reichelt, S., Spelbrink, J. N., Walker, J. E., & Holt, I. J. (2007). The AAA+ protein ATAD3 has displacement loop binding properties and is involved in mitochondrial nucleoid organization. *The Journal of Cell Biology*, *176*(2), 141–146. https://doi.org/10.1083/jcb.200609158

Helsen, N., Van den Wyngaert, T., Carp, L., & Stroobants, S. (2018). FDG-PET/CT for treatment response assessment in head and neck squamous cell carcinoma: A systematic review and meta-analysis of diagnostic performance. *European Journal of Nuclear Medicine and Molecular Imaging*, *45*(6), 1063–1071. https://doi.org/10.1007/s00259-018-3978-3

Hill, R. M., Rocha, S., & Parsons, J. L. (2022). Overcoming the Impact of Hypoxia in Driving Radiotherapy Resistance in Head and Neck Squamous Cell Carcinoma. *Cancers*, *14*(17). https://doi.org/10.3390/cancers14174130

Hinsen, K., Vaitinadapoule, A., Ostuni, M. A., Etchebest, C., & Lacapere, J.-J. (2015). Construction and validation of an atomic model for bacterial TSPO from electron microscopy density,

evolutionary constraints, and biochemical and biophysical data. *Biochimica et Biophysica Acta*, *1848*(2), 568–580. https://doi.org/10.1016/j.bbamem.2014.10.028

Hirsch, J. D., Beyer, C. F., Malkowitz, L., Beer, B., & Blume, A. J. (1989). Mitochondrial benzodiazepine receptors mediate inhibition of mitochondrial respiratory control. *Molecular Pharmacology*, *35*(1), 157–163.

Hohenstein, N. A., Chan, J. W., Wu, S. Y., Tahir, P., & Yom, S. S. (2020). Diagnosis, Staging, Radiation Treatment Response Assessment, and Outcome Prognostication of Head and Neck Cancers Using PET Imaging: A Systematic Review. *PET Clinics*, *15*(1), 65–75. https://doi.org/10.1016/j.cpet.2019.08.010

Hristozova, T., Konschak, R., Stromberger, C., Fusi, A., Liu, Z., Weichert, W., Stenzinger, A., Budach, V., Keilholz, U., & Tinhofer, I. (2011). The presence of circulating tumor cells (CTCs) correlates with lymph node metastasis in nonresectable squamous cell carcinoma of the head and neck region (SCCHN). *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *22*(8), 1878–1885. https://doi.org/10.1093/annonc/mdr130

Huang, C., Chen, L., Savage, S. R., Eguez, R. V., Dou, Y., Li, Y., da Veiga Leprevost, F., Jaehnig, E. J., Lei, J. T., Wen, B., Schnaubelt, M., Krug, K., Song, X., Cieślik, M., Chang, H.-Y., Wyczalkowski, M. A., Li, K., Colaprico, A., Li, Q. K., … Zhang, B. (2021). Proteogenomic insights into the biology and treatment of HPV-negative head and neck squamous cell carcinoma. *Cancer Cell*, *39*(3), 361-379.e16. https://doi.org/10.1016/j.ccell.2020.12.007

Huang, R.-X., & Zhou, P.-K. (2020). DNA damage response signaling pathways and targets for radiotherapy sensitization in cancer. *Signal Transduction and Targeted Therapy*, *5*(1), 60. https://doi.org/10.1038/s41392-020-0150-x

Huang, S. H., & O'Sullivan, B. (2017). Overview of the 8th Edition TNM Classification for Head and Neck Cancer. *Current Treatment Options in Oncology*, *18*(7), 40. https://doi.org/10.1007/s11864-017-0484-y

Huber, M. A., & Tantiwongkosi, B. (2014). Oral and oropharyngeal cancer. *The Medical Clinics of North America*, *98*(6), 1299–1321. https://doi.org/10.1016/j.mcna.2014.08.005

Hutchinson, M.-K. N. D., Mierzwa, M., & D'Silva, N. J. (2020). Radiation resistance in head and neck squamous cell carcinoma: Dire need for an appropriate sensitizer. *Oncogene*, *39*(18), 3638–3649. https://doi.org/10.1038/s41388-020-1250-3

Hussein, A. A., Helder, M. N., de Visscher, J. G., Leemans, C. R., Braakhuis, B. J., de Vet, H. C. W., & Forouzanfar, T. (2017). Global incidence of oral and oropharynx cancer in patients younger than 45 years versus older patients: A systematic review. *European journal of cancer (Oxford, England : 1990)*, *82*, 115–127. https://doi.org/10.1016/j.ejca.2017.05.026

Ikawa, M., Lohith, T. G., Shrestha, S., Telu, S., Zoghbi, S. S., Castellano, S., Taliani, S., Da Settimo, F., Fujita, M., Pike, V. W., & Innis, R. B. (2017). ^{11}C-ER176, a Radioligand for 18-kDa Translocator Protein, Has Adequate Sensitivity to Robustly Image All Three Affinity Genotypes in Human Brain. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *58*(2), 320–325. https://doi.org/10.2967/jnumed.116.178996

Jaakkola, P., Mole, D. R., Tian, Y. M., Wilson, M. I., Gielbert, J., Gaskell, S. J., von Kriegsheim, A., Hebestreit, H. F., Mukherji, M., Schofield, C. J., Maxwell, P. H., Pugh, C. W., & Ratcliffe, P. J. (2001). Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science (New York, N.Y.)*, *292*(5516), 468–472. https://doi.org/10.1126/science.1059796

Jäckel, M. C., Martin, A., & Steiner, W. (2007). Twenty-five years experience with laser surgery for head and neck tumors: Report of an international symposium, Göttingen, Germany, 2005. *European Archives of Oto-Rhino-Laryngology: Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS): Affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery*, *264*(6), 577–585. https://doi.org/10.1007/s00405-007-0280-6

Jaipuria, G., Leonov, A., Giller, K., Vasa, S. K., Jaremko, Ł., Jaremko, M., Linser, R., Becker, S., & Zweckstetter, M. (2017). Cholesterol-mediated allosteric regulation of the mitochondrial translocator protein structure. *Nature Communications*, *8*, 14893. https://doi.org/10.1038/ncomms14893

Jamin, N., Neumann, J.-M., Ostuni, M. A., Vu, T. K. N., Yao, Z.-X., Murail, S., Robert, J.-C., Giatzakis, C., Papadopoulos, V., & Lacapère, J.-J. (2005). Characterization of the cholesterol recognition amino acid consensus sequence of the peripheral-type benzodiazepine receptor. *Molecular Endocrinology (Baltimore, Md.)*, *19*(3), 588–594. https://doi.org/10.1210/me.2004-0308

Jaremko, L., Jaremko, M., Giller, K., Becker, S., & Zweckstetter, M. (2014). Structure of the mitochondrial translocator protein in complex with a diagnostic ligand. *Science (New York, N.Y.)*, *343*(6177), 1363–1366. https://doi.org/10.1126/science.1248725

Jaremko, M., Jaremko, Ł., Jaipuria, G., Becker, S., & Zweckstetter, M. (2015). Structure of the mammalian TSPO/PBR protein. *Biochemical Society Transactions*, *43*(4), 566–571. https://doi.org/10.1042/BST20150029

Jayakumar, A. R., Panickar, K. S., & Norenberg, M. D. (2002). Effects on free radical generation by ligands of the peripheral benzodiazepine receptor in cultured neural cells. *Journal of Neurochemistry*, *83*(5), 1226–1234. https://doi.org/10.1046/j.1471-4159.2002.01261.x

Johnson, D. E., Burtness, B., Leemans, C. R., Lui, V. W. Y., Bauman, J. E., & Grandis, J. R. (2020). Head and neck squamous cell carcinoma. *Nature Reviews Disease Primers*, *6*(1). https://doi.org/10.1038/s41572-020-00224-3

Joo, H. K., Lee, Y. R., Lim, S. Y., Lee, E. J., Choi, S., Cho, E. J., Park, M. S., Ryoo, S., Park, J. B., & Jeon, B. H. (2012). Peripheral benzodiazepine receptor regulates vascular endothelial activations via suppression of the voltage-dependent anion channel-1. *FEBS Letters*, *586*(9), 1349–1355. https://doi.org/10.1016/j.febslet.2012.03.049

Joo, H. K., Lee, Y. R., Kang, G., Choi, S., Kim, C.-S., Ryoo, S., Park, J. B., & Jeon, B. H. (2015). The 18-kDa Translocator Protein Inhibits Vascular Cell Adhesion Molecule-1 Expression via Inhibition of Mitochondrial Reactive Oxygen Species. *Molecules and Cells*, *38*(12), 1064–1070. https://doi.org/10.14348/molcells.2015.0165

Joseph-Liauzun, E., Delmas, P., Shire, D., & Ferrara, P. (1998). Topological analysis of the peripheral benzodiazepine receptor in yeast mitochondrial membranes supports a five-transmembrane structure. *The Journal of Biological Chemistry*, *273*(4), 2146–2152. https://doi.org/10.1074/jbc.273.4.2146

Junck, L., Olson, J. M., Ciliax, B. J., Koeppe, R. A., Watkins, G. L., Jewett, D. M., McKeever, P. E., Wieland, D. M., Kilbourn, M. R., & Starosta-Rubinstein, S. (1989). PET imaging of human gliomas with ligands for the peripheral benzodiazepine binding site. *Annals of Neurology*, *26*(6), 752–758. https://doi.org/10.1002/ana.410260611

Jung, Y.-S., Najy, A. J., Huang, W., Sethi, S., Snyder, M., Sakr, W., Dyson, G., Hüttemann, M., Lee, I., Ali-Fehmi, R., Franceschi, S., Struijk, L., Kim, H. E., Kato, I., & Kim, H.-R. C. (2017). HPV-associated differential regulation of tumor metabolism in oropharyngeal head and neck cancer. *Oncotarget*, *8*(31), 51530–51541. https://doi.org/10.18632/oncotarget.17887

Kalyankrishna, S., & Grandis, J. R. (2006). Epidermal growth factor receptor biology in head and neck cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *24*(17), 2666–2672. https://doi.org/10.1200/JCO.2005.04.8306

Kang, H., Kiess, A., & Chung, C. H. (2015). Emerging biomarkers in head and neck cancer in the era of genomics. *Nature Reviews Clinical Oncology*, *12*(1), 11–26. https://doi.org/10.1038/nrclinonc.2014.192

Katz, Y., Eitan, A., Amiri, Z., & Gavish, M. (1988). Dramatic increase in peripheral benzodiazepine binding sites in human colonic adenocarcinoma as compared to normal colon. *European Journal of Pharmacology*, *148*(3), 483–484. https://doi.org/10.1016/0014-2999(88)90135-5

Katz, Y., Eitan, A., & Gavish, M. (1990). Increase in peripheral benzodiazepine binding sites in colonic adenocarcinoma. *Oncology*, *47*(2), 139–142. https://doi.org/10.1159/000226806

Kazemi-Sefat, G. E., Keramatipour, M., Talebi, S., Kavousi, K., Sajed, R., Kazemi-Sefat, N. A., & Mousavizadeh, K. (2021). The importance of CDC27 in cancer: Molecular pathology and clinical aspects. *Cancer Cell International*, *21*(1), 160. https://doi.org/10.1186/s12935-021-01860-9

Keller, T., Krzyczmonik, A., Forsback, S., Picón, F. R. L., Kirjavainen, A. K., Takkinen, J., Rajander, J., Cacheux, F., Damont, A., Dollé, F., Rinne, J. O., Haaparanta-Solin, M., & Solin, O. (2017). Radiosynthesis and Preclinical Evaluation of [^{18}F]F-DPA, A Novel Pyrazolo[1,5a]pyrimidine

Acetamide TSPO Radioligand, in Healthy Sprague Dawley Rats. *Molecular Imaging and Biology*, *19*(5), 736–745. https://doi.org/10.1007/s11307-016-1040-z

Keller, T., López-Picón, F. R., Krzyczmonik, A., Forsback, S., Takkinen, J. S., Rajander, J., Teperi, S., Dollé, F., Rinne, J. O., Haaparanta-Solin, M., & Solin, O. (2020). Comparison of high and low molar activity TSPO tracer [(18)F]F-DPA in a mouse model of Alzheimer's disease. *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *40*(5), 1012–1020. https://doi.org/10.1177/0271678X19853117

Kelly-Hershkovitz, E., Weizman, R., Spanier, I., Leschiner, S., Lahav, M., Weisinger, G., & Gavish, M. (1998). Effects of peripheral-type benzodiazepine receptor antisense knockout on MA-10 Leydig cell proliferation and steroidogenesis. *The Journal of Biological Chemistry*, *273*(10), 5478–5483. https://doi.org/10.1074/jbc.273.10.5478

Kerawala, C. J. (2010). Complications of head and neck cancer surgery—Prevention and management. *Oral Oncology*, *46*(6), 433–435. https://doi.org/10.1016/j.oraloncology.2010.03.013

Kim, W., Lee, S., Seo, D., Kim, D., Kim, K., Kim, E., Kang, J., Seong, K. M., Youn, H., & Youn, B. (2019). Cellular Stress Responses in Radiotherapy. *Cells*, *8*(9). https://doi.org/10.3390/cells8091105

Kimple, R. J., Harari, P. M., Torres, A. D., Yang, R. Z., Soriano, B. J., Yu, M., Armstrong, E. A., Blitzer, G. C., Smith, M. A., Lorenz, L. D., Lee, D., Yang, D. T., McCulloch, T. M., Hartig, G. K., & Lambert, P. F. (2013). Development and characterization of HPV-positive and HPV-negative head and neck squamous cell carcinoma tumorgrafts. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *19*(4), 855–864. https://doi.org/10.1158/1078-0432.CCR-12-2746

Klubo-Gwiezdzinska, J., Jensen, K., Bauer, A., Patel, A., Costello, J. J., Burman, K. D., Wartofsky, L., Hardwick, M. J., & Vasko, V. V. (2012). The expression of translocator protein in human thyroid cancer and its role in the response of thyroid cancer cells to oxidative stress. *The Journal of Endocrinology*, *214*(2), 207–216. https://doi.org/10.1530/JOE-12-0081

Kluza, J., Peugnet, V., Daunou, B., Laine, W., Kervoaze, G., Rémy, G., Loyens, A., Maboudou, P., Fovez, Q., Grangette, C., Wolowczuk, I., Gosset, P., Garçon, G., Marchetti, P., Pinet, F., Pichavant, M., & Dubois-Deruy, E. (2021). A New Strategy to Preserve and Assess Oxygen Consumption in Murine Tissues. *International Journal of Molecular Sciences*, *23*(1). https://doi.org/10.3390/ijms23010109

Kokoszka, J. E., Waymire, K. G., Levy, S. E., Sligh, J. E., Cai, J., Jones, D. P., MacGregor, G. R., & Wallace, D. C. (2004). The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature*, *427*(6973), 461–465. https://doi.org/10.1038/nature02229

Kong, L., & Birkeland, A. C. (2021). Liquid Biopsies in Head and Neck Cancer: Current State and Future Challenges. *Cancers*, *13*(8). https://doi.org/10.3390/cancers13081874

Königsrainer, I., Vogel, U. F., Beckert, S., Sotlar, K., Coerper, S., Braun, A., Lembert, N., Steurer, W., Königsrainer, A., & Kupka, S. (2007). Increased translocator protein (TSPO) mRNA levels in colon but not in rectum carcinoma. *European Surgical Research*, *39*(6), 359–363. https://doi.org/10.1159/000106380

Kramer, I. R., Lucas, R. B., Pindborg, J. J., & Sobin, L. H. (1978). Definition of leukoplakia and related lesions: An aid to studies on oral precancer. *Oral Surgery, Oral Medicine, and Oral Pathology*, *46*(4), 518–539.

Kreimer, A. R., Clifford, G. M., Boyle, P., & Franceschi, S. (2005). Human papillomavirus types in head and neck squamous cell carcinomas worldwide: A systematic review. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *14*(2), 467–475. https://doi.org/10.1158/1055-9965.EPI-04-0551

Kreisl, W. C., Kim, M.-J., Coughlin, J. M., Henter, I. D., Owen, D. R., & Innis, R. B. (2020). PET imaging of neuroinflammation in neurological disorders. *The Lancet. Neurology*, *19*(11), 940–950. https://doi.org/10.1016/S1474-4422(20)30346-X

Krueger, K. E., & Papadopoulos, V. (1990). Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *The Journal of Biological Chemistry*, *265*(25), 15015–15022.

Kugler, W., Veenman, L., Shandalov, Y., Leschiner, S., Spanier, I., Lakomek, M., & Gavish, M. (2008). Ligands of the mitochondrial 18 kDa translocator protein attenuate apoptosis of human glioblastoma cells exposed to erucylphosphohomocholine. *Cellular Oncology: The Official Journal of the International Society for Cellular Oncology*, *30*(5), 435–450. https://doi.org/10.3233/clo-2008-0431

Kuhlmann, A. C., & Guilarte, T. R. (2000). Cellular and subcellular localization of peripheral benzodiazepine receptors after trimethyltin neurotoxicity. *Journal of Neurochemistry*, *74*(4), 1694–1704. https://doi.org/10.1046/j.1471-4159.2000.0741694.x

Kühn, J. P., Schmid, W., Körner, S., Bochen, F., Wemmert, S., Rimbach, H., Smola, S., Radosa, J. C., Wagner, M., Morris, L. G. T., Bozzato, V., Bozzato, A., Schick, B., & Linxweiler, M. (2021). HPV Status as Prognostic Biomarker in Head and Neck Cancer-Which Method Fits the Best for Outcome Prediction? *Cancers*, *13*(18). https://doi.org/10.3390/cancers13184730

Kulangara, K., Zhang, N., Corigliano, E., Guerrero, L., Waldroup, S., Jaiswal, D., Ms, M. J., Shah, S., Hanks, D., Wang, J., Lunceford, J., Savage, M. J., Juco, J., & Emancipator, K. (2019). Clinical Utility of the Combined Positive Score for Programmed Death Ligand-1 Expression and the Approval of Pembrolizumab for Treatment of Gastric Cancer. *Archives of Pathology & Laboratory Medicine*, *143*(3), 330–337. https://doi.org/10.5858/arpa.2018-0043-OA

Kurth, I., Hein, L., Mäbert, K., Peitzsch, C., Koi, L., Cojoc, M., Kunz-Schughart, L., Baumann, M., & Dubrovska, A. (2015). Cancer stem cell related markers of radioresistance in head and neck squamous cell carcinoma. *Oncotarget*, *6*(33), 34494–34509. https://doi.org/10.18632/oncotarget.5417

Kurumaji, A., & Toru, M. (1996). Postnatal development of peripheral-type benzodiazepine receptors in rat brain and peripheral tissues. *Brain Research. Developmental Brain Research*, *97*(1), 148–151. https://doi.org/10.1016/s0165-3806(96)00150-2

Lacapère, J. J., Delavoie, F., Li, H., Péranzi, G., Maccario, J., Papadopoulos, V., & Vidic, B. (2001). Structural and functional study of reconstituted peripheral benzodiazepine receptor. *Biochemical and Biophysical Research Communications*, *284*(2), 536–541. https://doi.org/10.1006/bbrc.2001.4975

Lanfranca, M. P., Lazarus, J., Shao, X., Nathan, H., Di Magliano, M. P., Zou, W., Piert, M., & Frankel, T. L. (2018). Tracking Macrophage Infiltration in a Mouse Model of Pancreatic Cancer with the Positron Emission Tomography Tracer [^{11}C]PBR28. *The Journal of Surgical Research*, *232*, 570–577. https://doi.org/10.1016/j.jss.2018.07.015

Langen, B., Rudqvist, N., Spetz, J., Swanpalmer, J., Helou, K., & Forssell-Aronsson, E. (2016). Non-targeted transcriptomic effects upon thyroid irradiation: Similarity between in-field and out-of-field responses varies with tissue type. *Scientific Reports*, *6*, 30738. https://doi.org/10.1038/srep30738

Langendijk, J. A., & Psyrri, A. (2010). The prognostic significance of p16 overexpression in oropharyngeal squamous cell carcinoma: Implications for treatment strategies and future clinical studies. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*, *21*(10), 1931–1934. https://doi.org/10.1093/annonc/mdq439

Larcher, J. C., Vayssiere, J. L., Le Marquer, F. J., Cordeau, L. R., Keane, P. E., Bachy, A., Gros, F., & Croizat, B. P. (1989). Effects of peripheral benzodiazepines upon the O_2 consumption of neuroblastoma cells. *European Journal of Pharmacology*, *161*(2–3), 197–202. https://doi.org/10.1016/0014-2999(89)90843-1

Lawrence, M. S., Sougnez, C., Lichtenstein, L., Cibulskis, K., Lander, E., Gabriel, S. B., Getz, G., Ally, A., Balasundaram, M., Birol, I., Bowlby, R., Brooks, D., Butterfield, Y. S. N., Carlsen, R., Cheng, D., Chu, A., Dhalla, N., Guin, R., Holt, R. A., … Pham, M. (2015). Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*, *517*(7536), 576–582. https://doi.org/10.1038/nature14129

Le Fur, G., Perrier, M. L., Vaucher, N., Imbault, F., Flamier, A., Benavides, J., Uzan, A., Renault, C., Dubroeucq, M. C., & Guérémy, C. (1983a). Peripheral benzodiazepine binding sites: Effect of PK 11195, 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinolinecarboxamide. I. In vitro studies. *Life Sciences*, *32*(16), 1839–1847. https://doi.org/10.1016/0024-3205(83)90062-0

Le Fur, G., Guilloux, F., Rufat, P., Benavides, J., Uzan, A., Renault, C., Dubroeucq, M. C., & Guérémy, C. (1983b). Peripheral benzodiazepine binding sites: Effect of PK 11195, 1-(2-chlorophenyl)-N-methyl-(1-methylpropyl)-3 isoquinolinecarboxamide. II. In vivo studies. *Life Sciences*, *32*(16), 1849–1856. https://doi.org/10.1016/0024-3205(83)90063-2

Le Fur, G., Vaucher, N., Perrier, M. L., Flamier, A., Benavides, J., Renault, C., Dubroeucq, M. C., Guérémy, C., & Uzan, A. (1983c). Differentiation between two ligands for peripheral benzodiazepine binding sites, [^{3}H]RO5-4864 and [^{3}H]PK 11195, by thermodynamic studies. *Life Sciences*, *33*(5), 449–457. https://doi.org/10.1016/0024-3205(83)90794-4

Leemans, C. R., Braakhuis, B. J. M., & Brakenhoff, R. H. (2011). The molecular biology of head and neck cancer. *Nature Reviews. Cancer*, *11*(1), 9–22. https://doi.org/10.1038/nrc2982

Lejri, I., Grimm, A., Hallé, F., Abarghaz, M., Klein, C., Maitre, M., Schmitt, M., Bourguignon, J.-J., Mensah-Nyagan, A. G., Bihel, F., & Eckert, A. (2019). TSPO Ligands Boost Mitochondrial Function and Pregnenolone Synthesis. *Journal of Alzheimer's Disease: JAD*, *72*(4), 1045–1058. https://doi.org/10.3233/JAD-190127

Levin, E., Premkumar, A., Veenman, L., Kugler, W., Leschiner, S., Spanier, I., Weisinger, G., Lakomek, M., Weizman, A., Snyder, S. H., Pasternak, G. W., & Gavish, M. (2005). The peripheral-type benzodiazepine receptor and tumorigenicity: Isoquinoline binding protein (IBP) antisense knockdown in the C6 glioma cell line. *Biochemistry*, *44*(29), 9924–9935. https://doi.org/10.1021/bi050150s

Li, F., Liu, J., Zheng, Y., Garavito, R. M., & Ferguson-Miller, S. (2015). Protein structure. Crystal structures of translocator protein (TSPO) and mutant mimic of a human polymorphism. *Science (New York, N.Y.)*, *347*(6221), 555–558. https://doi.org/10.1126/science.1260590

Li, H., & Papadopoulos, V. (1998). Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. *Endocrinology*, *139*(12), 4991–4997. https://doi.org/10.1210/endo.139.12.6390

Li, H., Yao, Z., Degenhardt, B., Teper, G., & Papadopoulos, V. (2001). Cholesterol binding at the cholesterol recognition/ interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(3), 1267–1272. https://doi.org/10.1073/pnas.98.3.1267

Li, J. S. Z., Abbasi, A., Kim, D. H., Lippman, S. M., Alexandrov, L. B., & Cleveland, D. W. (2023). Chromosomal fragile site breakage by EBV-encoded EBNA1 at clustered repeats. *Nature*, *616*(7957), 504–509. https://doi.org/10.1038/s41586-023-05923-x

Li, N., Chamkha, I., Verma, G., Swoboda, S., Lindstedt, M., Greiff, L., Elmér, E., & Ehinger, J. (2023). Human papillomavirus-associated head and neck squamous cell carcinoma cells rely on glycolysis and display reduced oxidative phosphorylation. *Frontiers in Oncology*, *13*, 1304106. https://doi.org/10.3389/fonc.2023.1304106

Li, W., Hardwick, M. J., Rosenthal, D., Culty, M., & Papadopoulos, V. (2007). Peripheral-type benzodiazepine receptor overexpression and knockdown in human breast cancer cells indicate its prominent role in tumor cell proliferation. *Biochemical Pharmacology*, *73*(4), 491–503. https://doi.org/10.1016/j.bcp.2006.10.025

Liberzon, A., Subramanian, A., Pinchback, R., Thorvaldsdóttir, H., Tamayo, P., & Mesirov, J. P. (2011). Molecular signatures database (MSigDB) 3.0. *Bioinformatics*, *27*(12), 1739–1740. https://doi.org/10.1093/bioinformatics/btr260

Liberzon, A., Birger, C., Ghandi, M., Jill, P., Tamayo, P., Jolla, L., & Jolla, L. (2016). *Collection*. *1*(6), 417–425. https://doi.org/10.1016/j.cels.2015.12.004.The

Licitra, L., Bernier, J., Grandi, C., Merlano, M., Bruzzi, P., & Lefebvre, J.-L. (2002). Cancer of the oropharynx. *Critical Reviews in Oncology/Hematology*, *41*(1), 107–122. https://doi.org/10.1016/s1040-8428(01)00129-9

Licitra, L., Bernier, J., Grandi, C., Locati, L., Merlano, M., Gatta, G., & Lefebvre, J.-L. (2003). Cancer of the larynx. *Critical Reviews in Oncology/Hematology*, *47*(1), 65–80. https://doi.org/10.1016/s1040-8428(03)00017-9

Liebthal, M., Kushwah, M. S., Kukura, P., & Dietz, K.-J. (2021). Single molecule mass photometry reveals the dynamic oligomerization of human and plant peroxiredoxins. *iScience*, *24*(11), 103258. https://doi.org/10.1016/j.isci.2021.103258

Lin, R., Angelin, A., Da Settimo, F., Martini, C., Taliani, S., Zhu, S., & Wallace, D. C. (2014). Genetic analysis of dTSPO, an outer mitochondrial membrane protein, reveals its functions in apoptosis, longevity, and Ab42-induced neurodegeneration. *Aging Cell*, *13*(3), 507–518. https://doi.org/10.1111/acel.12200

Lin, Y.-M., Sung, W.-W., Hsieh, M.-J., Tsai, S.-C., Lai, H.-W., Yang, S.-M., Shen, K.-H., Chen, M.-K., Lee, H., Yeh, K.-T., & Chen, C.-J. (2015). High PD-L1 Expression Correlates with Metastasis and Poor Prognosis in Oral Squamous Cell Carcinoma. *PloS One*, *10*(11), e0142656. https://doi.org/10.1371/journal.pone.0142656

Lindel, K., Beer, K. T., Laissue, J., Greiner, R. H., & Aebersold, D. M. (2001). Human papillomavirus positive squamous cell carcinoma of the oropharynx: a radiosensitive subgroup of head and neck carcinoma. *Cancer*, *92*(4), 805–813. https://doi.org/10.1002/1097-0142(20010815)92:4<805::aid-cncr1386>3.0.co;2-9

Lindquist, D., Romanitan, M., Hammarstedt, L., Näsman, A., Dahlstrand, H., Lindholm, J., Onelöv, L., Ramqvist, T., Ye, W., Munck-Wikland, E., & Dalianis, T. (2007). Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. *Molecular oncology*, *1*(3), 350–355. https://doi.org/10.1016/j.molonc.2007.08.005

Liu, G. J., Middleton, R. J., Kam, W. W. Y., Chin, D. Y., Hatty, C. R., Chan, R. H. Y., & Banati, R. B. (2017). Functional gains in energy and cell metabolism after TSPO gene insertion. *Cell Cycle*, *16*(5), 436–447. https://doi.org/10.1080/15384101.2017.1281477

Liu, J., Li, H., & Papadopoulos, V. (2003). PAP7, a PBR/PKA-RIalpha-associated protein: A new element in the relay of the hormonal induction of steroidogenesis. *The Journal of Steroid Biochemistry and Molecular Biology*, *85*(2–5), 275–283. https://doi.org/10.1016/s0960-0760(03)00213-9

Lo, Y. M., Chan, L. Y., Lo, K. W., Leung, S. F., Zhang, J., Chan, A. T., Lee, J. C., Hjelm, N. M., Johnson, P. J., & Huang, D. P. (1999). Quantitative analysis of cell-free Epstein-Barr virus DNA in plasma of patients with nasopharyngeal carcinoma. *Cancer Research*, *59*(6), 1188–1191.

Lo, Y. M., Leung, S. F., Chan, L. Y., Lo, K. W., Zhang, J., Chan, A. T., Lee, J. C., Hjelm, N. M., Johnson, P. J., & Huang, D. P. (2000). Plasma cell-free Epstein-Barr virus DNA quantitation in patients with nasopharyngeal carcinoma. Correlation with clinical staging. *Annals of the New York Academy of Sciences*, *906*, 99–101. https://doi.org/10.1111/j.1749-6632.2000.tb06597.x

Lui, V. W. Y., Hedberg, M. L., Li, H., Vangara, B. S., Pendleton, K., Zeng, Y., Lu, Y., Zhang, Q., Du, Y., Gilbert, B. R., Freilino, M., Sauerwein, S., Peyser, N. D., Xiao, D., Diergaarde, B., Wang, L., Chiosea, S., Seethala, R., Johnson, J. T., … Grandis, J. R. (2013). Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. *Cancer Discovery*, *3*(7), 761–769. https://doi.org/10.1158/2159-8290.CD-13-0103

Lydiatt, W. M., Patel, S. G., O'Sullivan, B., Brandwein, M. S., Ridge, J. A., Migliacci, J. C., Loomis, A. M., & Shah, J. P. (2017). Head and Neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. *CA: A Cancer Journal for Clinicians*, *67*(2), 122–137. https://doi.org/10.3322/caac.21389

Maaser, K., Höpfner, M., Jansen, A., Weisinger, G., Gavish, M., Kozikowski, A. P., Weizman, A., Carayon, P., Riecken, E. O., Zeitz, M., & Scherübl, H. (2001). Specific ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in human colorectal cancer cells. *British Journal of Cancer*, *85*(11), 1771–1780. https://doi.org/10.1054/bjoc.2001.2181

Maaser, K., Grabowski, P., Sutter, A. P., Hopfner, M., Foss, H. D., Stein, H., Berger, G., Gavish, M., Zeitz, M., & Scherubl, H. (2002). Overexpression of the peripheral benzodiazepine receptor is a relevant prognostic factor in stage III colorectal cancer. *Clinical Cancer Research*, *8*(10), 3205–3209.

Magnes, T., Wagner, S., Kiem, D., Weiss, L., Rinnerthaler, G., Greil, R., & Melchardt, T. (2021). Prognostic and predictive factors in advanced head and neck squamous cell carcinoma. *International Journal of Molecular Sciences*, *22*(9). https://doi.org/10.3390/ijms22094981

Mantione, C. R., Goldman, M. E., Martin, B., Bolger, G. T., Lueddens, H. W., Paul, S. M., & Skolnick, P. (1988). Purification and characterization of an endogenous protein modulator of radioligand binding to "peripheral-type" benzodiazepine receptors and dihydropyridine Ca^{2+}-channel antagonist binding sites. *Biochemical Pharmacology*, *37*(2), 339–347. https://doi.org/10.1016/0006-2952(88)90738-1

Marangos, P. J., Patel, J., Boulenger, J. P., & Clark-Rosenberg, R. (1982). Characterization of peripheral-type benzodiazepine binding sites in brain using [^{3}H]Ro 5-4864. *Molecular Pharmacology*, *22*(1), 26–32.

Marur, S., & Forastiere, A. A. (2016). Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. *Mayo Clinic Proceedings*, *91*(3), 386–396. https://doi.org/10.1016/j.mayocp.2015.12.017

Mascarella, M. A., Mannard, E., Silva, S. D., & Zeitouni, A. (2018). Neutrophil-to-lymphocyte ratio in head and neck cancer prognosis: A systematic review and meta-analysis. *Head & Neck*, *40*(5), 1091–1100. https://doi.org/10.1002/hed.25075

Mattner, F., Katsifis, A., Bourdier, T., Loc'h, C., Berghofer, P., Fookes, C., Hung, T.-T., Jackson, T., Henderson, D., Pham, T., Lee, B. J., Shepherd, R., Greguric, I., Wyatt, N., Le, T., Poon, J., Power, C., & Fulham, M. (2021). Synthesis and pharmacological evaluation of [(18)F]PBR316: A novel PET ligand targeting the translocator protein 18 kDa (TSPO) with low binding sensitivity to human single nucleotide polymorphism rs6971. *RSC Medicinal Chemistry*, *12*(7), 1207–1221. https://doi.org/10.1039/d1md00035g

McEnery, M. W., Snowman, A. M., Trifiletti, R. R., & Snyder, S. H. (1992). Isolation of the mitochondrial benzodiazepine receptor: Association with the voltage-dependent anion channel and the adenine nucleotide carrier. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(8), 3170–3174. https://doi.org/10.1073/pnas.89.8.3170

McIlwain, W. R., Sood, A. J., Nguyen, S. A., & Day, T. A. (2014). Initial symptoms in patients with HPV-positive and HPV-negative oropharyngeal cancer. *JAMA otolaryngology-- head & neck surgery*, *140*(5), 441–447. https://doi.org/10.1001/jamaoto.2014.141

McLaughlin-Drubin, M. E., & Münger, K. (2009). The human papillomavirus E7 oncoprotein. *Virology*, *384*(2), 335–344. https://doi.org/10.1016/j.virol.2008.10.006

Mehanna, H., Taberna, M., von Buchwald, C., Tous, S., Brooks, J., Mena, M., Morey, F., Grønhøj, C., Rasmussen, J. H., Garset-Zamani, M., Bruni, L., Batis, N., Brakenhoff, R. H., Leemans, C. R., Baatenburg de Jong, R. J., Klussmann, J. P., Wuerdemann, N., Wagner, S., Dalianis, T., … Alemany, L. (2023). Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): A multicentre, multinational, individual patient data analysis. *The Lancet. Oncology*, *24*(3), 239–251. https://doi.org/10.1016/S1470-2045(23)00013-X

Meng, Y., Tian, M., Yin, S., Lai, S., Zhou, Y., Chen, J., He, M., & Liao, Z. (2020). Downregulation of TSPO expression inhibits oxidative stress and maintains mitochondrial homeostasis in cardiomyocytes subjected to anoxia/reoxygenation injury. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *121*, 109588. https://doi.org/10.1016/j.biopha.2019.109588

Miettinen, H., Kononen, J., Haapasalo, H., Helén, P., Sallinen, P., Harjuntausta, T., Helin, H., & Alho, H. (1995). Expression of Peripheral-Type Benzodiazepine Receptor and Diazepam Binding Inhibitor in Human Astrocytomas: Relationship to Cell Proliferation. *Cancer Research*, *55*(12), 2691–2695.

Milenkovic, V. M., Slim, D., Bader, S., Koch, V., Heinl, E. S., Alvarez-Carbonell, D., Nothdurfter, C., Rupprecht, R., & Wetzel, C. H. (2019). CRISPR-cas9 mediated TSPO gene knockout alters respiration and cellular metabolism in human primary microglia cells. *International Journal of Molecular Sciences*, *20*(13). https://doi.org/10.3390/ijms20133359

Mims, J., Bansal, N., Bharadwaj, M. S., Chen, X., Molina, A. J., Tsang, A. W., & Furdui, C. M. (2015). Energy metabolism in a matched model of radiation resistance for head and neck squamous cell cancer. *Radiation Research*, *183*(3), 291–304. https://doi.org/10.1667/RR13828.1

Miranda-Filho, A., & Bray, F. (2020). Global patterns and trends in cancers of the lip, tongue and mouth. *Oral Oncology*, *102*, 104551. https://doi.org/10.1016/j.oraloncology.2019.104551

Miyakawa, A., Wang, X. L., Nakanishi, H., Imai, F. L., Shiiba, M., Miya, T., Imai, Y., & Tanzawa, H. (1998). Allelic loss on chromosome 22 in oral cancer: Possibility of the existence of a tumor

suppressor gene on 22q13. *International Journal of Oncology*, *13*(4), 705–709. https://doi.org/10.3892/ijo.13.4.705

Mizrahi, R., Rusjan, P. M., Kennedy, J., Pollock, B., Mulsant, B., Suridjan, I., De Luca, V., Wilson, A. A., & Houle, S. (2012). Translocator protein (18 kDa) polymorphism (rs6971) explains in-vivo brain binding affinity of the PET radioligand [(18)F]-FEPPA. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *32*(6), 968–972. https://doi.org/10.1038/jcbfm.2012.46

Mody, M. D., Rocco, J. W., Yom, S. S., Haddad, R. I., & Saba, N. F. (2021). Head and neck cancer. *The Lancet*, *398*(10318), 2289–2299. https://doi.org/10.1016/S0140-6736(21)01550-6

Moeller, B. J., & Dewhirst, M. W. (2006). HIF-1 and tumour radiosensitivity. *British Journal of Cancer*, *95*(1), 1–5. https://doi.org/10.1038/sj.bjc.6603201

Möhler, H., & Okada, T. (1977). Benzodiazepine receptor: Demonstration in the central nervous system. *Science (New York, N.Y.)*, *198*(4319), 849–851. https://doi.org/10.1126/science.918669

Mokoala, K. M. G., & Sathekge, M. M. (2024). Non-FDG hypoxia tracers. *Seminars in Nuclear Medicine*, *54*(6), 827–844. https://doi.org/10.1053/j.semnuclmed.2024.10.001

Montero, P. H., & Patel, S. G. (2015). Cancer of the oral cavity. *Surgical Oncology Clinics of North America*, *24*(3), 491–508. https://doi.org/10.1016/j.soc.2015.03.006

Moody, C. A., & Laimins, L. A. (2010). Human papillomavirus oncoproteins: Pathways to transformation. *Nature Reviews. Cancer*, *10*(8), 550–560. https://doi.org/10.1038/nrc2886

Morohaku, K., Pelton, S. H., Daugherty, D. J., Butler, W. R., Deng, W., & Selvaraj, V. (2014). Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology*, *155*(1), 89–97. https://doi.org/10.1210/en.2013-1556

Moskovitz, J., Moy, J., & Ferris, R. L. (2018). Immunotherapy for Head and Neck Squamous Cell Carcinoma. *Current Oncology Reports*, *20*(2), 22. https://doi.org/10.1007/s11912-018-0654-5

Mukherjee, S., & Das, S. K. (1989). Subcellular distribution of "peripheral type" binding sites for [^{3}H]Ro5-4864 in guinea pig lung. Localization to the mitochondrial inner membrane. *The Journal of Biological Chemistry*, *264*(28), 16713–16718.

Mukhin, A. G., Papadopoulos, V., Costa, E., & Krueger, K. E. (1989). Mitochondrial benzodiazepine receptors regulate steroid biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(24), 9813–9816. https://doi.org/10.1073/pnas.86.24.9813

Murail, S., Robert, J.-C., Coïc, Y.-M., Neumann, J.-M., Ostuni, M. A., Yao, Z.-X., Papadopoulos, V., Jamin, N., & Lacapère, J.-J. (2008). Secondary and tertiary structures of the transmembrane domains of the translocator protein TSPO determined by NMR. Stabilization of the TSPO tertiary fold upon ligand binding. *Biochimica et Biophysica Acta*, *1778*(6), 1375–1381. https://doi.org/10.1016/j.bbamem.2008.03.012

Mylly, M., Nissi, L., Huusko, T., Routila, J., Vaittinen, S., Irjala, H., Leivo, I., & Ventelä, S. (2022). Epidemiological Study of p16 Incidence in Head and Neck Squamous Cell Carcinoma 2005–2015 in a Representative Northern European Population. *Cancers*, *14*(22). https://doi.org/10.3390/cancers14225717

Nagler, R., Ben-Izhak, O., Savulescu, D., Krayzler, E., Akrish, S., Leschiner, S., Otradnov, I., Zeno, S., Veenman, L., & Gavish, M. (2010). Oral cancer, cigarette smoke and mitochondrial 18kDa translocator protein (TSPO)—In vitro, in vivo, salivary analysis. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1802*(5), 454–461. https://doi.org/10.1016/j.bbadis.2010.01.008

Nagler, R., Cohen, S., & Gavish, M. (2015). The Effect of Cigarette Smoke on the Translocator Protein (TSPO) in Cultured Lung Cancer Cells. *Journal of Cellular Biochemistry*, *116*(12), 2786–2792. https://doi.org/10.1002/jcb.25221

Neville, B. W., & Day, T. A. (2002). Oral cancer and precancerous lesions. *CA: A Cancer Journal for Clinicians*, *52*(4), 195–215. https://doi.org/10.3322/canjclin.52.4.195

Ngamphaiboon, N., Chureemas, T., Siripoon, T., Arsa, L., Trachu, N., Jiarpinitnun, C., Pattaranutaporn, P., Sirachainan, E., & Larbcharoensub, N. (2019). Characteristics and impact of programmed death-ligand 1 expression, CD8+ tumor-infiltrating lymphocytes, and p16 status in head and neck squamous cell carcinoma. *Medical Oncology (Northwood, London, England)*, *36*(2), 21. https://doi.org/10.1007/s12032-018-1241-1

Noh, J. K., Woo, S. R., Kong, M., Lee, M. K., Lee, J. W., Lee, Y. C., Ko, S. G., & Eun, Y. G. (2023). Gene signature predicting recurrence in oral squamous cell carcinoma is characterized by increased oxidative phosphorylation. *Molecular Oncology*, *17*(1), 134–149. https://doi.org/10.1002/1878-0261.13328

Nordsmark, M., Bentzen, S. M., Rudat, V., Brizel, D., Lartigau, E., Stadler, P., Becker, A., Adam, M., Molls, M., Dunst, J., Terris, D. J., & Overgaard, J. (2005). Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology*, *77*(1), 18–24. https://doi.org/10.1016/j.radonc.2005.06.038

Nordsmark, M., Overgaard, M., & Overgaard, J. (1996). Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of the head and neck. *Radiotherapy and Oncology: Journal of the European Society for Therapeutic Radiology and Oncology*, *41*(1), 31–39. https://doi.org/10.1016/s0167-8140(96)91811-3

Notter, T., Coughlin, J. M., Gschwind, T., Weber-Stadlbauer, U., Wang, Y., Kassiou, M., Vernon, A. C., Benke, D., Pomper, M. G., Sawa, A., & Meyer, U. (2018). Translational evaluation of translocator protein as a marker of neuroinflammation in schizophrenia. *Molecular Psychiatry*, *23*(2), 323–334. https://doi.org/10.1038/mp.2016.248

O'Beirne, G. B., & Williams, D. C. (1988). The subcellular location in rat kidney of the peripheral benzodiazepine acceptor. *European Journal of Biochemistry*, *175*(2), 413–421. https://doi.org/10.1111/j.1432-1033.1988.tb14211.x

Obid, R., Redlich, M., & Tomeh, C. (2019). The Treatment of Laryngeal Cancer. *Oral and Maxillofacial Surgery Clinics of North America*, *31*(1), 1–11. https://doi.org/10.1016/j.coms.2018.09.001

Okada, H., & Mak, T. W. (2004). Pathways of apoptotic and non-apoptotic death in tumour cells. *Nature Reviews. Cancer*, *4*(8), 592–603. https://doi.org/10.1038/nrc1412

Oke, B. O., Suarez-Quian, C. A., Riond, J., Ferrara, P., & Papadopoulos, V. (1992). Cell surface localization of the peripheral-type benzodiazepine receptor (PBR) in adrenal cortex. *Molecular and Cellular Endocrinology*, *87*(1–3), R1-6. https://doi.org/10.1016/0303-7207(92)90248-5

Olson, J. M., Ciliax, B. J., Mancini, W. R., & Young, A. B. (1988). Presence of peripheral-type benzodiazepine binding sites on human erythrocyte membranes. *European Journal of Pharmacology*, *152*(1–2), 47–53. https://doi.org/10.1016/0014-2999(88)90834-5

Omami, G., Tamimi, D., & Branstetter, B. F. (2014). Basic principles and applications of (18)F-FDG-PET/CT in oral and maxillofacial imaging: A pictorial essay. *Imaging Science in Dentistry*, *44*(4), 325–332. https://doi.org/10.5624/isd.2014.44.4.325

Ortiz-Cuaran, S., Bouaoud, J., Karabajakian, A., Fayette, J., & Saintigny, P. (2021). Precision Medicine Approaches to Overcome Resistance to Therapy in Head and Neck Cancers. *Frontiers in Oncology*, *11*, 614332. https://doi.org/10.3389/fonc.2021.614332

Ostuni, M. A., Ducroc, R., Péranzi, G., Tonon, M.-C., Papadopoulos, V., & Lacapere, J.-J. (2007). Translocator protein (18 kDa) ligand PK 11195 induces transient mitochondrial Ca^{2+} release leading to transepithelial Cl- secretion in HT-29 human colon cancer cells. *Biology of the Cell*, *99*(11), 639–647. https://doi.org/10.1042/BC20070048

O'Sullivan, B., Huang, S. H., Su, J., Garden, A. S., Sturgis, E. M., Dahlstrom, K., Lee, N., Riaz, N., Pei, X., Koyfman, S. A., Adelstein, D., Burkey, B. B., Friborg, J., Kristensen, C. A., Gothelf, A. B., Hoebers, F., Kremer, B., Speel, E. J., Bowles, D. W., … Xu, W. (2016). Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): A multicentre cohort study. *The Lancet Oncology*, *17*(4), 440–451. https://doi.org/10.1016/S1470-2045(15)00560-4

Owen, D. R. J., Gunn, R. N., Rabiner, E. A., Bennacef, I., Fujita, M., Kreisl, W. C., Innis, R. B., Pike, V. W., Reynolds, R., Matthews, P. M., & Parker, C. A. (2011). Mixed-affinity binding in humans with 18-kDa translocator protein ligands. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *52*(1), 24–32. https://doi.org/10.2967/jnumed.110.079459

Owen, D. R., Fan, J., Campioli, E., Venugopal, S., Midzak, A., Daly, E., Harlay, A., Issop, L., Libri, V., Kalogiannopoulou, D., Oliver, E., Gallego-Colon, E., Colasanti, A., Huson, L., Rabiner, E. A., Suppiah, P., Essagian, C., Matthews, P. M., & Papadopoulos, V. (2017). TSPO mutations in rats

and a human polymorphism impair the rate of steroid synthesis. *The Biochemical Journal*, *474*(23), 3985–3999. https://doi.org/10.1042/BCJ20170648

Owen, F., Poulter, M., Waddington, J. L., Mashal, R. D., & Crow, T. J. (1983). [^{3}H]R05-4864 and [^{3}H]flunitrazepam binding in kainate-lesioned rat striatum and in temporal cortex of brains from patients with senile dementia of the Alzheimer type. *Brain Research*, *278*(1–2), 373–375. https://doi.org/10.1016/0006-8993(83)90276-7

Pai, S. I., & Westra, W. H. (2009). Molecular pathology of head and neck cancer: Implications for diagnosis, prognosis, and treatment. *Annual Review of Pathology*, *4*, 49–70. https://doi.org/10.1146/annurev.pathol.4.110807.092158

Palzur, E., Edelman, D., Sakas, R., & Soustiel, J. F. (2021). Etifoxine Restores Mitochondrial Oxidative Phosphorylation and Improves Cognitive Recovery Following Traumatic Brain Injury. *International Journal of Molecular Sciences*, *22*(23). https://doi.org/10.3390/ijms222312881

Pan, J.-H., Kang, Y.-Q., Li, Q., Xing, W., Chen, Y.-H., Yan, Y., Luo, D.-X., Qiu, Y., Yuan, Y.-F., Zeng, W.-A., & Chen, D.-T. (2023). TSPO is a novel biomarker for prognosis that regulates cell proliferation through influencing mitochondrial functions in HCC. *Heliyon*, *9*(12), e22590. https://doi.org/10.1016/j.heliyon.2023.e22590

Panagopoulos, N. T., Papadopoulos, G. C., & Matsokis, N. A. (1991). Dopaminergic innervation and binding in the rat cerebellum. *Neuroscience Letters*, *130*(2), 208–212. https://doi.org/10.1016/0304-3940(91)90398-d

Pang, Y., Chen, X., Ji, T., Cheng, M., Wang, R., Zhang, C., Liu, M., Zhang, J., & Zhong, C. (2023). The Chromatin Remodeler ATRX: Role and Mechanism in Biology and Cancer. *Cancers*, *15*(8). https://doi.org/10.3390/cancers15082228

Panier, S., & Boulton, S. J. (2014). Double-strand break repair: 53BP1 comes into focus. *Nature Reviews. Molecular Cell Biology*, *15*(1), 7–18. https://doi.org/10.1038/nrm3719

Papadopoulos, V., Mukhin, A. G., Costa, E., & Krueger, K. E. (1990). The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis. *The Journal of Biological Chemistry*, *265*(7), 3772–3779.

Papadopoulos, V., Guarneri, P., Kreuger, K. E., Guidotti, A., & Costa, E. (1992). Pregnenolone biosynthesis in C6-2B glioma cell mitochondria: Regulation by a mitochondrial diazepam binding inhibitor receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(11), 5113–5117. https://doi.org/10.1073/pnas.89.11.5113

Papadopoulos, V., Boujrad, N., Ikonomovic, M. D., Ferrara, P., & Vidic, B. (1994). Topography of the Leydig cell mitochondrial peripheral-type benzodiazepine receptor. *Molecular and Cellular Endocrinology*, *104*(1), R5-9. https://doi.org/10.1016/0303-7207(94)90061-2

Papadopoulos, V., Amri, H., Li, H., Boujrad, N., Vidic, B., & Garnier, M. (1997a). Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. *The Journal of Biological Chemistry*, *272*(51), 32129–32135. https://doi.org/10.1074/jbc.272.51.32129

Papadopoulos, V., Amri, H., Boujrad, N., Cascio, C., Culty, M., Garnier, M., Hardwick, M., Li, H., Vidic, B., Brown, A. S., Reversa, J. L., Bernassau, J. M., & Drieu, K. (1997b). Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids*, *62*(1), 21–28. https://doi.org/10.1016/S0039-128X(96)00154-7

Papadopoulos, V., Baraldi, M., Guilarte, T. R., Knudsen, T. B., Lacapère, J.-J., Lindemann, P., Norenberg, M. D., Nutt, D., Weizman, A., Zhang, M.-R., & Gavish, M. (2006). Translocator protein (18kDa): New nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends in Pharmacological Sciences*, *27*(8), 402–409. https://doi.org/10.1016/j.tips.2006.06.005

Papesch, M., & Watkins, R. (2001). Epstein-Barr virus infectious mononucleosis. *Clinical Otolaryngology and Allied Sciences*, *26*(1), 3–8. https://doi.org/10.1046/j.1365-2273.2001.00431.x

Parola, A. L., & Laird, H. E. 2nd. (1991). The bovine peripheral-type benzodiazepine receptor: A receptor with low affinity for benzodiazepines. *Life Sciences*, *48*(8), 757–764. https://doi.org/10.1016/0024-3205(91)90090-x

Pastorino, J. G., Simbula, G., Gilfor, E., Hoek, J. B., & Farber, J. L. (1994). Protoporphyrin IX, an endogenous ligand of the peripheral benzodiazepine receptor, potentiates induction of the mitochondrial permeability transition and the killing of cultured hepatocytes by rotenone. *Journal of Biological Chemistry*, *269*(49), 31041–31046. https://doi.org/10.1016/s0021-9258(18)47387-5

Pastorino, J. G., Simbula, G., Yamamoto, K., Glascott, P. A. J., Rothman, R. J., & Farber, J. L. (1996). The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. *The Journal of Biological Chemistry*, *271*(47), 29792–29798. https://doi.org/10.1074/jbc.271.47.29792

Pavese, N., Gerhard, A., Tai, Y. F., Ho, A. K., Turkheimer, F., Barker, R. A., Brooks, D. J., & Piccini, P. (2006). Microglial activation correlates with severity in Huntington disease: A clinical and PET study. *Neurology*, *66*(11), 1638–1643. https://doi.org/10.1212/01.wnl.0000222734.56412.17

Penninckx, S., Pariset, E., Cekanaviciute, E., & Costes, S. V. (2021). Quantification of radiation-induced DNA double strand break repair foci to evaluate and predict biological responses to ionizing radiation. *NAR Cancer*, *3*(4), zcab046. https://doi.org/10.1093/narcan/zcab046

Perrone, M., Moon, B. S., Park, H. S., Laquintana, V., Jung, J. H., Cutrignelli, A., Lopedota, A., Franco, M., Kim, S. E., Lee, B. C., & Denora, N. (2016). A Novel PET Imaging Probe for the Detection and Monitoring of Translocator Protein 18 kDa Expression in Pathological Disorders. *Scientific Reports*, *6*, 20422. https://doi.org/10.1038/srep20422

Pfister, D. G., Spencer, S., Adelstein, D., Adkins, D., Anzai, Y., Brizel, D. M., Bruce, J. Y., Busse, P. M., Caudell, J. J., Cmelak, A. J., Colevas, A. D., Eisele, D. W., Fenton, M., Foote, R. L., Galloway, T., Gillison, M. L., Haddad, R. I., Hicks, W. L., Hitchcock, Y. J., … Darlow, S. D. (2020). Head and Neck Cancers, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network : JNCCN*, *18*(7), 873–898. https://doi.org/10.6004/jnccn.2020.0031

Porceddu, S. V., Scotté, F., Aapro, M., Salmio, S., Castro, A., Launay-Vacher, V., & Licitra, L. (2019). Treating Patients With Locally Advanced Squamous Cell Carcinoma of the Head and Neck Unsuitable to Receive Cisplatin-Based Therapy. *Frontiers in Oncology*, *9*, 1522. https://doi.org/10.3389/fonc.2019.01522

Prusinkiewicz, M. A., Gameiro, S. F., Ghasemi, F., Dodge, M. J., Zeng, P. Y. F., Maekebay, H., Barrett, J. W., Nichols, A. C., & Mymryk, J. S. (2020). Survival-associated metabolic genes in human papillomavirus-positive head and neck cancers. *Cancers*, *12*(1), 1–17. https://doi.org/10.3390/cancers12010253

Qin, T., Mullan, B., Ravindran, R., Messinger, D., Siada, R., Cummings, J. R., Harris, M., Muruganand, A., Pyaram, K., Miklja, Z., Reiber, M., Garcia, T., Tran, D., Danussi, C., Brosnan-Cashman, J., Pratt, D., Zhao, X., Rehemtulla, A., Sartor, M. A., … Koschmann, C. (2022). ATRX loss in glioma results in dysregulation of cell-cycle phase transition and ATM inhibitor radio-sensitization. *Cell Reports*, *38*(2), 110216. https://doi.org/10.1016/j.celrep.2021.110216

Quach, S., Holzgreve, A., Kaiser, L., Unterrainer, M., Dekorsy, F. J., Nelwan, D. V., Bartos, L. M., Kirchleitner, S. V., Weller, J., Weidner, L., Niyazi, M., Ruf, V. C., Herms, J., Stöcklein, S., Wetzel, C., Riemenschneider, M. J., V Baumgarten, L., Thon, N., Brendel, M., … Albert, N. L. (2023). TSPO PET signal using [(18)F]GE180 is associated with survival in recurrent gliomas. *European Journal of Nuclear Medicine and Molecular Imaging*, *50*(3), 859–869. https://doi.org/10.1007/s00259-022-06006-1

Ramakrishnan, R., Liu, H., Donahue, H., Malovannaya, A., Qin, J., & Rice, A. P. (2012). Identification of novel CDK9 and Cyclin T1-associated protein complexes (CCAPs) whose siRNA depletion enhances HIV-1 Tat function. *Retrovirology*, *9*, 90. https://doi.org/10.1186/1742-4690-9-90

Rasey, J. S., Koh, W. J., Evans, M. L., Peterson, L. M., Lewellen, T. K., Graham, M. M., & Krohn, K. A. (1996). Quantifying regional hypoxia in human tumors with positron emission tomography of [^{18}F]fluoromisonidazole: A pretherapy study of 37 patients. *International Journal of Radiation Oncology, Biology, Physics*, *36*(2), 417–428. https://doi.org/10.1016/s0360-3016(96)00325-2

Rechichi, M., Salvetti, A., Chelli, B., Costa, B., Da Pozzo, E., Spinetti, F., Lena, A., Evangelista, M., Rainaldi, G., Martini, C., Gremigni, V., & Rossi, L. (2008). TSPO over-expression increases motility, transmigration and proliferation properties of C6 rat glioma cells. *Biochimica et Biophysica Acta*, *1782*(2), 118–125. https://doi.org/10.1016/j.bbadis.2007.12.001

Rehmani, H. S., & Issaeva, N. (2020). EGFR in head and neck squamous cell carcinoma: Exploring possibilities of novel drug combinations. *Annals of Translational Medicine*, *8*(13), 813. https://doi.org/10.21037/atm.2020.04.07

Reid, P. A., Wilson, P., Li, Y., Marcu, L. G., & Bezak, E. (2017). Current understanding of cancer stem cells: Review of their radiobiology and role in head and neck cancers. *Head & Neck*, *39*(9), 1920–1932. https://doi.org/10.1002/hed.24848

Reis, P. P., Rogatto, S. R., Kowalski, L. P., Nishimoto, I. N., Montovani, J. C., Corpus, G., Squire, J. A., & Kamel-Reid, S. (2002). Quantitative real-time PCR identifies a critical region of deletion on 22q13 related to prognosis in oral cancer. *Oncogene*, *21*(42), 6480–6487. https://doi.org/10.1038/sj.onc.1205864

Richards, A. L., Eckhardt, M., & Krogan, N. J. (2021). Mass spectrometry-based protein-protein interaction networks for the study of human diseases. *Molecular Systems Biology*, *17*(1), e8792. https://doi.org/10.15252/msb.20188792

Ricken, S., Leipziger, J., Greger, R., & Nitschke, R. (1998). Simultaneous measurements of cytosolic and mitochondrial Ca^{2+} transients in HT29 cells. *The Journal of Biological Chemistry*, *273*(52), 34961–34969. https://doi.org/10.1074/jbc.273.52.34961

Rieckmann, T., Tribius, S., Grob, T. J., Meyer, F., Busch, C. J., Petersen, C., Dikomey, E., & Kriegs, M. (2013). HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. *Radiotherapy and Oncology*, *107*(2), 242–246. https://doi.org/10.1016/j.radonc.2013.03.013

Riond, J., Mattei, M. G., Kaghad, M., Dumont, X., Guillemot, J. C., Le Fur, G., Caput, D., & Ferrara, P. (1991). Molecular cloning and chromosomal localization of a human peripheral-type benzodiazepine receptor. *European Journal of Biochemistry*, *195*(2), 305–311. https://doi.org/10.1111/j.1432-1033.1991.tb15707.x

Ritta, M. N., & Calandra, R. S. (1989). Testicular interstitial cells as targets for peripheral benzodiazepines. *Neuroendocrinology*, *49*(3), 262–266. https://doi.org/10.1159/000125126

Rone, M. B., Midzak, A. S., Issop, L., Rammouz, G., Jagannathan, S., Fan, J., Ye, X., Blonder, J., Veenstra, T., & Papadopoulos, V. (2012). Identification of a dynamic mitochondrial protein complex driving cholesterol import, trafficking, and metabolism to steroid hormones. *Molecular Endocrinology (Baltimore, Md.)*, *26*(11), 1868–1882. https://doi.org/10.1210/me.2012-1159

Routila, J., Qiao, X., Weltner, J., Rantala, J. K., Carpén, T., Hagström, J., Mäkitie, A., Leivo, I., Ruuskanen, M., Söderlund, J., Rintala, M., Hietanen, S., Irjala, H., Minn, H., Westermarck, J., & Ventelä, S. (2022). Cisplatin overcomes radiotherapy resistance in OCT4-expressing head and neck squamous cell carcinoma. *Oral Oncology*, *127*, 105772. https://doi.org/10.1016/j.oraloncology.2022.105772

Ru, B., Wong, C. N., Tong, Y., Zhong, J. Y., Zhong, S. S. W., Wu, W. C., Chu, K. C., Wong, C. Y., Lau, C. Y., Chen, I., Chan, N. W., & Zhang, J. (2019). TISIDB: An integrated repository portal for tumor-immune system interactions. *Bioinformatics*, *35*(20), 4200–4202. https://doi.org/10.1093/bioinformatics/btz210

Rupprecht, R., Papadopoulos, V., Rammes, G., Baghai, T. C., Fan, J., Akula, N., Groyer, G., Adams, D., & Schumacher, M. (2010). Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nature Reviews. Drug Discovery*, *9*(12), 971–988. https://doi.org/10.1038/nrd3295

Rusthoven, K. E., Koshy, M., & Paulino, A. C. (2004). The role of fluorodeoxyglucose positron emission tomography in cervical lymph node metastases from an unknown primary tumor. *Cancer*, *101*(11), 2641–2649. https://doi.org/10.1002/cncr.20687

Ryu, J. K., Choi, H. B., & McLarnon, J. G. (2005). Peripheral benzodiazepine receptor ligand PK11195 reduces microglial activation and neuronal death in quinolinic acid-injected rat striatum. *Neurobiology of Disease*, *20*(2), 550–561. https://doi.org/10.1016/j.nbd.2005.04.010

Schägger, H., & von Jagow, G. (1991). Blue native electrophoresis for isolation of membrane protein complexes in enzymatically active form. *Analytical Biochemistry*, *199*(2), 223–231. https://doi.org/10.1016/0003-2697(91)90094-a

Schoemaker, H., Bliss, M., & Yamamura, H. I. (1981). Specific high-affinity saturable binding of [^{3}H] R05-4864 to benzodiazepine binding sites in the rat cerebral cortex. *European Journal of Pharmacology*, *71*(1), 173–175. https://doi.org/10.1016/0014-2999(81)90405-2

Schoemaker, H., Morelli, M., Deshmukh, P., & Yamamura, H. I. (1982). [^{3}H]Ro5-4864 benzodiazepine binding in the kainate lesioned striatum and Huntington's diseased basal ganglia. *Brain Research*, *248*(2), 396–401. https://doi.org/10.1016/0006-8993(82)90602-3

Schoemaker, H., & Nickolson, V. J. (1983). Dopamine uptake by rat striatal synaptosomes: Time- and temperature-dependent decay and protection by dithiothreitol and dopamine. *Journal of Neurochemistry*, *40*(4), 922–928. https://doi.org/10.1111/j.1471-4159.1983.tb08075.x

Schweitzer, P. J., Fallon, B. A., Mann, J. J., & Kumar, J. S. D. (2010). PET tracers for the peripheral benzodiazepine receptor and uses thereof. *Drug Discovery Today*, *15*(21–22), 933–942. https://doi.org/10.1016/j.drudis.2010.08.012

Secretan, B., Straif, K., Baan, R., Grosse, Y., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L., & Cogliano, V. (2009). A review of human carcinogens—Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *The Lancet. Oncology*, *10*(11), 1033–1034. https://doi.org/10.1016/s1470-2045(09)70326-2

Sedaghat, A. R., Zhang, Z., Begum, S., Palermo, R., Best, S., Ulmer, K. M., Levine, M., Zinreich, E., Messing, B. P., Gold, D., Wu, A. A., Niparko, K. J., Kowalski, J., Hirata, R. M., Saunders, J. R., Westra, W. H., & Pai, S. I. (2009). Prognostic significance of human papillomavirus in oropharyngeal squamous cell carcinomas. *The Laryngoscope*, *119*(8), 1542–1549. https://doi.org/10.1002/lary.20533

Semenza, G. L., & Wang, G. L. (1992). A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Molecular and Cellular Biology*, *12*(12), 5447–5454. https://doi.org/10.1128/mcb.12.12.5447-5454.1992

Semenza, G. L. (2001). HIF-1 and mechanisms of hypoxia sensing. *Current Opinion in Cell Biology*, *13*(2), 167–171. https://doi.org/10.1016/s0955-0674(00)00194-0

Shah, A., Malik, A., Garg, A., Mair, M., Nair, S., & Chaturvedi, P. (2017). Oral sex and human papilloma virus-related head and neck squamous cell cancer: A review of the literature. *Postgraduate Medical Journal*, *93*(1105), 704–709. https://doi.org/10.1136/postgradmedj-2016-134603

Shah, F., Hume, S. P., Pike, V. W., Ashworth, S., & McDermott, J. (1994). Synthesis of the enantiomers of [N-methyl-^{11}C]PK 11195 and comparison of their behaviours as radioligands for PK binding sites in rats. *Nuclear Medicine and Biology*, *21*(4), 573–581. https://doi.org/10.1016/0969-8051(94)90022-1

Shen, M., Hu, P., Donskov, F., Wang, G., Liu, Q., & Du, J. (2014). Tumor-associated neutrophils as a new prognostic factor in cancer: A systematic review and meta-analysis. *PloS One*, *9*(6), e98259. https://doi.org/10.1371/journal.pone.0098259

Shimizu, K., Inoue, H., Saitoh, M., Ohtsuki, N., Ishida, H., Makino, K., Amatsu, M., & Nibu, K. (2006). Distribution and impact of lymph node metastases in oropharyngeal cancer. *Acta oto-laryngologica*, *126*(8), 872–877. https://doi.org/10.1080/00016480500504259

Shoukrun, R., Veenman, L., Shandalov, Y., Leschiner, S., Spanier, I., Karry, R., Katz, Y., Weisinger, G., Weizman, A., & Gavish, M. (2008). The 18-kDa translocator protein, formerly known as the peripheral-type benzodiazepine receptor, confers proapoptotic and antineoplastic effects in a human colorectal cancer cell line. *Pharmacogenetics and Genomics*, *18*(11), 977–988. https://doi.org/10.1097/FPC.0b013e3283117d52

Signes, A., & Fernandez-Vizarra, E. (2018). Assembly of mammalian oxidative phosphorylation complexes I-V and supercomplexes. *Essays in Biochemistry*, *62*(3), 255–270. https://doi.org/10.1042/EBC20170098

Šileikytė, J., Blachly-Dyson, E., Sewell, R., Carpi, A., Menabò, R., Di Lisa, F., Ricchelli, F., Bernardi, P., & Forte, M. (2014). Regulation of the mitochondrial permeability transition pore by the outer membrane does not involve the peripheral benzodiazepine receptor (Translocator Protein of 18 kDa (TSPO)). *The Journal of Biological Chemistry*, *289*(20), 13769–13781. https://doi.org/10.1074/jbc.M114.549634

Singh, P., Adhikari, A., Singh, D., Gond, C., & Tiwari, A. K. (2022). The 18-kDa Translocator Protein PET Tracers as a Diagnostic Marker for Neuroinflammation: Development and Current Standing. *ACS Omega*, *7*(17), 14412–14429. https://doi.org/10.1021/acsomega.2c00588

Snyder, S. H., Verma, A., & Trifiletti, R. R. (1987). The peripheral-type benzodiazepine receptor: A protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *1*(4), 282–288. https://doi.org/10.1096/fasebj.1.4.2820823

Sprengel, R., Werner, P., Seeburg, P. H., Mukhin, A. G., Santi, M. R., Grayson, D. R., Guidotti, A., & Krueger, K. E. (1989). Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *The Journal of Biological Chemistry*, *264*(34), 20415–20421.

Squatrito, M., Vanoli, F., Schultz, N., Jasin, M., & Holland, E. C. (2012). 53BP1 is a haploinsufficient tumor suppressor and protects cells from radiation response in glioma. *Cancer Research*, *72*(20), 5250–5260. https://doi.org/10.1158/0008-5472.CAN-12-0045

Squires, R. F., & Brastrup, C. (1977). Benzodiazepine receptors in rat brain. *Nature*, *266*(5604), 732–734. https://doi.org/10.1038/266732a0

Sroussi, H. Y., Epstein, J. B., Bensadoun, R.-J., Saunders, D. P., Lalla, R. V., Migliorati, C. A., Heaivilin, N., & Zumsteg, Z. S. (2017). Common oral complications of head and neck cancer radiation therapy: Mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. *Cancer Medicine*, *6*(12), 2918–2931. https://doi.org/10.1002/cam4.1221

Steuer, C. E., El-Deiry, M., Parks, J. R., Higgins, K. A., & Saba, N. F. (2017). An update on larynx cancer. *CA: A Cancer Journal for Clinicians*, *67*(1), 31–50. https://doi.org/10.3322/caac.21386

Stoebner, P. E., Carayon, P., Casellas, P., Portier, M., Lavabre-Bertrand, T., Cuq, P., Cano, J. P., Meynadier, J., & Meunier, L. (2001). Transient protection by peripheral benzodiazepine receptors during the early events of ultraviolet light-induced apoptosis. *Cell Death and Differentiation*, *8*(7), 747–753. https://doi.org/10.1038/sj.cdd.4400861

Stransky, N., Egloff, A. M., Tward, A. D., Kostic, A. D., Cibulskis, K., Sivachenko, A., Kryukov, G. V., Lawrence, M. S., Sougnez, C., McKenna, A., Shefler, E., Ramos, A. H., Stojanov, P., Carter, S. L., Voet, D., Cortés, M. L., Auclair, D., Berger, M. F., Saksena, G., … Grandis, J. R. (2011). The mutational landscape of head and neck squamous cell carcinoma. *Science (New York, N.Y.)*, *333*(6046), 1157–1160. https://doi.org/10.1126/science.1208130

Strong, M. S., & Jako, G. J. (1972). Laser surgery in the larynx. Early clinical experience with continuous CO 2 laser. *The Annals of Otology, Rhinology, and Laryngology*, *81*(6), 791–798. https://doi.org/10.1177/000348947208100606

Su, Z., Roncaroli, F., Durrenberger, P. F., Coope, D. J., Karabatsou, K., Hinz, R., Thompson, G., Turkheimer, F. E., Janczar, K., Du Plessis, D., Brodbelt, A., Jackson, A., Gerhard, A., & Herholz, K. (2015). The 18-kDa mitochondrial translocator protein in human gliomas: An ^{11}C-(R)PK11195 PET imaging and neuropathology study. *Journal of Nuclear Medicine: Official Publication, Society of Nuclear Medicine*, *56*(4), 512–517. https://doi.org/10.2967/jnumed.114.151621

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(43), 15545–15550. https://doi.org/10.1073/pnas.0506580102

Sun, T., Zou, K., Yuan, Z., Yang, C., Lin, X., & Xiong, B. (2017). Clinicopathological and prognostic significance of circulating tumor cells in patients with head and neck cancer: A meta-analysis. *OncoTargets and Therapy*, *10*, 3907–3916. https://doi.org/10.2147/OTT.S136530

Swartz, J. E., Pothen, A. J., Stegeman, I., Willems, S. M., & Grolman, W. (2015). Clinical implications of hypoxia biomarker expression in head and neck squamous cell carcinoma: A systematic review. *Cancer Medicine*, *4*(7), 1101–1116. https://doi.org/10.1002/cam4.460

Tanimoto, Y., Onishi, Y., Sato, Y., & Kizaki, H. (1999). Benzodiazepine receptor agonists modulate thymocyte apoptosis through reduction of the mitochondrial transmembrane potential. *Japanese Journal of Pharmacology*, *79*(2), 177–183. https://doi.org/10.1254/jjp.79.177

Tantawy, M. N., Charles Manning, H., Peterson, T. E., Colvin, D. C., Gore, J. C., Lu, W., Chen, Z., & Chad Quarles, C. (2018). Translocator Protein PET Imaging in a Preclinical Prostate Cancer Model. *Molecular Imaging and Biology*, *20*(2), 200–204. https://doi.org/10.1007/s11307-017-1113-7

Tawk, B., Debus, J., & Abdollahi, A. (2021). Evolution of a Paradigm Switch in Diagnosis and Treatment of HPV-Driven Head and Neck Cancer-Striking the Balance Between Toxicity and Cure. *Frontiers in Pharmacology*, *12*, 753387. https://doi.org/10.3389/fphar.2021.753387

Tham, T., Bardash, Y., Herman, S. W., & Costantino, P. D. (2018). Neutrophil-to-lymphocyte ratio as a prognostic indicator in head and neck cancer: A systematic review and meta-analysis. *Head & Neck*, *40*(11), 2546–2557. https://doi.org/10.1002/hed.25324

Thomaidou, A. C., Batsaki, P., Adamaki, M., Goulielmaki, M., Baxevanis, C. N., Zoumpourlis, V., & Fortis, S. P. (2022). Promising Biomarkers in Head and Neck Cancer: The Most Clinically Important miRNAs. *International Journal of Molecular Sciences*, *23*(15). https://doi.org/10.3390/ijms23158257

Thomas, J., & Primeaux, T. (2012). Is p16 immunohistochemistry a more cost-effective method for identification of human papilloma virus-associated head and neck squamous cell carcinoma?. *Annals of diagnostic pathology*, *16*(2), 91–99. https://doi.org/10.1016/j.anndiagpath.2011.09.002

Tonon, M. C., Désy, L., Nicolas, P., Vaudry, H., & Pelletier, G. (1990). Immunocytochemical localization of the endogenous benzodiazepine ligand octadecaneuropeptide (ODN) in the rat brain. *Neuropeptides*, *15*(1), 17–24. https://doi.org/10.1016/0143-4179(90)90155-r

Tu, L. N., Zhao, A. H., Hussein, M., Stocco, D. M., & Selvaraj, V. (2016). Translocator Protein (TSPO) Affects Mitochondrial Fatty Acid Oxidation in Steroidogenic Cells. *Endocrinology*, *157*(3), 1110–1121. https://doi.org/10.1210/en.2015-1795

Underwood, E., Redell, J. B., Zhao, J., Moore, A. N., & Dash, P. K. (2020). A method for assessing tissue respiration in anatomically defined brain regions. *Scientific Reports*, *10*(1), 13179. https://doi.org/10.1038/s41598-020-69867-2

van der Schroeff, M. P., & Baatenburg de Jong, R. J. (2009). Staging and prognosis in head and neck cancer. *Oral Oncology*, *45*(4–5), 356–360. https://doi.org/10.1016/j.oraloncology.2008.05.022

Vasaikar, S. V., Straub, P., Wang, J., & Zhang, B. (2018). LinkedOmics: Analyzing multi-omics data within and across 32 cancer types. *Nucleic Acids Research*, *46*(D1), D956–D963. https://doi.org/10.1093/nar/gkx1090

Vasdev, N., Green, D. E., Vines, D. C., McLarty, K., McCormick, P. N., Moran, M. D., Houle, S., Wilson, A. A., & Reilly, R. M. (2013). Positron-emission tomography imaging of the TSPO with [(18)F]FEPPA in a preclinical breast cancer model. *Cancer Biotherapy & Radiopharmaceuticals*, *28*(3), 254–259. https://doi.org/10.1089/cbr.2012.1196

Vaupel, P. (2004). Tumor microenvironmental physiology and its implications for radiation oncology. *Seminars in Radiation Oncology*, *14*(3), 198–206. https://doi.org/10.1016/j.semradonc.2004.04.008

Veenman, L., Levin, E., Weisinger, G., Leschiner, S., Spanier, I., Snyder, S. H., Weizman, A., & Gavish, M. (2004). Peripheral-type benzodiazepine receptor density and in vitro tumorigenicity of glioma cell lines. *Biochemical Pharmacology*, *68*(4), 689–698. https://doi.org/10.1016/j.bcp.2004.05.011

Veenman, L., & Gavish, M. (2006). The peripheral-type benzodiazepine receptor and the cardiovascular system. Implications for drug development. *Pharmacology & Therapeutics*, *110*(3), 503–524. https://doi.org/10.1016/j.pharmthera.2005.09.007

Venturini, I., Alho, H., Podkletnova, I., Corsi, L., Rybnikova, E., Pellicci, R., Baraldi, M., Pelto-Huikko, M., Helén, P., & Zeneroli, M. L. (1999). Increased expression of peripheral benzodiazepine receptors and diazepam binding inhibitor in human tumors sited in the liver. *Life Sciences*, *65*(21), 2223–2231. https://doi.org/10.1016/s0024-3205(99)00487-7

Verma, A., Nye, J. S., & Snyder, S. H. (1987). Porphyrins are endogenous ligands for the mitochondrial (peripheral-type) benzodiazepine receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *84*(8), 2256–2260. https://doi.org/10.1073/pnas.84.8.2256

Vigneswaran, N., & Williams, M. D. (2014). Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral and Maxillofacial Surgery Clinics of North America*, *26*(2), 123–141. https://doi.org/10.1016/j.coms.2014.01.001

Viviano, M., Barresi, E., Siméon, F. G., Costa, B., Taliani, S., Da Settimo, F., Pike, V. W., & Castellano, S. (2022). Essential Principles and Recent Progress in the Development of TSPO PET Ligands for

Neuroinflammation Imaging. *Current Medicinal Chemistry*, *29*(28), 4862–4890. https://doi.org/10.2174/0929867329666220329204054

Vyas, A., Harbison, R. A., Faden, D. L., Kubik, M., Palmer, D., Zhang, Q., Osmanbeyoglu, H. U., Kiselyov, K., Méndez, E., & Duvvuri, U. (2021). Recurrent Human Papillomavirus-Related Head a1. Vyas A, Harbison RA, Faden DL, Kubik M, Palmer D, Zhang Q, et al. Recurrent Human Papillomavirus-Related Head and Neck Cancer Undergoes Metabolic Reprogramming and Is Driven by Oxidative Phosphorylation. Cli. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *27*(22), 6250–6264. https://doi.org/10.1158/1078-0432.CCR-20-4789

Wadsworth, H., Jones, P. A., Chau, W.-F., Durrant, C., Fouladi, N., Passmore, J., O'Shea, D., Wynn, D., Morisson-Iveson, V., Ewan, A., Thaning, M., Mantzilas, D., Gausemel, I., Khan, I., Black, A., Avory, M., & Trigg, W. (2012). [^{18}F]GE-180: A novel fluorine-18 labelled PET tracer for imaging Translocator protein 18 kDa (TSPO). *Bioorganic & Medicinal Chemistry Letters*, *22*(3), 1308–1313. https://doi.org/10.1016/j.bmcl.2011.12.084

Walker, F. M., Sobral, L. M., Danis, E., Sanford, B., Donthula, S., Balakrishnan, I., Wang, D., Pierce, A., Karam, S. D., Kargar, S., Serkova, N. J., Foreman, N. K., Venkataraman, S., Dowell, R., Vibhakar, R., & Dahl, N. A. (2024). Rapid P-TEFb-dependent transcriptional reorganization underpins the glioma adaptive response to radiotherapy. *Nature Communications*, *15*(1), 4616. https://doi.org/10.1038/s41467-024-48214-3

Wang, F., Huynh, P. M., & An, Y. A. (2024). Protocol for Seahorse analysis of ex vivo mouse brown and white adipose tissues. *STAR Protocols*, *5*(2), 103042. https://doi.org/10.1016/j.xpro.2024.103042

Wang, G. L., & Semenza, G. L. (1995). Purification and characterization of hypoxia-inducible factor 1. *The Journal of Biological Chemistry*, *270*(3), 1230–1237. https://doi.org/10.1074/jbc.270.3.1230

Wang, H., Zhai, K., Xue, Y., Yang, J., Yang, Q., Fu, Y., Hu, Y., Liu, F., Wang, W., Cui, L., Chen, H., Zhang, J., & He, W. (2016). Global deletion of TSPO does not affect the viability and gene expression profile. *PLoS ONE*, *11*(12), 8–10. https://doi.org/10.1371/journal.pone.0167307

Wang, H., Wang, B., Wei, J., Meng, L., Zhang, Q., Qu, C., Xin, Y., & Jiang, X. (2020). Molecular mechanisms underlying increased radiosensitivity in human papillomavirus-associated oropharyngeal squamous cell carcinoma. *International Journal of Biological Sciences*, *16*(6), 1035–1043. https://doi.org/10.7150/ijbs.40880

Wang, Z., Valera, J. C., Zhao, X., Chen, Q., & Gutkind, J. S. (2017). mTOR co-targeting strategies for head and neck cancer therapy. *Cancer Metastasis Reviews*, *36*(3), 491–502. https://doi.org/10.1007/s10555-017-9688-7

Warburg, O. (1956). On the origin of cancer cells. *Science (New York, N.Y.)*, *123*(3191), 309–314. https://doi.org/10.1126/science.123.3191.309

Warnakulasuriya, S., Johnson, N. W., & van der Waal, I. (2007). Nomenclature and classification of potentially malignant disorders of the oral mucosa. *Journal of Oral Pathology & Medicine: Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *36*(10), 575–580. https://doi.org/10.1111/j.1600-0714.2007.00582.x

Watkins, G. L., Jewett, D. M., Mulholland, G. K., Kilbourn, M. R., & Toorongian, S. A. (1988). A captive solvent method for rapid N-[^{11}C]methylation of secondary amides: Application to the benzodiazepine, 4'-chlorodiazepam (RO5-4864). *International Journal of Radiation Applications and Instrumentation. Part A, Applied Radiation and Isotopes*, *39*(5), 441–444. https://doi.org/10.1016/0883-2889(88)90077-9

Wedman, J., Pruim, J., Roodenburg, J. L. N., Halmos, G. B., Langedijk, J. A., Dierckx, R. A. J. O., & van der Laan, B. F. A. M. (2013). Alternative PET tracers in head and neck cancer. A review. *European Archives of Oto-Rhino-Laryngology: Official Journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS): Affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery*, *270*(10), 2595–2601. https://doi.org/10.1007/s00405-012-2316-9

Weisinger, G., Kelly-Hershkovitz, E., Veenman, L., Spanier, I., Leschiner, S., & Gavish, M. (2004). Peripheral benzodiazepine receptor antisense knockout increases tumorigenicity of MA-10 Leydig cells in vivo and in vitro. *Biochemistry*, *43*(38), 12315–12321. https://doi.org/10.1021/bi030251v

Werry, E. L., Bright, F. M., Piguet, O., Ittner, L. M., Halliday, G. M., Hodges, J. R., Kiernan, M. C., Loy, C. T., Kril, J. J., & Kassiou, M. (2019). Recent Developments in TSPO PET Imaging as A Biomarker of Neuroinflammation in Neurodegenerative Disorders. *International Journal of Molecular Sciences*, *20*(13). https://doi.org/10.3390/ijms20133161

West, L. A., Horvat, R. D., Roess, D. A., Barisas, B. G., Juengel, J. L., & Niswender, G. D. (2001). Steroidogenic acute regulatory protein and peripheral-type benzodiazepine receptor associate at the mitochondrial membrane. *Endocrinology*, *142*(1), 502–505. https://doi.org/10.1210/endo.142.1.8052

Wilms, H., Claasen, J., Röhl, C., Sievers, J., Deuschl, G., & Lucius, R. (2003). Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: Evidence from activated microglial cells in vitro. *Neurobiology of Disease*, *14*(3), 417–424. https://doi.org/10.1016/j.nbd.2003.07.002

Wongso, H., Kurniawan, A., Setiadi, Y., Kusumaningrum, C. E., Widyasari, E. M., Wibawa, T. H. A., Mahendra, I., Febrian, M. B., Sriyani, M. E., Halimah, I., Daruwati, I., Gunawan, R., Achmad, A., Nugraha, D. H., Lesmana, R., & Nugraha, A. S. (2024). Translocator Protein 18 kDa (TSPO): A Promising Molecular Target for Image-Guided Surgery of Solid Cancers. *Advanced Pharmaceutical Bulletin*, *14*(1), 86–104. https://doi.org/10.34172/apb.2024.015

Woods, M. J., & Williams, D. C. (1996). Multiple forms and locations for the peripheral-type benzodiazepine receptor. *Biochemical Pharmacology*, *52*(12), 1805–1814. https://doi.org/10.1016/s0006-2952(96)00558-8

Wu, X., & Gallo, K. A. (2013). The 18-kDa Translocator Protein (TSPO) Disrupts Mammary Epithelial Morphogenesis and Promotes Breast Cancer Cell Migration. *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0071258

Xie, Y., Wang, H., Wang, S., Feng, Y., Feng, Y., Fan, S., Hu, C., Liu, X., & Hou, T. (2021). Clinicopathological Significance of ATRX Expression in Nasopharyngeal Carcinoma Patients: A Retrospective Study. *Journal of Cancer*, *12*(23), 6931–6936. https://doi.org/10.7150/jca.63333

Yamamoto, V. N., Thylur, D. S., Bauschard, M., Schmale, I., & Sinha, U. K. (2016). Overcoming radioresistance in head and neck squamous cell carcinoma. *Oral Oncology*, *63*, 44–51. https://doi.org/10.1016/j.oraloncology.2016.11.002

Yang, W.-F., Wong, M. C. M., Thomson, P. J., Li, K.-Y., & Su, Y.-X. (2018). The prognostic role of PD-L1 expression for survival in head and neck squamous cell carcinoma: A systematic review and meta-analysis. *Oral Oncology*, *86*, 81–90. https://doi.org/10.1016/j.oraloncology.2018.09.016

Yeliseev, A. A., Krueger, K. E., & Kaplan, S. (1997). A mammalian mitochondrial drug receptor functions as a bacterial "oxygen" sensor. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(10), 5101–5106. https://doi.org/10.1073/pnas.94.10.5101

Yim, E.-K., & Park, J.-S. (2005). The role of HPV E6 and E7 oncoproteins in HPV-associated cervical carcinogenesis. *Cancer Research and Treatment*, *37*(6), 319–324. https://doi.org/10.4143/crt.2005.37.6.319

Zanoni, D. K., Patel, S. G., & Shah, J. P. (2019). Changes in the 8th Edition of the American Joint Committee on Cancer (AJCC) Staging of Head and Neck Cancer: Rationale and Implications. *Current Oncology Reports*, *21*(6), 52. https://doi.org/10.1007/s11912-019-0799-x

Zanotti-Fregonara, P., Pascual, B., Rizzo, G., Yu, M., Pal, N., Beers, D., Carter, R., Appel, S. H., Atassi, N., & Masdeu, J. C. (2018). Head-to-Head Comparison of (11)C-PBR28 and (18)F-GE180 for Quantification of the Translocator Protein in the Human Brain. *Journal of Nuclear Medicine : Official Publication, Society of Nuclear Medicine*, *59*(8), 1260–1266. https://doi.org/10.2967/jnumed.117.203109

Zeineh, N., Denora, N., Laquintana, V., Franco, M., Weizman, A., & Gavish, M. (2020). Efficaciousness of Low Affinity Compared to High Affinity TSPO Ligands in the Inhibition of Hypoxic Mitochondrial Cellular Damage Induced by Cobalt Chloride in Human Lung H1299 Cells. *Biomedicines*, *8*(5). https://doi.org/10.3390/biomedicines8050106

Zeineh, N., Nagler, R. M., Gabay, M., Obeid, F., Kahana, M., Weizman, A., & Gavish, M. (2021). The TSPO Ligands MGV-1 and 2-Cl-MGV-1 Differentially Inhibit the Cigarette Smoke-Induced Cytotoxicity to H1299 Lung Cancer Cells. *Biology*, *10*(5). https://doi.org/10.3390/biology10050395

Zeng, J., Guareschi, R., Damre, M., Cao, R., Kless, A., Neumaier, B., Bauer, A., Giorgetti, A., Carloni, P., & Rossetti, G. (2018). Structural Prediction of the Dimeric Form of the Mammalian Translocator Membrane Protein TSPO: A Key Target for Brain Diagnostics. *International Journal of Molecular Sciences*, *19*(9). https://doi.org/10.3390/ijms19092588

Zeno, S., Zaaroor, M., Leschiner, S., Veenman, L., & Gavish, M. (2009). CoCl(2) induces apoptosis via the 18 kDa translocator protein in U118MG human glioblastoma cells. *Biochemistry*, *48*(21), 4652–4661. https://doi.org/10.1021/bi900064t

Zhang, D., Man, D., Lu, J., Jiang, Y., Ding, B., Su, R., Tong, R., Chen, J., Yang, B., Zheng, S., Chen, D., & Wu, J. (2023). Mitochondrial TSPO Promotes Hepatocellular Carcinoma Progression through Ferroptosis Inhibition and Immune Evasion. *Advanced Science*, *2206669*, 1–13. https://doi.org/10.1002/advs.202206669

Zhang, H., Tan, H., Mao, W.-J., Zhou, J., Fu, Z.-Q., Hu, Y., Xiao, J., Lin, Q.-Y., Shi, H.-C., & Cheng, D.-F. (2019). ^{18}F-PBR06 PET/CT imaging of inflammation and differentiation of lung cancer in mice. *Nuclear Science and Techniques*, *30*(5), 83. https://doi.org/10.1007/s41365-019-0597-z

Zhang, J., Yan, Z., Wang, Y., Wang, Y., Guo, X., Jing, J., Dong, X., Dong, S., Liu, X., Yu, X., & Wu, C. (2021). Cancer-associated 53BP1 mutations induce DNA damage repair defects. *Cancer Letters*, *501*, 43–54. https://doi.org/10.1016/j.canlet.2020.12.033

Zhang, L., Hu, K., Shao, T., Hou, L., Zhang, S., Ye, W., Josephson, L., Meyer, J. H., Zhang, M.-R., Vasdev, N., Wang, J., Xu, H., Wang, L., & Liang, S. H. (2021). Recent developments on PET radiotracers for TSPO and their applications in neuroimaging. *Acta Pharmaceutica Sinica. B*, *11*(2), 373–393. https://doi.org/10.1016/j.apsb.2020.08.006

Zhao, A. H., Tu, L. N., Mukai, C., Sirivelu, M. P., Pillai, V. V., Morohaku, K., Cohen, R., & Selvaraj, V. (2016). Mitochondrial Translocator Protein (TSPO) Function Is Not Essential for Heme Biosynthesis. *The Journal of Biological Chemistry*, *291*(4), 1591–1603. https://doi.org/10.1074/jbc.M115.686360

Zheng, J., Boisgard, R., Siquier-Pernet, K., Decaudin, D., Dollé, F., & Tavitian, B. (2011). Differential expression of the 18 kDa translocator protein (TSPO) by neoplastic and inflammatory cells in mouse tumors of breast cancer. *Molecular Pharmaceutics*, *8*(3), 823–832. https://doi.org/10.1021/mp100433c

Zheng, J., Winkeler, A., Peyronneau, M.-A., Dollé, F., & Boisgard, R. (2016). Evaluation of PET Imaging Performance of the TSPO Radioligand [^{18}F]DPA-714 in Mouse and Rat Models of Cancer and Inflammation. *Molecular Imaging and Biology*, *18*(1), 127–134. https://doi.org/10.1007/s11307-015-0877-x

Zhu, X., Zhang, F., Zhang, W., He, J., Zhao, Y., & Chen, X. (2013). Prognostic role of epidermal growth factor receptor in head and neck cancer: A meta-analysis. *Journal of Surgical Oncology*, *108*(6), 387–397. https://doi.org/10.1002/jso.23406

zur Hausen, H. (1996). Papillomavirus infections—A major cause of human cancers. *Biochimica et Biophysica Acta*, *1288*(2), F55-78. https://doi.org/10.1016/0304-419x(96)00020-0

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2025

ISBN 978-952-02-0058-9 (PRINT)
ISBN 978-952-02-0059-6 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)