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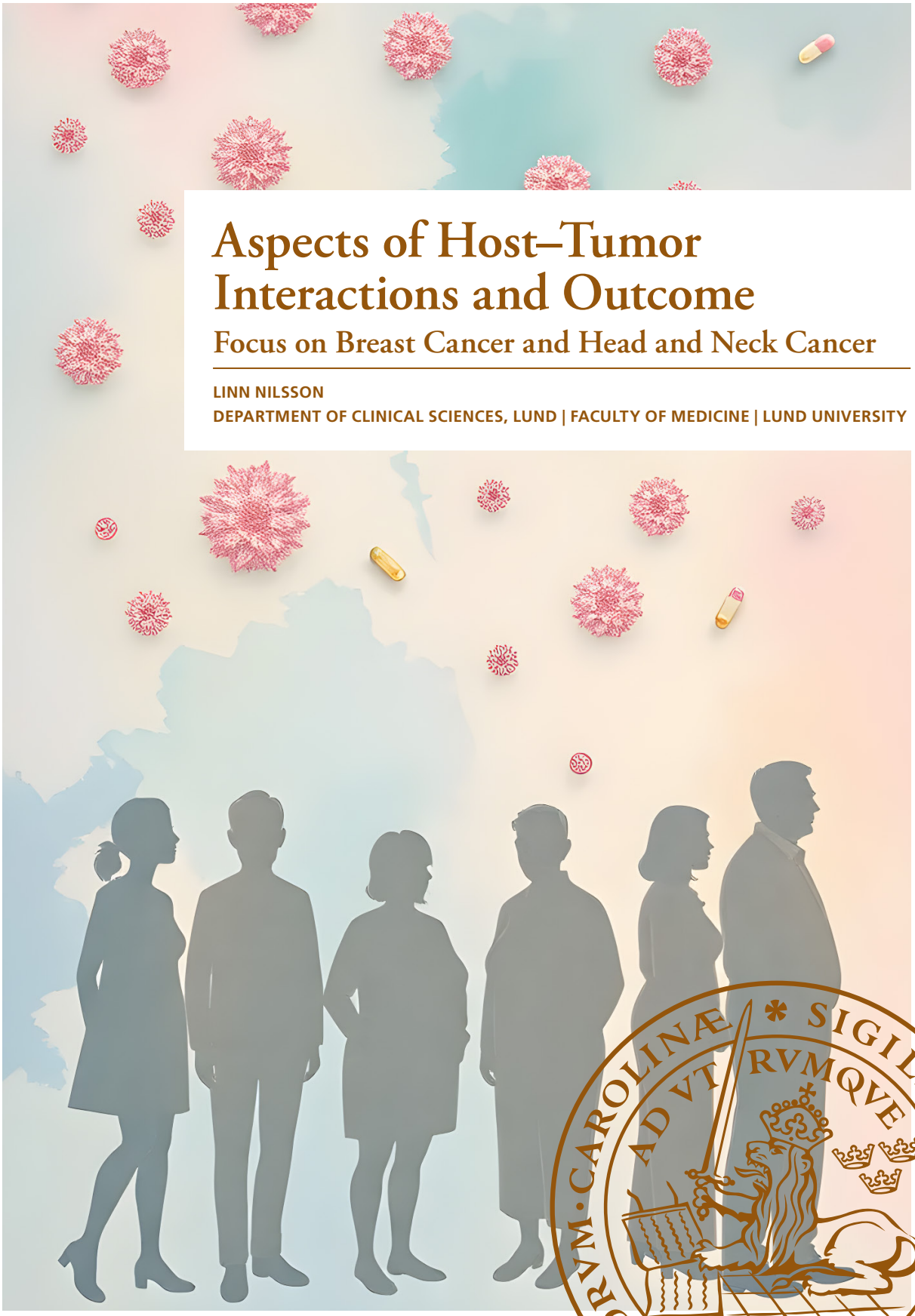

Aspects of Host–Tumor Interactions and Outcome

Focus on Breast Cancer and Head and Neck Cancer

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Linn Nilsson

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on the 24th of October 2025 at 13.00 in Torsten Landberg Hall, the Radiotherapy building, Klinikgatan 5, Lund

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Abstract: Many factors influence clinical outcomes in an oncological setting, and in order to improve personalized treatment, more knowledge of host–tumor interactions and prognostic factors is needed. This thesis explores the associations between patient and tumor characteristics in relation to clinical outcome from three different perspectives. **Study I:** The signal transducer and activator of transcription 3 (STAT3) is involved in inflammation and is a key regulator in involution following breastfeeding. Activated STAT3 (pSTAT3^{Y705}) was evaluated in early breast cancer using tumor tissue microarrays (TMAs) from 867 patients. The results showed that higher levels of pSTAT3^{Y705} were associated with younger age, smaller body constitution, any alcohol consumption, smaller tumors, lower histological grade, lymph-node negativity, progesterone receptor positivity, and human epidermal growth factor 2 (HER2) negativity. High pSTAT3^{Y705} was associated with a lower incidence of early breast cancer events and distant metastasis at 5 years but was not an independent prognostic factor. **Study II:** Antioxidants may interact with oncological treatments by scavenging free radicals that would normally target the tumor. The aryl hydrocarbon receptor (AHR) is an environmental sensor that is involved in antioxidant regulation and estrogen metabolism. In patients with early breast cancer, pre- and postoperative self-reported use of antioxidants (vitamins A, C, E, carotenoids, and Q10) and multivitamins was evaluated (Lund n = 1855, Helsingborg n = 478). Two breast cancer cell lines were used to explore the activation of AHR by vitamin C using western blot. Antioxidant users (about 10%) were more often prior users of menopausal hormone therapy. TMA for AHR (available for 915 patients in Lund) showed that nuclear positivity was twice as common among antioxidant users compared to non-users. *In vitro* studies indicated AHR activation after exposure of vitamin C by increasing levels of downstream cytochrome P450 1B1 (CYP1B1). Associations with antioxidant use and clinical outcome differed according to the type of supplement used, timing of use, and activation of AHR. **Study III**: Low muscle mass is an indicator of sarcopenia and worse clinical outcome for patients with head and neck squamous cell carcinoma (HNSCC). It is less well known whether these associations also exist for patients with fewer comorbidities. This study used data from patients treated within the ARTSCAN III trial, which randomized patients with HNSCC to curative treatment with either cisplatin or cetuximab in combination with radiation therapy. For these patients (n = 290), a skeletal muscle index (SMI) was based on skeletal muscles at the level of the third cervical vertebra from treatment planning computed tomography scans. The results showed that a low SMI was associated with older age, lower body mass index, current smoking, tumors more often localized in the hypopharynx than the oropharynx, and worse overall survival and progression-free survival in univariable models. In multivariable models, the associations with outcome remained only for men. **Conclusion:** Lifestyle factors can influence tumor characteristics and treatment outcome in different ways, which makes it important to consider them in oncological research and treatment.

Key words: breast cancer, signal transducer and activator of transcription 3, antioxidants, aryl hydrocarbon receptor, head and neck squamous cell carcinoma, sarcopenia, muscle mass

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MADE IN SWEDEN

To my grandmother Stina (1916–2009),
whose dream of becoming a nurse was unjustly denied out of the fear
that education would render her arrogant

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Thesis at a glance

	Study I	Study II	Study III
Research questions	Is pSTAT3^{Y705} associated with patient and tumor characteristics? Are there associations between pSTAT3^{Y705} and clinical outcome in breast cancer?	Is antioxidant use associated with patient and tumor characteristics and clinical outcome? Can vitamin C activate AHR in breast cancer cells?	Is low skeletal muscle index (SMI_{low}) associated with patient and tumor characteristics, treatment adherence, and clinical outcome in HNSCC?
Methods	BC-Blood cohort; Immunohistochemical evaluation of pSTAT3^{Y705} on TMA in 867 patients. Outcome was evaluated in relation to BCFI, DMFI, and OS.	BC-Blood cohort; Self-reported antioxidant use was evaluated in relation to BCFI. Western blot analysis of activation of AHR and CYP1B1 in MCF-7 and MDA-MB-231 cells following vitamin C exposure.	ARTSCAN III cohort; skeletal muscle mass delineation on treatment-planning CT scans at the level of C3. SMI_{low} was evaluated as continuous and dichotomized variable.
Results	High pSTAT3^{Y705} levels were associated with younger age, smaller body constitution, smaller tumors, lower histological grade, and HER2^{-}. pSTAT3^{Y705} was not an independent prognostic factor for improved outcome. A subgroup analysis of ER^{+} chemonaïve patients indicated interaction between pSTAT3^{Y705} negativity and tamoxifen treatment for BCFI and DMFI, where patients treated with tamoxifen had better prognosis than those receiving aromatase inhibitors or no endocrine treatment.	Antioxidant use was more common among women using MHT. Among patients with AHR information, activation was twice as common among antioxidant users. Multivitamin use was not associated with clinical outcome. Clinical outcome for antioxidant use differed according to timing of use and AHR activation. Cell lines exposed to vitamin C showed AHR activation by increased CYP1B1 levels.	SMI_{low} was associated with older age, lower BMI, tumors in hypopharynx and less often in oropharynx, and current smoking. Patients with SMI_{low} had worse OS and PFS in univariable models. In multivariable models, associations with prognosis remained only for men. There was an interaction between SMI_{low} and randomized treatment for PFS, suggesting that SMI_{low} primarily influenced outcome for patients treated with cetuximab.
Conclusion	High levels of pSTAT3^{Y705} were associated with favorable prognostic characteristics, but pSTAT3^{Y705} was not an independent prognostic marker.	Antioxidants can have other roles besides scavenging free radicals in breast cancer and may influence outcome.	SMI_{low} influenced treatment outcome in patients with HNSCC treated in a randomized trial.

Abbreviations: pSTAT3^{Y705}: phosphorylated signal transducer and activator of transcript 3 at residue tyrosine 705, TMA: tissue microarray, BCFI: breast cancer-free interval, DMFI: distant metastasis-free interval, OS: overall survival, HER2^{-}: human epidermal growth factor receptor 2 negativity, ER^{+}: estrogen receptor positive, BCFI: breast cancer-free interval, AHR: aryl hydrocarbon receptor, CYP1B1: cytochrome P-450 1B1 enzyme, MHT: menopausal hormone therapy, SMI_{low}: low skeletal muscle mass index, BC-Blood: Breast cancer and blood, C3: cervical vertebra 3, PFS: progression-free survival

List of Papers

Paper I

Nilsson L, Khazaei S, Tryggvadottir H, Nodin B, Jirström K, Borgquist S, Isaksson K, Jernström H. Patient Characteristics Influence Activated Signal Transducer and Activator of Transcription 3 (STAT3) Levels in Primary Breast Cancer—Impact on Prognosis. Frontiers in Oncology 2020; 10: 1278 eCollection 2020

Paper II

Nilsson L, Khazaei S, Tryggvadottir H, Björner S, Bressan A, Jirström K, Adrian G, Falck A-K, Borgquist S, Isaksson K, Jernström H. Pre- and Postoperative Antioxidant Use, Aryl Hydrocarbon Receptor (AhR) Activation and Clinical Outcome in Different Treatment Groups of Breast Cancer Patients. Clinical Breast Cancer 2024; 24: e152-e166

Paper III

Nilsson L, Engström P, Adrian G, Gebre-Medhin M. Low skeletal muscle mass is associated with outcome in head and neck squamous cell carcinoma patients treated within a prospective chemoradiotherapy trial. *Manuscript*

Author's contribution to the papers

Paper I

Linn Nilsson is the sole first author of the study. I evaluated tumor-specific pSTAT3^{Y705} in TMAs of breast cancer. I contributed to the study design, statistical analysis, data analysis, interpretation, and manuscript writing. I contributed to obtaining funding for the project.

Paper II

Linn Nilsson is the sole first author of the study. I collected data on antioxidant constituents in reported supplements from patients with breast cancer in the BC-Blood study. I contributed to the study design, statistical analysis, data analysis, interpretation, and manuscript writing. I contributed to obtaining funding for the project.

Paper III

Linn Nilsson is the sole first author of the study. I collected data by delineating skeletal muscle mass on computed tomography (CT) scans of patients treated in the ARTSCAN III trial. I contributed to the study design, statistical analysis, data analysis, interpretation, and manuscript writing. I contributed to obtaining funding for the project.

Populärvetenskaplig sammanfattning

Cancersjukdomar har blivit vanligare, både i Sverige och i övriga delar av världen. En av anledningarna till ökningen är att vi lever allt längre och därmed utsätts för risk under en längre tid. En annan orsak är så kallade livsstilsfaktorer, exempelvis rökning, alkoholkonsumtion och kost, som kan både öka och minska risken för en cancersjukdom. För vissa cancersjukdomar påverkar livsstilsfaktorer egenskaper hos tumören, vilka i sin tur kan påverka möjligheten att bli botad. Livsstilsfaktorer kan även påverka behandlingens resultat och patientens möjlighet att genomgå en cancerbehandling. Trots stora framgångar inom cancervården finns ett stort behov av att bättre kunna anpassa cancerbehandlingen utifrån tumörens och patientens egenskaper, både för att undvika underbehandling, men även överbehandling. Studierna som ingår i den här avhandlingen undersöker samband mellan patienten, livsstilsfaktorer, egenskaper hos tumören och behandlingsresultat.

De två första studierna i avhandlingen handlar om bröstcancer, som diagnostiseras hos nära 9000 kvinnor i Sverige per år. Amning minskar risken att drabbas av bröstcancer, men omorganiseringen av vävnaden i bröstet efter amning har setts som en kritisk tidpunkt för utveckling av bröstcancer. En viktig aktör i den här processen är proteinet STAT3 som bland annat styr celldöd, sårläkning och inflammationer i kroppen. Det är däremot oklart vilken roll STAT3 har i bröstcancer och cellstudier har exempelvis visat att tumörer kan bli mindre känsliga för behandling om STAT3 är aktiverat/aktiveras. Behandlingar som ges vid cancer kan också öka nivån av inflammation i kroppen och på så sätt påverka bröstcancern som utnyttjar inflammationen för att överleva. I den första studien kunde vi se att höga nivåer av aktivt STAT3 i bröstcancern var vanligare i bröstcancrar som var mindre och som inte lika ofta spred sig till andra delar av kroppen. Höga nivåer av aktivt STAT3 var vanligare hos kvinnor som var yngre, som rapporterade någon form av intag av alkohol och som inte var överviktiga. Det fanns ett samband mellan höga nivåer av aktivt STAT3 och färre återfall av bröstcancern de första fem åren, men det sambandet fanns inte kvar när hänsyn togs till andra kända faktorer som också påverkar risken för att sjukdomen ska komma tillbaka. I en analys där enbart patienter som inte behandlades med cytostatika ingick skiljde sig behandlingsresultatet åt för de patienter som saknade aktivt STAT3 i sin bröstcancer och som påverkades av vilket hormonsänkande läkemedel som patienten fått. Det här sambandet har inte rapporterats tidigare och behöver bekräftas i andra studier.

Användning av kosttillskott och antioxidanter är vanligt hos allmänheten och bland patienter med bröstcancer. Antioxidanter är ämnen som kan påverka cancerns egenskaper men även cancerbehandlingen. Proteinet AHR är en receptor som aktiveras av exempelvis rökning, men som också styr reglering av antioxidanter och östrogen. I studie II undersökte vi självrapporterad användning av multivitaminer och kosttillskott som innehöll någon av antioxidanterna vitamin A, C, E, Q10, och betakaroten. Överlag var det färre som använde antioxidanter (ca 10 %) jämfört med

andra studier vilket gjorde att grupperna som jämfördes blev små och därmed ger osäkrare resultat. Användning av antioxidanter var vanligare bland kvinnor som tidigare använt hormonbehandling för klimakteriebesvär. Dessutom sågs ett samband mellan att bröstcancern var mindre och användning av antioxidanter. Det var också dubbelt så vanligt att patienter som rapporterade att de använde antioxidanter hade högre nivåer av AHR i sin bröstcancer. I laboratorieförsök med två olika cellinjer från bröstcancer kunde vi visa att vitamin C aktiverar ett ämne som bryter ner hormonet östrogen. Det fanns olika samband mellan användning av antioxidanter och cancerbehandlingens resultat. De här sambanden påverkades både av vilken typ av kosttillskott med antioxidanter som patienterna använt, men även när de använts och ifall AHR var aktiv eller inte. Kommande studier behöver undersöka olika antioxidanters samspel med AHR och hur aktiveringen påverkas av till exempel rökning.

Den tredje studien fokuserade på huvud- och halscancer, en cancerdiagnos som drabbar cirka 1700 personer varje år i Sverige. För många patienter med huvud- och halscancer är strålbehandling tillsammans med cytostatika den bästa behandlingen för att patienten ska bli botad. Behandlingen är mycket påfrestande, därför behövs information som kan hjälpa till att avgöra vilka patienter som tål och har bäst nytta av behandlingen. Olika vävnader i kroppen åldras olika snabbt och redan i tidig vuxen ålder börjar muskler få sämre funktion och storlek. Andra orsaker som exempelvis cancersjukdom eller andra sjukdomar, lågt intag av näring ifrån kosten och låg grad av fysisk aktivitet påverkar också musklerna. Tidigare studier har visat att patienter med cancer som samtidigt har en låg nivå av muskelmassa får sämre resultat av sin behandling och därför större risk att få tillbaka sin sjukdom eller dö av sjukdomen. Få studier har undersökt om det här sambandet även gäller patienter som får behandling i forskningsstudier där patienter som ska få botande behandling deltar. Dessa patienter har ofta färre andra sjukdomar och är oftare yngre än patienter som inte deltar i forskningsstudier. I studie III användes information från ARTSCAN III-studien, där patienterna behandlades med strålbehandling i kombination med två olika läkemedel - antingen cisplatin eller cetuximab. Patienternas muskelmassa bedömdes på förberedande röntgenbilder, som tagits inför patienternas strålbehandling. Resultaten i studien visade att patienter med lägre nivå av muskelmassa var äldre, hade lägre BMI (body mass index), var oftare rökare, och deras cancer satt oftare i den nedre delen av svalget och mindre ofta i mellansvalget. Patienter med låg nivå av muskelmassa överlevde en kortare tid och fick oftare tillbaka sin cancersjukdom. Men, när hänsyn togs till andra kända faktorer som också påverkar samband med resultatet efter en cancerbehandling fanns sambandet med muskelmassa bara kvar hos männen. Resultaten antydde även att det framför allt var patienter som blivit behandlade med cetuximab och som samtidigt hade en låg muskelmassa som hade sämst effekt av behandlingen. Det här sambandet har inte visats tidigare och behöver undersökas i fler studier.

Abbreviations

ADCC	antibody-dependent cell mediated cytotoxicity
AHR	aryl hydrocarbon receptor
AHR^{cyt}	cytoplasmic AHR
AHR^{nuc}	nuclear AHR
AHRR	aryl hydrocarbon receptor repressor
AI	aromatase inhibitor
ALDH1	aldehyde dehydrogenase 1 family, member A1
ALND	axillary lymph node dissection
AREG	amphiregulin
ARNT	aryl hydrocarbon receptor nuclear translocator
ATM	ataxia telangiectasia mutated
AUDIT	Alcohol Use Disorders Identification Test
AUDIT-C	Short Version of AUDIT
BC-Blood	Breast cancer and blood
BCFI	breast cancer-free interval
BCS	breast-conserving surgery
BMI	body mass index
BRCA1	breast cancer gene 1
BRCA2	breast cancer gene 2
BSA	bovine serum albumin
C3	cervical vertebra 3
$CD8^+$ T-cells	T-cells expressing the CD8 receptor
CD	cluster of differentiation
$CD56^{dim}$	CD56 diminished
CHECK2	checkpoint kinase 2
Chemo-RT	chemo- and radiation therapy combined
CSA	cross-sectional area
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	cytotoxic T-lymphocyte antigen 4
CYP	cytochrome P-450
DAG	directed acyclic graphs

DATECAN	Definitions for the Assessment of Time-to-Event Endpoints in CANcer trials
DFS	disease-free survival
DMFI	distant metastasis-free interval
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
E1	estrone
E2	estradiol-17β
EBCTCG	Early Breast Cancer Trialists' Group
ECOG-PS	Eastern Cooperative Oncology Group performance status
EGFR	epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer
ER	estrogen receptor
ERα	estrogen receptor subtype α
ERβ	estrogen receptor subtype β
ErbB	erythroblastic leukemia viral oncogene
FBS	fetal bovine serum
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GI	gastrointestinal
GRADE	Grading of Recommendations Assessment, Development and Evaluation
Gy	Gray
HER2	human epidermal growth factor receptor 2
HIF-α	hypoxia inducible factor subunit alpha
HNC	head and neck cancer
HNCUP	head and neck cancer of unknown primary
HNSCC	head and neck squamous cell carcinoma
HPV	human papillomavirus
HR	hazard ratio
H-score	Histo-score
HSP-90	heat-shock protein 90
HU	Hounsfield unit
IARC	International Agency for Research on Cancer
IGF-1	insulin growth factor 1

IHC	immunohistochemistry
L3	lumbar vertebra 3
IL-6	interleukin 6
IL-15	interleukin 15
IMRT	intensity modulated radiation therapy
INF-γ	interferon gamma
JAK	Janus kinase
KM	Kaplan-Meier
Ki67	Kiel 67
MHT	menopausal hormonal therapy
MRI	magnetic resonance imaging
NF-κβ	nuclear factor kappa-light-chain enhancer of B cells
NFR-2	nuclear factor erythroid 2p45-related factor
NHG	Nottingham histological grade
NK-cells	natural killer cells
Non-MV antioxidant	non-multivitamin antioxidant
OTT	overall treatment time
PAH	polycyclic aromatic hydrocarbons
PALB	partner and localizer of *BRCA2*
PAM50	Prosigna breast cancer prognostic gene signature assay
PARP	poly ADP ribose polymerase
PBS	phosphate buffered saline
PD-L1	programmed cell death ligand 1
PET-CT	positron emission tomography – CT
PEtH	phosphatidylethanols
PI3K	phosphoinositide-3-kinase
PIAS	protein inhibitor of activated STAT
PR	progesterone receptor
pSTAT3	phosphorylated STAT3
$pSTAT3^{Y705}$	STAT3 phosphorylated at residue tyrosine 705
$pSTAT3^{S727}$	STAT3 phosphorylated at residue serine 727
P-value	probability value
pRb	retinoblastoma protein
REMARK	reporting recommendations for tumor marker prognostic studies

RIPA	radioimmunoprecipitation assay
ROR	Risk of recurrence
ROS	reactive oxygen species
RPM	revolutions per minute
RPMI	Roswell Park Memorial Institute
RR	relative risk
RT	radiation therapy
RTC	randomized clinical trial
RTOG	Radiation Therapy Oncology Group
SER727	serine 727
SERM	selective estrogen receptor modulator
SH2	Src homology 2
SMI	skeletal muscle index
SMI_{low}	low skeletal muscle index
SOCS	suppressor of cytokine signaling
STAT3	signal transducer and activator of transcript 3
STAT3α	STAT3 alpha isoform
STATβ	STAT3 beta isoform
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TCDD	2,3,7,8 – tetrachlorodibenzo-p-dioxin
T-cells	T lymphocytes
TCGA	The Cancer Genome Atlas
TDLU	terminal ductal lobular unit
TMA	tissue microarray
TNBC	triple negative breast cancer
TNF-α	tumor necrosis factor alpha
TNM	Tumor, Node, Metastasis classification
TYR705	tyrosine 705
UICC	Union for International Cancer Control
VIF	variance of inflation
VMAT	volumetric modulated arc therapy
WHR	waist-to-hip ratio
XAP2	X-associated protein 2
XREs	xenobiotic responsive elements

Introduction

The goals of the 2030 Agenda for Sustainable Development formulated by the World Health Organization are aimed at ensuring healthy lives and promoting wellbeing for people of all ages (1). In Europe, the incidence and mortality burden of cancer is disproportionately high in relation to the population size (2,3). Established treatments have remarkably decreased cancer recurrences and mortality rates for many patient groups, but others still have poor prognoses (2).

Tumor formation is an evolutionary process with a natural selection of oncogenic variants that can ultimately lead to cancer. The process involves a complex interplay between tumor cells, surrounding cells and tissues, and the immune system (4). Many lifestyle factors influence cancer risk (5) and tumor characteristics (6-8). Lifestyle factors can also influence treatment tolerability (9,10) and treatment outcome (11). Therefore, it is important to incorporate lifestyle factors in oncological research.

This thesis is based on two distinct cancer diagnoses: breast cancer in women and head and neck squamous cell carcinoma (HNSCC) in both men and women. Study I evaluated tumor-specific levels of activation of signal transducer and activator of transcript 3 (STAT3) in breast cancer in relation to lifestyle, other tumor characteristics, and prognosis in different treatment groups. Activation of STAT3 is a key regulator in the apoptosis of breast tissue following cessation of breastfeeding (12) and inflammation (13). In different cancers, activation of STAT3 has primarily been associated with treatment resistance (13,14) and poor prognosis (15).

The aim of study II was to contribute more knowledge to the patient-derived question of whether antioxidant supplements can be used during treatment. The study explores the associations and possible influence of antioxidants on the aryl hydrocarbon receptor (AHR). AHR is a transcription factor that is highly sensitive to chemicals and environmental changes and is important for estrogen metabolism.

Study III evaluates whether pre-treatment imaging that is otherwise used for treatment planning for radiation therapy (RT) could be informative in regard to treatment adherence and outcome. This was accomplished by the use of skeletal muscle mass as an indicator for sarcopenia (16). Sarcopenia increases with increasing age and can also arise due to poor nutrition or a sedentary lifestyle, and studies have indicated that it has a negative influence on prognosis in different types of cancers (17).

Background

The breast and breast cancer

Physiology of the breast

The breast is a highly dynamic organ, which makes it sensitive to carcinogenic exposure throughout life (18). The initial ductal tree of the breast is established during the fetal period (19), while further development is controlled by ovarian hormones (20). Thelarche, the initial appearance of breast development (20), is influenced by ethnicity and environmental factors and currently starts around the age of 10 years (21).

During the peripubertal period, there is an extensive increase in both glandular and stromal tissue in the breast. The increase of glandular tissue is due to growth and division of small bundles of primary and secondary ducts, which eventually end in a terminal ductal lobular unit (TDLU) (20,22). Estrogen and progesterone are essential for normal duct development, and the presence of these hormones is related to the proliferation rate in breast tissue (20,23). In addition, growth hormones, insulin growth factor-1 (IGF-1), adipocytes, fibroblasts, and resident macrophages are involved in the morphogenesis of the mammary glands (19,22).

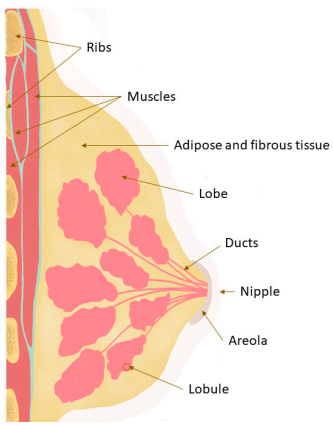

Figure 1. Schematic image of the breast. The breast consists of 15–20 lobes and connective ducts surrounded by adipose and fibrous connective tissue.

The mammary ducts are composed of an inner layer with luminal cells and an outer layer of basal cells that are able to differentiate into myoepithelial cells, which enable contraction and thus the transportation of milk during breastfeeding (19). In the normal mammary epithelium, the proliferating cells are predominantly found in the epithelium lining ducts and lobules (20). The TDLU is highly vascularized to ensure supply of nutrients, hormones, and the presence of immune cells (19). It is believed that the majority of breast tumors arise from the TDLU (20).

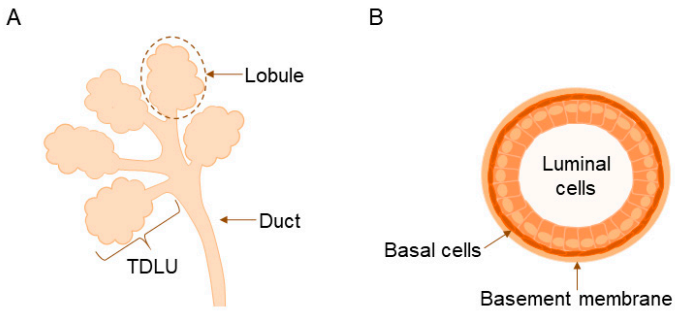

Figure 2. A Part of the glandular tissue of the breast showing lobules, the duct, and the terminal ductal lobular unit (TDLU) that comprise a lobule and an end duct. The TDLU is the most common location for breast cancers to arise. **B**. Cells in breast lobules and ducts. An inner layer of luminal cells and outer layer of basal cells are surrounded by the basement membrane.

Four different types of TDLU have been defined in the breast according to the branching structure (20), and an increase in the amount of acini per lobule distinguishes lobule types 1, 2, and 3 (24-26). Differentiation from lobule type 1 to 2 and 3 occurs under hormonal influences during the menstrual cycle in preparation for a potential pregnancy (19,20,22), although some studies report low intraindividual variations (24). The interindividual variation of lobular type composition is high, particularly in nulliparous women (22,24). Type 1 lobules have typically been described more commonly in young females (20) compared to mature women, in which type 2 is more common (20,22). Type 1 lobules are less differentiated, they have the highest proliferation rate, and they are also believed to be more susceptible to carcinogenic agents (20).

Type 3 lobules are the dominant type in parous women and peak in the early reproductive years (20). Interestingly, this type has also been reported to be present in nulliparous women, although to a lesser extent (24,26). In a recent study using volumetric imaging, parity in association with higher age was not linked to lobular type (22). Type 4 lobules are the predominant type at the end of pregnancy and during lactation (20,24,26). This type has a secretory phenotype (24,26) and is regarded as fully differentiated (24). Although long-lasting lobular type 3 in the TDLU has been reported in women who have given birth at a young age (20),

similarities with the lobular type composition of parous women and nulliparous women have been reported as early as three months (24) to 18 months post-partum (24).

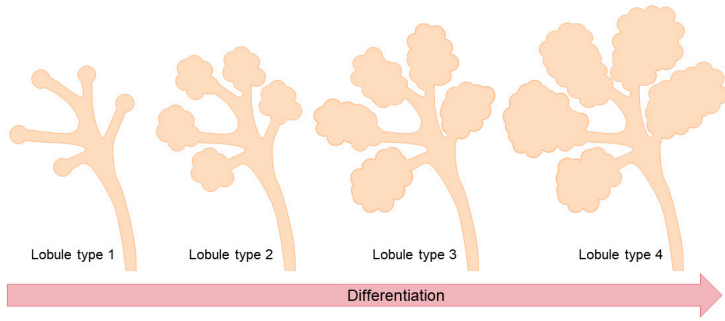

Figure 3. Different types of terminal duct lobular units in the female breast that change with age, menstrual cycle, pregnancy, and lactation. Type 1 is the least differentiated, and type 4 is considered fully differentiated.

Involution

Upon termination of breastfeeding, the glandular and stromal tissue in the breast is reorganized in a controlled process called involution (27), which is initiated by decreased levels of prolactin (19) and milk stasis in the ducts (28). The transcription factor STAT3 is a key regulator of involution, which consists of an initial phase of programmed cell death of the secretory epithelium (12,24,27) and a second phase with active wound healing, which is regulated by STAT3 (28). The second phase involves immune-cell infiltration (24,26,29) of antigen-presenting dendritic cells, followed by macrophage and lymphocyte infiltration (29), tissue remodeling of matrix metalloproteinases, and adipogenesis (19). The involution process is believed to influence breast-tumor development and histological changes, and sometimes, prolonged activity of phosphorylated STAT3 at residue tyrosine 705 ($pSTAT3^{Y705}$) has been reported *in vivo* (27).

A second type of involution is age-related involution, which is characterized by regression of type 2 and 3 lobules to type 1 with different characteristics than in nulliparous women (25,30), along with replacement of the intralobular stroma with more dense collagen (30). Over time, the fibroglandular tissue is replaced with adipose tissue (19,31). Hence, the breast of postmenopausal women generally has a higher degree of adipose tissue compared to premenopausal women (32).

The progression of age-related involution has been associated with lower levels of $pSTAT3^{Y705}$ and higher levels of plasminogen, whereas no association with matrix metalloproteinase 9 (important for collagen degradation) has been observed (33) in comparison to post-lactation involution. Age-related involution is more common in postmenopausal women (25,30,31) but can be partially present in women younger

than 40 years and still ongoing in women older than 70 years (30). Incomplete age-related involution has been reported to be more common in women with a family history of breast cancer and nulliparity (30,31), while associations with exogenous hormone use are inconsistent (23,25,30,31,33). Breast tissues with high levels of pro-inflammatory markers (besides $pSTAT3^{Y705}$) (25) and environmental pollutants (34) have been suggested to delay age-related involution. In women with benign breast disease, age-related involution has been associated with a reduced risk for breast cancer (30).

Breast cancer epidemiology

With 2.3 million new cases reported globally in 2022, breast cancer is ranked as the world's second most commonly diagnosed cancer (2). The age-standardized incidence is far higher in countries with a high or very high human developmental index compared to countries with low or medium levels (incidence rate: 54.1 vs. 30.8 per 100,000 women). The highest incidence rates are observed in France, Australia, New Zealand, North America, and Northern Europe, while Middle Africa and South Central Asia have the lowest reported rates (2). In Sweden, breast cancer is the most commonly diagnosed cancer among females, with more than 8700 new patients diagnosed in 2021 (3).

The median age at breast cancer diagnosis has been rising (35) and is 66 years in Sweden. One in five female patients with breast cancer is diagnosed before 50 years of age (3). An increase in breast cancer among women younger than 50 years has been observed in various countries, such as Canada (36) and the USA (37). The global mortality burden of breast cancer is 6.9%, and breast cancer is the fourth most common cause of death from cancer. However, the mortality burden varies between global regions (2) and probably reflects the access to health care, as well as economic and social differences (38). In Europe, many countries report improved survival rates for breast cancer (38), and in Sweden, the 10-year overall survival (OS) is almost 90% (3).

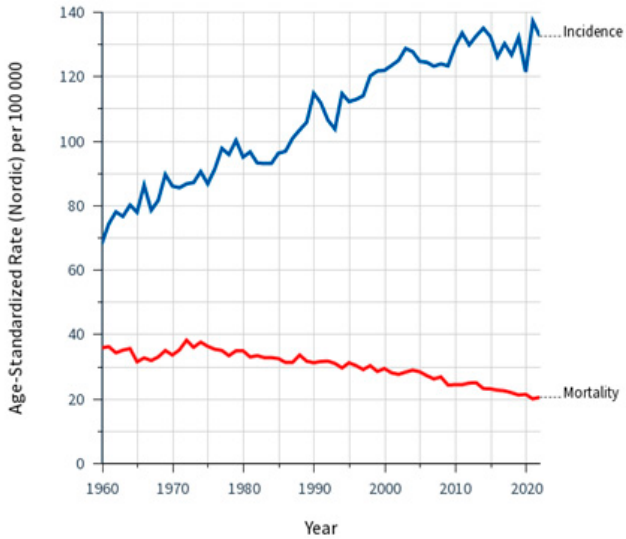

Figure 4. Age-standardized incidence and mortality rate of breast cancer in Sweden between 1960 and 2022. © NORDCAN database, provided by the International Agency for Research and Cancer.

Breast carcinogenesis

Gene-expression studies have given insight into the heterogeneity of breast cancer (39-41), suggesting that multiple factors influence tumor initiation and progression, and many of them are related to lifestyle (19,42). Cancer stem cells in the breast tissue are capable of tumor initiation, progression, and metastasis (43). Germline or acquired mutations involved in mechanisms such as deoxyribonucleic acid (DNA) repair can result in uncontrolled cellular proliferation, metabolic alterations, or aberrant control of the cell cycle and apoptosis (20). Breast cancer cells also influence and are influenced by stromal cells (42,44) in the tumor microenvironment, and tumor progression leads to vascularization and remodeling of stromal compartments.

Crosstalk between the breast cancer cells and fibroblasts (called cancer-associated fibroblasts) can induce secretion of matrix metalloproteinases, which promote extracellular matrix degradation, which enables cell migration into the surrounding tissue (19). In addition, resident macrophages are influenced to acquire a pro-tumorigenic phenotype (tumor-associated macrophages) (4), which facilitate immune evasion (19). Together, the cancer cells and the tumor microenvironment shape pro-inflammatory conditions that facilitate disease progression (44).

Risk factors for breast cancer

Historical observations of higher frequencies of nuns having breast cancer (45) drove attention to the female sex and hormonal and reproductive factors in early case-report studies in the 1920s (46). These factors are now well-established risk factors for breast cancer (47). Together with age, which is the strongest risk factor for breast cancer, other factors related to heritability, previous benign breast disease, body composition, and lifestyle have been identified (47). The influence of lifestyle can be measured using the population attributable factor, which is an estimate of the percentage of a disease that could have been prevented if risk factors were removed from the population.

The Nurse's Health Study used data from 1980 and examined the total population's attributable risk percentage, including the modifiable factors of weight, alcohol consumption, breastfeeding, and refraining from hormone use. According to this study, removing these factors would reduce the risk by almost 40% for ER^+ breast cancer and 28% for ER^- breast cancer (47). A Swedish report from the Institute for Health Economics evaluated different types of cancer. 15% of breast cancer cases in Sweden were attributed to lifestyle factors in a model combining smoking, alcohol consumption, physical inactivity, dietary habits, overweight, infections, and sun exposure (48). The papers included in this thesis focus on lifestyle and associations with patient and tumor characteristics in addition to prognosis, and some common risk factors for breast cancer are presented below.

Estrogens and estrogen metabolism

Estrogens have important roles in breast cancers and have both proliferative and carcinogenic properties in breast tissue (20,49). Estradiol-17β (E_2) and estrone (E_1) are the most common endogenous estrogens (20). Activation is mediated by estrogen receptors alpha (ERα) and beta (ERβ), which belong to the nuclear superfamily of transcription factors (20). Upon activation, ERα dimerizes and is transported to the nucleus, where it binds to estrogen-responsive elements in the DNA. Activation can also involve interactions with other transcription factors, such as epidermal growth factor (EGFR) ligand amphiregulin (AREG) (23) and AHR (23,49,50).

ERα is essential for cells' responsiveness to estrogen in the target tissue (20), hence the ERα has been investigated and characterized more extensively than ERβ in various physiological and pathological processes (23). Invalidation of the use of antibodies for ERβ have led to many published results about the role of ERβ in the breast and in breast cancer being questioned (51,52). In breast cells, higher levels of estrogen have been correlated with increased transcripts of AREG, while levels of ERβ and AREG have shown negative correlations. Hereafter, ERα will be referred to as ER throughout the text.

The metabolism of estrogen is controlled by tissue-specific cytochrome P-450 (CYP) enzymes and involves the generation of metabolites with genotoxic abilities. Most of the metabolism occurs in the liver, but other tissues also metabolize estrogens, such as breast and endometrial tissue (49). Hydroxylation by CYP1A1, CYP1A2, and CYP3A generates 2-hydroxycatechol estrogen, while CYP1B1 generates 4-hydroxycathecol estrogen. The catechol estrogens have short half-lives due to inactivation by catechole-*O*-methyltransferase (20), yet 4-hydroxycathecol estrogen has genotoxic properties due to the generation of free radicals in tissues such as liver, lung, breast, kidney, uterus, and ovary tissues (49).

Exposure to hormones

Early onset of menarche and late onset of menopause expose the breast tissue to endogenous hormones for a longer period of time (20). Pregnancy has been associated with a short temporary increase in breast cancer risk, possibly due to unique hormone exposure, immune changes, or the involution process (as described above) (53). Parity, on the other hand, is associated with reduced breast cancer risk. In a study by The Collaborative Group on Hormonal Factors in Breast Cancer, each birth was associated with a relative risk (RR) reduction of 7% (54). In the Women's Health study, nulliparity was mainly associated with an increased risk for ER^+ disease, and reduced risk from parity was mainly apparent among women giving birth at the age of 25 years or earlier (47).

Breastfeeding is associated with reduced breast cancer risk (54,55). In a study by Islami *et al.,* reduced breast cancer risk upon breastfeeding was largely apparent in patients diagnosed with triple negative breast cancer (TNBC; see section "*Breast cancer classification"*) (55). In addition to the morphological changes in the breast during breastfeeding (20), the associated weight change may also influence the reduction in breast cancer risk (56).

Exogenous hormones increase breast cancer risk. Menopausal hormonal therapy (MHT) increases the risk for breast cancer (8,57), and the risk is dependent on the duration of use and type of therapy (8). Combined estrogen–progestogen treatments increase breast cancer risk to a larger extent than regimens that only contain estrogen (RR 60% and 17%, respectively, after 1–4 years of use). Regarding tumor characteristics, tumors from MHT users are more often ER^+ and of the lobular type among women using MHT for 5–14 years (8).

In a Danish study, Mørch et al. followed 1.8 million women for an average of 10.9 years, and current or recent hormonal contraceptive users had a relative increase in risk by 20% compared to those who had never used it, and the increased risk was related to a longer duration of use. However, the absolute increased risk was low (0.013% per year of using hormonal contraceptives) (58). Exposure to endocrine-disruptive chemicals has also been associated with an increased risk for breast cancer (18,59).

Breast density

High mammographic breast density is associated with increased risk of breast cancer (60). Dense breast tissue appears white in mammograms (61) and can mask tumor appearance. Lower breast density as a result of preventive tamoxifen treatment can reduce the incidence of breast cancer (62). In premenopausal women, blocking ER by tamoxifen decreases the proliferation rate of epithelial cells and epithelial tissue expressing ER and progesterone receptor (PR), while in postmenopausal women the breast density did not change significantly with tamoxifen treatment (32). Smoking and alcohol consumption have been reported to influence breast density by modulating the non-dense tissue of the breast in non-obese women (63). One study has reported an association between high breast density and higher levels of $pSTAT3^{Y705}$ (64).

Body composition

Overweight and obesity are established risk factors for postmenopausal breast cancer (47,56). The increased risk is primarily related to elevated levels of estrogen (23) produced by adipose tissue through the conversion of androgens to estrogens by CYP19A1 (65). Insulin resistance is more common among overweight and obese individuals and is also associated with increased breast cancer risk (66). Increased visceral fat is related to both higher age and increased body mass index (BMI) (67).

A study assessing normal breast tissue showed that increased trunk fat was associated with larger adipocytes in the breast, higher levels of leptin, and increased expression of *CYP19A1* (65). Additionally, higher levels of reactive oxygen species (ROS) scores, immune scores, and gene expression of *Interleukin-6* (*IL6)* suggest that increased trunk fat can change the breast tissue to an immunosuppressant pro-inflammatory state with higher levels of oxidative stress (65). In contrast, a lower BMI at the age of 18 years has been associated with an increased risk for postmenopausal breast cancer, particularly ER^- breast cancer (47). Other factors have also been associated with increased breast cancer risk, such as birth weight (68), birth length (69), and increased adult height (47).

Alcohol and smoking

Alcohol is a determined risk factor for breast cancer (47), even at lower levels and at all ages (70). The global age-standardized incidence rate for breast cancer due to alcohol is 2.2 per 100,000 females, which corresponds to a population attributable fraction of 4.4% (71), although there are regional differences (70). Meta-analyses assessing the increased relative risk from alcohol consumption show that one drink (12.5 g of alcohol per day) increases the relative risk of breast cancer by 5% compared to non-drinkers (5).

There are several potential mechanisms through which alcohol could influence breast cancer risk, such as acetaldehyde promoting tumor initiation, ethanol

metabolism-induced oxidative stress, altered metabolism, impaired immunity, and increased aromatization of androgens to estrogens (70). Heavy drinking is more common among smokers (72), which may be one explanation why the association between smoking and breast cancer is unclear (73). A second explanation involves the crosstalk between ER and the environmental sensor AHR, which can generate both estrogenic and anti-estrogenic effects (23,49,50). Nevertheless, two recent meta-analyses assessing breast cancer risk among smokers and second-hand smokers reported that both the duration and intensity of smoking and second-hand smoke exposure linearly increase breast cancer risk (74,75).

Physical activity/inactivity

Studies indicate that physical activity is associated with a reduced breast cancer risk (76-78), but reports have not been consistent (47). Most studies include postmenopausal women, and the type of exercise may influence the study results (66,77). In a Swedish cohort study, women engaging in a high level of physical activity (>1 h daily walking) had a 23% lower long-term breast cancer risk overall compared to women reporting low levels of physical activity (76).

Physical activity could influence breast cancer risk in various ways, such as by reducing body fat, thereby reducing circulating estrogens, increasing the sensitivity to insulin, and improving immune functions (66). Additionally, reduced levels of pro-inflammatory markers such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) have been observed after engaging in physical activity (79,80). *In vivo* studies of obese mice performing spontaneous physical activity display slower tumor growth with increased levels of the anti-inflammatory hormone adiponectin, as well as decreased antioxidant response in tumors compared to tumors in sedentary mice (81).

Dietary supplements and antioxidants

Dietary supplements

Health benefits from nutrients and phytochemicals are predominantly observed when consumed in the diet (82-86). Malnutrition is uncommon in the general population (87), and only certain circumstances require dietary supplements among individuals who consume a varied diet (88). Nevertheless, dietary supplements represent a large and increasing market (89,90), and a wide range of products are commonly used in the general population (89,91-93). The reasons for use not based on general recommendations include proposed improvement of overall health (88,94-97), improved physical performance, mobility (94), an improved immune system (97), and relief of menopausal symptoms (93). Women are more likely to use dietary supplements than men, and the types of supplement differ between age groups (95). High socioeconomic status (95), higher levels of physical activity (95), higher education (86), and a healthy diet are more common among supplement users (86,96).

Since dietary supplements are classified as food, no proven effect of the supplements is necessary. In Europe, dietary supplements are regulated by Food Supplements Directive 2002/46/EC. This regulation requires supplement constituents to be safe and bioavailable before allowance as ingredients in dietary supplements (89). However, the responsibility lies with the producer of the product (89). In Sweden, no authority is responsible for regulating or controlling products that are available on the market (94). Generally, dietary supplements contain higher doses of nutrients compared to food (92), which increases the risk for adverse effects (89,94) and interactions with medical treatments (93,94). Contaminations in dietary supplements have also been reported (94).

Antioxidants and antioxidant supplementation

A common group of dietary supplements comprises those with antioxidant properties (92). Antioxidants are substances that are capable of delaying or preventing oxidative damage caused by ROS (82). ROS are highly reactive molecules that are generated from oxygen and have at least one unpaired electron in the outer shell that renders them highly reactive (98). The main source of ROS is cell metabolism, and a higher metabolism (often present in cancer cells) generates higher levels of ROS (98).

In normal conditions, ROS are important signaling molecules in several cellular regulating processes and the innate immune system (98), but excessive amounts increase the risk for cellular and DNA damage (82). To avoid oxidative stress, several potent endogenous antioxidants are present in the body (e.g., superoxide dismutase, catalase, and glutathione) (4,85,99), while exogenous antioxidants like vitamin C and E, carotenoids, and polyphenols need to be obtained from the diet (100). An important difference between antioxidants obtained from the diet compared to supplements is the higher level of antioxidant capacity. Regular multivitamins have higher antioxidant capacity compared to a regular diet (101), while single-ingredient supplements and supplements beyond regular multivitamins often have even higher levels (92). Studies also indicate that antioxidants can influence tumor characteristics (102-105).

Several medical conditions are caused by or lead to oxidative stress (88). This observation has led to several randomized trials aiming to prevent cancer (106), but unexpectedly, supplementation with antioxidants increased the risk of some cancers, such as prostate (106) and lung cancer (88,107). Consequently, organizations like the U.S. Preventive Service Task Force advices against use of beta-carotenes and vitamin E for prevention purposes against cardiovascular disease or cancer (88).

A French population-based study reported that vitamin C intake from food modified the association between supplement use and postmenopausal breast cancer risk. Users in the highest quartile of vitamin C intake from food had increased risk for breast cancer if they also reported supplement use (86). However, these results were

not confirmed in the Nurses' Health Study, in which the effect of vitamin C on breast cancer risk varied depending on family history of breast cancer. In that study, a reduced breast cancer risk for women in the highest quintile of vitamin C intake was borderline significant for women with a family history of breast cancer (108).

Clinical aspects

Hereditary breast cancer and syndromes

Approximately 10% of all breast cancers have a hereditary component for breast cancer, of which 6% constitute known pathogenic variants, and 4% may be due to genetic, environmental, or a combination of these factors. Germline pathogenic variants in *BRCA1/2* (breast cancer gene 1/2) and genes like *PALB2* (partner and localizer of *BRCA2*) are considered high-risk genes (109) with cumulative lifetime breast cancer risks of 72%, 50–69%, and 30% respectively. The proteins encoded by *BRCA1/2* and *PALB2* are all involved in homologous recombination, an important repair mechanism following DNA double strand breaks (110). Women with germline pathogenic variants in *BRCA1/2* usually inherit a single variant copy in the germline, thereby making them more susceptible to loss of function if the second copy is damaged (110).

ATM (ataxia-telangiectasia mutated) and *CHEK2* (checkpoint kinase 2) are associated with a moderate risk for breast cancer (109). The *CHEK2* gene is important for DNA repair, cell-cycle regulation, and apoptosis (110). The *ATM* gene is activated by DNA damage and regulates proteins involved in cell-cycle progression (*e.g.*, p53). Hallmarks of ataxia-telangiectasia include predisposition to cancer, hypersensitivity to ionizing radiation, immunodeficiency, and infertility (110). A study of TNBC reported a negative correlation between high levels of ATM and phosphorylated STAT3 (pSTAT3) (activation residue not reported) (111). Tumors from patients with genetic predisposition present earlier than sporadic cancers, hence genetic testing is recommended for younger patients (<40 years) and women with a known history of breast or ovarian cancer. Additionally, genetic testing can influence the choice of treatment in some cases (109).

Diagnostics

Breast cancers are diagnosed using triple diagnostic assessments comprising mammography (or other imaging modalities such as ultrasound), palpation, and core-needle biopsy or cytology. In Sweden, the majority of breast cancers are detected through the mammography screening program, where women between the ages of 40 and 74 years are invited for screening every 18–24 months. Screening was gradually implemented in the 1980s and 1990s in Sweden, and since 2014, it has been regulated by the national health screening program (112).

Among women included in the screening program who regularly attend, 60–70 % of breast cancers are detected by mammography. Cancers detected between mammography visits are called interval breast cancers. A core needle biopsy is essential to confirm malignancy (113) and to assess treatment-predictive and prognostic biomarkers (113). The core biopsy provides information on the expression of hormone receptors (ER, PR), human epidermal growth factor-2 (HER2), and Kiel-67 (Ki-67) (113). A second staining is performed on the surgical specimen (114), which also provides information about surgical margins and the presence of *in situ* components (115). The correlation between the different techniques is generally high with the exception of Ki-67, which often displays more heterogeneous expression (113).

Breast cancer classification

Classification of breast cancer is performed at different levels and provides an informed decision tool about the characteristics of the disease. The TNM (Tumor, Node, Metastasis) classification from the Union for International Cancer Control (UICC) (116) categorizes the tumor according to size and local growth characteristics (TI–4), axillary nodal involvement (N0–3), and distant metastasis (M0–M1) (116). By combining information from the T, N, and M status, a clinical stage is defined (I–IV) and used to guide treatment choices. Prefix annotations in the TNM stage are used to inform how the information is obtained (*e.g.,* "c" for clinical evaluation and "p" for pathological evaluation) (116).

Table 1. Clinical stage for breast cancer according to the UICC TNM classification of malignant tumors (pathological), 8th edition.

	T0	**T1**	**T2**	**T3**	**T4**
N0	-	IA	IIA	IIB	IIIB
N1mi	IB	IB	-	-	-
N1	IIA	IIA	IIB	IIIA	IIIB
N2	IIIA	IIIA	IIIA	IIIA	IIIB
N3	IIIC	IIIC	IIIC	IIIC	IIIC
M1	IV	IV	IV	IV	IV

Abbreviations: T: primary tumor, N: regional lymph nodes, mi: micro metastasis, M: distant metastasis

The next classification level is the histological type and grade. The most common histological type is "no special type" (formerly known as ductal carcinoma), which accounts for approximately 70–80% of breast tumors (114). The lobular type (15–20% of breast tumors) is characterized by a "single-file" growth pattern due to a loss or low expression of E-cadherin (114). Less common histological types include mucinous, tubular, and papillary types, invasive carcinoma with a medullary pattern, and adenoid cystic and metaplastic types (114). The morphology of the tumor is graded according to the Nottingham Histologic Grade (NHG) (116), which

combines information about tubule formation, mitotic activity, and nuclear pleomorphism to assign one of three different categories (NHG I–III) (114,117).

The third level of classification is related to tumor markers. In Sweden, the cutoff for ER^+ and PR^+ is defined as ≥10% nuclear staining (113,118). ER^+ breast cancers account for about 80% of all breast cancers (119). $HER2^+$ is defined by a score of 3^+ according to immunohistochemistry (IHC) analyses. Tumors with a HER2 score of 2^+ can be tested for gene amplification by fluorescence *in situ* hybridization (FISH) or silver-enhanced *in situ* hybridization and are denoted as positive if HER2 amplification is detected (113). Approximately 10–20% of tumors are $HER2^+$ (120).

Tumors with low levels of ER, PR, and HER2 expression are defined as TNBC and constitute about 10–15% of all breast cancers (41,121). Many countries use 1% positivity as a cutoff for ER^+ (109,122). In an updated version from the American Society of Clinical Oncology/College of American Pathologists, ER^+ expression of 1 to 10% is denoted as ER-low (122).

The cutoff for ER positivity influences the treatment choice and is still controversial (109). In a recent Swedish population-based study, ER-low tumors (defined as ER^+ expression of 1 to 9%) constituted 10% of all TNBC (118). Mutations in the *BRCA1* gene are more frequently observed in TNBC (4). According to the International Ki67 in Breast Cancer Working Group, Ki67 staining has shown inter-observer/laboratory variability in the range of >5% to <30% (123). Thus, the Swedish cutoffs for low, intermediate, and high are ≤5%, 6–29%, and ≥30%, respectively (124).

In current clinical practice in Sweden, breast cancer is classified by IHC as one of five different intrinsic subgroups: luminal A-like, luminal B-like, $HER2^+$/luminal-like, $HER2^+$/non-luminal-like, and TNBC (124) (Figure 5). The intrinsic subgroups are clinico-pathological surrogate definitions of the intrinsic subgroups defined by gene expression (40) and were adopted at the St. Gallen consensus meeting in 2013 (121). There is 80% overlap between TNBC and the basal-like subtype, meaning that not all TNBCs have the same characteristics as those defined as basal-like breast cancer (121). Basal-like breast cancers (basal-like types 1 and 2) have high levels of genes involved in cell proliferation and DNA-damage response, such as cell-cycle check point loss (type 1) and EGFR and IGFR signaling (type 2) (41). The luminal cancers are a heterogeneous group, and for some patients with $ER^+/HER2^-$ breast cancers gene expression analysis (e.g., Prosigna breast cancer prognostic gene signature assay (PAM50), Oncotype DX, and risk of recurrence (ROR)) (121) can provide information on whether or not the patient will benefit from chemotherapy.

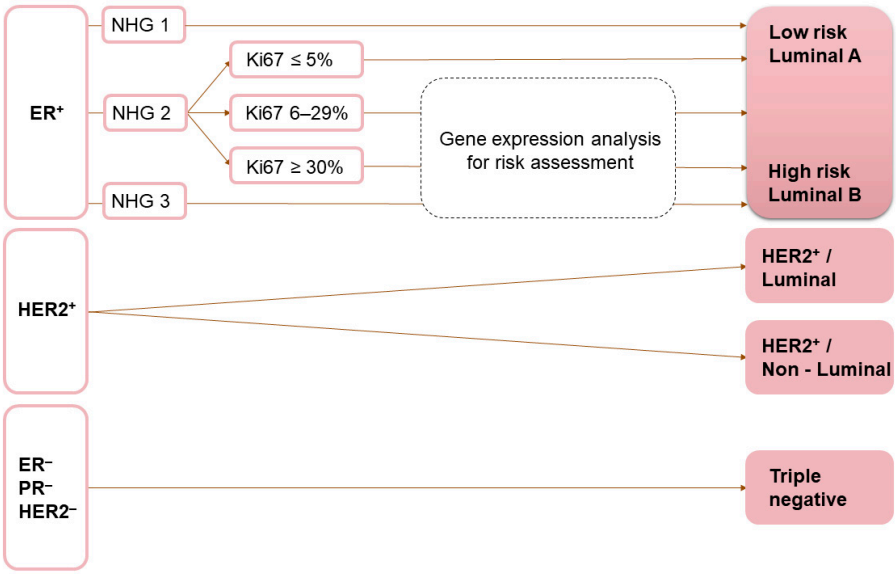

Figure 5. Classification of intrinsic subtypes according to the St. Gallen consensus. Abbreviations: ER: estrogen receptor, HER2: human epidermal growth factor 2, PR: progesterone receptor, NHG: Nottingham Histological Grade, Ki67: Kiel 67.

Tumor prognostic and predictive factors

Several factors influence clinical outcome. Prognostic factors provide information about the likely course or outcome of a disease, while treatment-predictive factors give information about the expected response to a specific treatment. In breast cancer, many of the prognostic factors are also treatment predictive. The 8th edition of the TNM Classification of Malignant Tumors classifies eight tumor-related factors as essential: ER status, HER2 status, histological grade, number and percentage of involved nodes, tumor size, presence of lymphatic or vascular invasion, and surgical resection-margin status (116). The essential patient-related prognostic factors are age and menopausal status (116).

The defined intrinsic subtypes provide prognostic information and show different time frames for recurrence. Basal-like breast cancers tend to have early relapses, while luminal cancers often display late recurrence (40). Prior to the introduction of anti-HER2 targeted treatments, HER2^{+} tumors also displayed early recurrence (40). Many of the prognostic factors are also associated with each other. For instance, a Danish study reported that tumor size, HER2^{+}, and younger age are associated with axillary lymph-node involvement (125). The choice of treatment is guided by the prognostic and predictive factors, as well as the patient's preferences, age, and general health (109).

Breast cancer surgery

In early breast cancer, the aim of surgery is to remove macroscopic tumor tissue. Tumor-free margins of normal tissue are of great importance (126). Therefore, historically, for many years, Halsted radical mastectomy was the first method of choice for breast cancer surgery (127). The less extensive total/modified mastectomy has shown equal disease-free survival (DFS) to the previous approach in studies performed in the 1970s, so it was adopted (127). An even less extensive surgery technique is breast-conserving surgery (BCS), in which only enough breast tissue is removed to ensure tumor-free margins (128). Several randomized clinical trials (RTCs) indicate that BCS combined with RT displays similar survival rates to that of mastectomy (127,129-131). These results are supported by population-based studies reporting improved OS among patients treated with BCS and RT compared to mastectomy (119,132), although residual confounding cannot be excluded (115).

Currently, the majority of patients with breast cancer are treated with BCS followed by RT (119). The development of oncoplastic breast surgery has rendered more patients eligible for BCS since it allows a more generous resection of breast tissue without a poor cosmetic result (133). Oncoplastic surgery includes both volume displacement or replacement and breast-reduction techniques (133). Mastectomy is indicated when BCS is not possible, as in cases of larger tumors or inflammatory breast cancer (109). Mastectomy may also be necessary in converted surgery or in cases of repeated surgery (126).

Elective bilateral risk-reducing mastectomy can be offered for pre- and postmenopausal patients with pathogenic variants of *BRCA1* and *BRCA2,* as well as premenopausal patients with pathogenic variants of *PALB2* (109). Traditionally, mastectomy has also been more common for elderly women (133,134). In a Swedish population-based study, the type of surgery was also associated with comorbidity and socioeconomic status (119).

The type of surgery can also influence the wellbeing of the patient. A recent Swedish study concluded that body image, breast satisfaction, and sexual functioning were higher among patients with partial mastectomy and oncoplastic partial mastectomy, while symptoms from the breast/chest wall were lower for patients with mastectomy one year after breast cancer surgery. Among patients treated with RT, patients who underwent both mastectomy and reconstruction reported the lowest levels of wellbeing (133).

Major post-operative surgical complications within 30 days, such as bleeding, impaired wound healing, and infections (135), may delay the initiation of adjuvant treatment in some cases (109) and influence survival (134,135). In a Swedish population-based study including women treated in 2008−2017, complications were less common after BCS (2.3%) compared to mastectomy with immediate reconstruction (7.3%) and without reconstruction (4.3%) (135). Among women who were 50 years or older and receiving the same type of surgery, associations with

major post-operative or medical complications were independent of age, but complications were more common among patients who were older than 70 years and had a mastectomy (134).

Axillary nodal status is an important prognostic tool. For patients with clinically node-negative disease, sentinel lymph node biopsy (SLNB) is a standard procedure for staging nodal status (109). However, SNLB underestimates the frequency of positive lymph nodes compared to axillary lymph node dissection (ALND), and the detailed information from ALND can be important to guide adjuvant treatments for some patients (136). Nevertheless, ALND is associated with a higher incidence of arm morbidity (137).

Attempts to de-escalate surgery for patients with breast cancer involve efforts to omit SLNB in early-stage breast cancer, such as by developing nomograms (138) or using preoperative negative ultrasonography (139). In the randomized multicenter SOUND trial, the omission of ALND following a negative ultrasonography for regional lymph nodes was non-inferior to SLNB for patients with early breast cancer (139). In relation to clinical outcome, omitting ALND for patients with T1–3 tumors with 1–2 positive SLNB who received standard of care, did not influence the 5-year OS or recurrence-free survival in the SENOMAC trial. The results from the SENOMAC trials support previous studies performed with patients who had lower tumor or nodal burden (AMAROS, ACOSOG Z0011) (109,140).

Chemotherapy in breast cancer

Chemotherapy in breast cancer is tailored to the disease characteristics, and the treatment combinations and approaches have changed over time (141-143). Studies on patients from the 1970s who were not treated with endocrine therapy demonstrated 21% lower mortality among patients treated with chemotherapy (cyclophosphamide, methotrexate, and fluorouracil) compared to those who received no chemotherapy (142). Currently, regimens consisting of anthracyclines (*e.g.,* doxorubicin or epirubicin) and taxanes (*e.g.,* paclitaxel or docetaxel) form the foundation of chemotherapy (124).

In the "Early Breast Cancer Trialists' Collaborative Group" (EBCTCG) meta-analysis from 2023, patients assigned anthracycline plus a taxane had 14% lower recurrence rates compared to patients given regimens without anthracyclines (141). The reported absolute benefit of adding taxanes was 2.6% (141). Dose-dense therapies and dose-intense schedules are more efficacious (141) and preferable for eligible patients (109). High cumulative doses of anthracyclines increase the risk for cardiovascular disease (143), therefore some patients with cardiovascular comorbidities are treated with non-anthracycline-based regimens (124).

Preoperative chemotherapy is indicated in inflammatory and inoperable breast cancers. For patients with stage II−III tumors, preoperative treatment can improve

surgical options, and the treatment response can guide the choice of adjuvant treatment (109). Systemic treatment is particularly important for TNBC and $HER2^+$ breast cancers, hence preoperative treatment can also be indicated for earlier stages (cT1cN0) (124). In TNBC, the addition of carboplatin is recommended, as is the PD-1 inhibitor pembrolizumab (109).

Adjuvant chemotherapy is decided based on tumor characteristics, nodal involvement, and younger age (124). Postoperative chemotherapy is recommended for patients with TNBC who are not receiving preoperative chemotherapy. In $HER2^+$ breast cancers, adjuvant chemotherapy is administered in combination with trastuzumab, along with the addition of endocrine treatment for hormone-receptor-positive breast cancer (109).

Radiation therapy for breast cancer

Postoperative RT is administered to reduce the risk for recurrence from microscopic foci or disseminated tumor cells (129). The target of RT depends on the type of surgery and clinical stage and may or may not include axillary or parasternal nodes (128,144). An EBCTCG meta-analysis published in 2011 including clinical trials performed between 1976 and 1999 demonstrated an absolute risk reduction for recurrences of 15.7% and reduced risk for death by almost 4% in RT treated patients compared patients not treated with RT at the 15-year follow-up (129). The study also reported greater benefit of local control in node-positive cancers compared to node-negative cancers (129).

Generally, the local recurrence risk following RT is low (129,145-147). In a Swedish retrospective study of patients treated between 2004 and 2008, the overall recurrence at 10 years was 7.1%, and local recurrences were more often represented by ER^- and $HER2^+$ breast cancers (148). The addition of a boost dose to the primary tumor site further reduces the risk of a local recurrence (149) but can increase the risk of fibrosis (149). Bartelink *et al*. examined patients between 1989 and 1996 and reported an absolute reduction of recurrences of 4%, but the additional gain of the boost strongly correlated with age (149). Therefore, a boost dose is recommended for younger women (≤50 years) and in cases of non-radical surgery (124).

The recent IMPORT HIGH trial evaluated simultaneous boost fractionation schedules. Ipsilateral breast recurrences at five years ranged between 1.9% and 3.2% in the three test groups, and there was no significant difference in moderate or marked late adverse events in normal tissue (147). The results of the IMPORT HIGH study indicate that use of a simultaneous boost is safe, while dose escalation for the tumor bed did not improve rates of locoregional recurrences (147).

Traditionally, whole-breast irradiation has been administered with 50 Gray (Gy) in 25 fractions with five fractions per week. A shift towards hypo-fractionated schedules of 40−42 Gy in 15−16 fractions occurred following studies performed by Haviland *et al*. in 2013 and Whelan *et al.* in 2010. These studies demonstrated

similar survival rates and toxicity profiles to those of conventional fractionation (145,146). During the COVID-19 pandemic, the ultra-hypofractionation schedule of 26 Gy in five fractions that was tested in the FAST FORWARD trial was introduced for older patients (150). This schedule has subsequently been implemented for women ≥ 51 years old following BCS without nodal involvement (124).

Compared to older studies, lower doses to organs at risk have been made possible in more recent studies due to knowledge about adverse events, improvements in target delineation, and treatment techniques such as CT-based treatment planning, tangential fields, and respirator gating (151). The SweBCGRT trial followed patients with early breast cancer over a 20-year period (1187 women; <76 years old, pTI–TIIA, N0), and patients treated with RT had similar cumulative incidence for cardiac mortality (12.4% vs. 13%), contralateral breast cancer (8.7% vs. 8.6%), and lung cancer (1.6% vs. 2.6%) compared to patients who were not treated with RT (152). These results are in line with those of a Danish population-based case-control study, which did not detect any significant increase in risk for cardiac events during the first 10 years following RT among patients treated with CT planning (153).

At the individual level, treatment targets and anatomical differences can still pose a risk for high doses to organs at risk. The systematic review by Taylor *et al*. estimated excess relative risks for cardiac mortality as 0.041 per Gy of whole-heart dose and 0.11 for lung cancer per Gy of whole-lung dose (151). These results were highly influenced by smoking status, with higher risk occurring among patients who continued smoking (151). Studies with high mean RT doses to the esophagus (primarily when the internal mammary nodes are included in the treatment target in older studies) have been associated with an increased risk for esophageal cancer (151). Skin toxicity includes erythema, edema, breast shrinkage, induration, fibrosis telangiectasia, and hyperpigmentation (146,147,154). Although there is individual variability, skin toxicities increase with increasing volume (154), dose (146,147,149), use of boluses (154), and smoking during RT (154).

Efforts to de-escalate or reduce RT include reducing treatment-target volumes (155) and forgoing RT for low-risk patients. The IMPORT LOW demonstrated that the target volume can be reduced to the primary tumor site for older women (>50 years) with low-risk tumors, which leads to comparable recurrence and survival rates to those obtained with whole-breast irradiation (156). Partial breast irradiation significantly reduces the radiation dose to organs at risk, such as the heart and lungs, and has been implemented in clinical practice (124) since 2020 in Sweden.

Kunkler *et al*. investigated the omission of RT between 2003 and 2009 with patients aged 65 years or older and TI–T2 tumors. The local recurrence rate was 9.5% in the no-RT group and 0.9% in the RT group (157). In that study, the survival rates were almost 81% in both groups, and most deaths were not due to breast cancer (157). In a subgroup analysis, patients discontinuing endocrine therapy in the no-RT group

had a more than a four-fold increase in risk for local recurrence (157). The ongoing T-REX trial is investigating the omission of RT for regional lymph nodes for patients with 1–2 sentinel-node macro-metastases (cN0, T1–2, ER^+, and $HER2^-$). Gene expression signatures for locoregional recurrences following RT are being developed, and future use may help to identify patients that can safely forgo RT (POLAR) (158) or have a higher risk of locoregional recurrence (ARCTIC) (159).

Endocrine treatment in breast cancer

Tamoxifen is a selective ER modulator (SERM) that antagonizes ER by competitive binding, thereby inhibiting transcription of estrogen-responsive elements (160). Tamoxifen treatment was introduced in the 1980s (161) and is primarily used for premenopausal women (109). In the large EBGTCG meta-analysis, tamoxifen treatment for five years reduced the relative risk for recurrence by 47% at the 15-year follow-up compared to no endocrine treatment (161).

Aromatase inhibitors (AIs) such as anastrozole, letrozole, and exemestane were approved in 2005 and subsequently introduced following RTCs (162). AIs block the aromatase enzyme that catalyzes adrenal androgens to estrone and estradiol (163). The conversion to estrogens primarily takes place in adipose tissue (65). Postmenopausal women receive adjuvant AIs or switch between 2 and 3 years of AI treatment followed by tamoxifen (109). This treatment sequence is the reverse of the one used when the regimen was introduced.

Premenopausal women with high-risk tumors are sometimes also candidates for AI treatment (109). The EBCTCG meta-analysis in 2015 compared AIs to tamoxifen in nine randomized trials. Five years of treatment with AI reduced recurrence risk (distant, local, and contralateral) and improved OS (162), while at 10 years, AI treatment led to 3.6% fewer recurrences and 2.1% reduced risk for death. Half of the deaths reported were not related to breast cancer (162). Compared to tamoxifen, AIs increase the risk for bone fracture, and the risk is increased for older women (162). Traditionally, endocrine treatment is given for a duration of five years, but an extended duration can reduce recurrence risk for high-risk patients (109).

Women younger than 40 years and high-risk premenopausal women benefit from combining endocrine treatment with ovarian suppression (109). The greatest benefit is seen with women who remain premenopausal following chemotherapy (164). The combined report of the TEXT and SOFT trials examined 4690 premenopausal women who were randomized to receive exemestane + ovarian suppression versus tamoxifen + ovarian suppression. The study reported a 4.6% absolute improvement in DFS for exemestane + ovarian suppression. The incidence of death was insignificant higher for exemestane + ovarian suppression during the first five years. There was no difference between treatments at later time intervals and no association with OS for any treatment at the 13-year follow-up (164).

The combined treatments showed a higher degree of grade 3 adverse events for musculoskeletal symptoms and osteoporosis, but rates for thrombosis or embolism were similar for tamoxifen with and without ovarian suppression (165). Bisphosphonates relieve the side-effects related to osteopenia/osteoporosis in postmenopausal women receiving AIs and in premenopausal women treated with ovarian suppression. Importantly, bisphosphonates also reduce the risk of breast cancer recurrence (109).

Anti-HER2 treatment in early breast cancer

The introduction of anti-HER2 treatments has improved the survival of patients with $HER2^+$ breast cancers (120,166). A meta-analysis examined seven RTCs performed in 2000−2005, and the addition of trastuzumab to the chemotherapy regimen was found to reduce the risk of recurrence by 9% and the 10-year mortality by 6% (120). Trastuzumab was the first registered drug to target HER2 and is a recombinant monoclonal antibody that is directed against the extracellular domain of the tyrosine kinase receptor HER2. The main effect of trastuzumab comes from direct inhibition of signal transduction upon binding. In addition, trastuzumab also acts through antibody-dependent cell-mediated cytotoxicity (ADCC) (167).

Pertuzumab is a fully humanized monoclonal antibody that blocks HER2 dimerization with other members of the erythroblastic leukemia viral oncogene (ErbB) family. Since trastuzumab and pertuzumab bind to different epitopes, their combination leads to an additive effect (167). The addition of pertuzumab to trastuzumab and chemotherapy was tested for early breast cancers in the APHINITY trial, but there was no improvement in OS. However, patients with lymph-node-positive disease had an absolute benefit of 4.9% regarding DFS. Despite increased risk of cardiac toxicities, the rate of cardiac events was below 1% in all treatment groups (168).

Targeted therapies in early breast cancer

Several ongoing studies are examining targeted therapies for breast cancer. Targeted drugs can be aimed at the tumor, as well as the TME and cellular energetics (166). In clinical practice, the CDK4/6 inhibitor abemaciclib can be offered to patients high-risk ER^+ breast cancers (109). In patients with germline pathological variants of *BRCA1/BRCA2*, OS can be improved by adjuvant treatment with the poly ADP ribose polymerase (PARP)-inhibitor olaparib (109). Nevertheless, despite remarkable improvements in breast cancer treatments, some patients still have poor prognoses, while de-escalation of treatments is warranted for others. To improve personalized treatment strategies, novel prognostic and treatment predictive markers are needed.

Signal transducer and activator of transcription 3

STAT3 belongs to a family of seven intracellular transcription factors that regulate gene expression (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, STAT6). STAT3 has a wide range of biological functions involving proliferation, cell differentiation, inflammation, and apoptosis (169), and it may influence treatment resistance (170) and immune modulation (171,172). The canonical STAT3 pathway is activated by several receptors, such as cytokine (*e.g.,* IL-6) receptors, growth factor receptors, G-coupled receptors, and Toll-like receptors. The most common phosphorylation site is at tyrosine residue 705, as shown in Figure 6. The non-canonical activation of STAT3 involves phosphorylation by serine 727 ($pSTAT3^{S727}$), un-phosphorylated STAT3, or mitochondrial regulation (169).

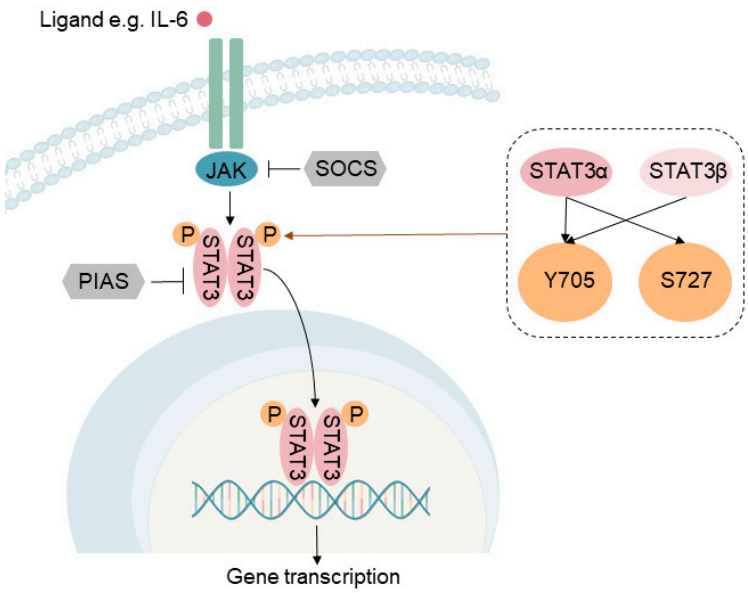

Figure 6. The canonical pathway of STAT3 activation. Activation of receptors recruits and activates intracellular tyrosine kinases of the JAK family, which exposes anchor sites for STAT3 binding by its SH2 domain, with subsequent phosphorylation of STAT3 (pSTAT3) at residue tyrosine 705 ($pSTAT3^{Y705}$). Following this, $pSTAT3^{Y705}$ dimerizes by either homo- or heterodimerization, translocates to the nucleus, and binds to responsive elements of the DNA. Both STAT3α and STAT3β isoforms can be activated by phosphorylation site Tyr705, whereas the STAT3β isoform lacks the Ser727 residue and cannot be phosphorylated at Ser727. The STAT3 pathway is negatively regulated by members of the SOCS and PIAS family. Abbreviations: STAT3: signal transducer and activator of transcript 3, JAK: Janus kinase, SH2: Src homology 2, pSTAT3: phosphorylation of STAT3, Y705; tyrosine residue 705, S727: serine residue 727, SOCS, suppressor of cytokine signaling, PIAS: protein inhibitor of activated STAT3

The two major isoforms of STAT3 are STAT3α and STAT3β, which lead to different responses upon activation. The STAT3α isoform contributes to a larger

portion of STAT3 signaling and mainly has tumor-promoting properties. An alternative splicing generates the STAT3β isoform, which lacks the Ser727 residue (169). The STAT3β isoform is believed to have anti-inflammatory properties, and mice depleted of this isoform are more susceptible to inflammation (173).

Overexpression of the splicing regulator factor poly(rC)-binding protein 1 in MCF-7 cells decreases the relative ratio between the STAT3α and β isoforms and downregulates B-cell lymphoma-extra large and survivin, which are important factors for the suppression of apoptosis (174). Important interplay between the two isoforms was recently demonstrated in esophageal squamous cell carcinoma, where enforced expression of STAT3β increased STAT3α *in vivo* and *in vitro*. The increased expression of STAT3β or knockdown of STAT3α decreased chemo resistance and clonogenic ability and was dependent on phosphorylation at the tyrosine 705 residue (175). An exclusive evaluation of the STAT3β isoform in breast cancer has not been performed so far.

In normal cells, the activation of STAT3 is strictly regulated by members of the SOCS and PIAS family and protein tyrosine phosphatases (15). Transient STAT3 activation is crucial for restoration of tissue integrity and has a regulatory role during post-lactation involution. In contrast, constitutive activation of STAT3 has been associated with mainly tumor-promoting capabilities (176), and compared to normal tissue, overexpression of STAT3 has been reported in many different types of cancer, including breast cancer (15). Gene alterations in STAT3 primarily consist of mutations or amplifications with a reported alteration frequency of less than 4% in breast cancer (15).

Activity of $pSTAT3^{Y705}$ is generally absent in normal healthy breast tissue (117), whereas low levels of $pSTAT^{S727}$ have been reported (42). In breast cancer cell lines, ethanol increases $pSTAT^{S727}$, which mediates increased cell proliferation by the IL-6/JAK/STAT pathway (MCF-7 and MDA-MB-231) and phosphoinositide-3-kinase (PI3K) (MDA-MB-231) (177). Data from The Cancer Genome Atlas (TCGA) database indicate a positive association between *pSTAT3* and *PD-L1* (programmed cell death ligand 1), and IL-6 treatment of MCF-7 increases protein levels of PD-L1 and $pSTAT3^{Y705}$ (171).

In TNBC, RT induces higher levels of $pSTAT3^{Y705}$, and RT-resistant TNBC cells with low levels of ROS display increased RT sensitivity upon $pSTAT3^{Y705}$ inhibition (170). Enhanced treatment resistance has also been observed in $HER2^{+}$ cell lines following RT-induced activation of $STAT3^{Y705}$ (178). Interestingly, although most cancer types with elevated levels of STAT3 are associated with an adverse prognosis (15), association with STAT3 in early breast cancer points towards an improved prognosis for most subgroups, but not all (117,179-181).

Antioxidant and oncological treatment

Patient-related factors may influence treatment outcome, such as the use of antioxidant supplements, which are commonly used by patients with cancer. (97,182,183). During active treatment, patients are generally advised to stop taking antioxidants (184,185) since there is no reliable evidence that such supplements do not interfere with oncological treatment (11,186). For patients treated with RT, 70% of the DNA-damaging effect is believed to arise from radicals (mainly the hydroxyl radical, OH●), which are produced by the interaction between the ionizing radiation and water (187). Most chemotherapeutic agents also rely on the generation of free radicals in addition to their main effect to trigger cell death (98). Anthracyclines, platinum-based chemotherapy, alkylating agents, camptothecins, arsenic agents, and topoisomerase inhibitors generate high levels of ROS, while lower levels have been reported for taxanes, vinca alkaloids, and antimetabolites (98).

The impact of antioxidant use on treatment can be influenced by comorbidities, other lifestyle factors (185), and smoking (11). Meyer *at al.* performed a randomized trial including 540 patients with HNSCC who were treated with RT. An effect modification between smoking and antioxidant supplementation during treatment was reported, where smokers had a 2-fold increased risk for recurrence or death if given supplements compared to non-smokers, who had no significant excess risk (11).

Antioxidants and breast cancer

Studies report that antioxidant use is common among patients with breast cancer (186,188-192). In line with data from the general population (96), most studies report that such patients that use supplements are less often smokers (182,188,193), have healthier eating habits (189), and are more likely to use MHT (182,190,191). High levels of supplement use have also been reported for women making active lifestyle changes post diagnosis (194). In the SWOG 0221 trial from 2003, some patients were recommended to use multivitamins or supplements during treatment, while others were not (195). A German study included young women with breast cancer who participated in a rehabilitation program, and 7 out of 10 participants used vitamins or minerals. The reported use of "antioxidants" was 16% while 9% reported use of supplements believed "to strengthen the immune system" (*e.g.,* vitamin E, ubiquinone, coenzyme Q10) (191). A recent Irish study reported that very few patients with breast cancer, particularly regular users of supplements, believed that supplements could increase the risk of a poor outcome (184).

Many studies report that multivitamins are among the most commonly used supplements among patients with breast cancer (182,184,192,194,195). A report from the Swedish Mammography Cohort examined data from 1997, and 25.5% of included women used multivitamins (182). So far, multivitamin use has not been associated with worse survival in observational studies (186,189,196). In contrast, studies report conflicting associations with outcome when highly potent antioxidant

supplements are evaluated separately (188,189) or in combination (186,190). It has also been proposed that prior use may influence the impact of the antioxidant if used during treatment (186).

The aryl hydrocarbon receptor

Antioxidant supplementation may also influence by other factors besides neutralization of ROS, such as the master regulator of estrogen metabolism, AHR. AHR is an environmental sensor that was first discovered to be induced by the herbicide 2,4,5-trichlorophenol by Alan Poland when investigating industrially acquired acne among factory workers (50). A multitude of compounds have been identified as AHR ligands, such as xenobiotics (*e.g.,* tobacco smoke), endocrine-disrupting chemicals (197,198), synthetic retinoids (199), omeprazole, and naturally occurring indoles and flavonoids (99).

AHR activation can occur through either the canonical pathway (Figure 7) involving gene expression or the non-canonical pathway, where AHR interacts with other signaling pathways (49,50). In the cell nucleus, AHR binds to xenobiotic-responsive elements (XREs) in the DNA with subsequent activation of CYP1 enzymes (*e.g.,* CYP1A1, CYP1B1), CYP19A1 (200-202), or antioxidants (4,99). Several mechanisms control AHR activity, including negative regulation by the AHR repressor (AHRR) (50,203,204).

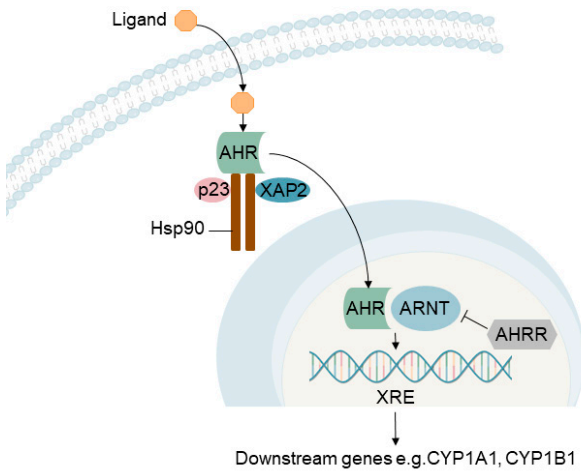

Figure 7. Inactive aryl hydrocarbon receptor (AHR) residing in the cytosol forms a complex with HSP-90, XAP2, and p23. Upon ligand binding, AHR is released from the complex, translocates to the nucleus, and forms a heterodimer with ARNT, which activates xenobiotic responsive elements such as CYP1A1 and CYP1B1. AHRR inhibits AHR function by competing with it for dimerization with ARNT. Abbreviations: AHR: aryl hydrocarbon receptor, ARNT: aryl hydrocarbon receptor translocator, HSP-90: heat-shock protein 90, XAP2 X-associated protein 2, XRE: xenobiotic responsive elements, CYP: cytochrome P-450 enzymes, AHRR: aryl hydrocarbon receptor repressor

Crosstalk between AHR and ER can result in estrogenic, anti-estrogenic, or anti-androgenic activity (50). The estrogenic effects are mediated by ligand-activated AHR binding to unliganded ER, while antiestrogenic effects involve increased estrogen metabolism and inhibition of binding to estrogen responsive elements in DNA (49,50,203). In addition, AHR activation can lead to enhanced degradation of ER (203) and androgen receptor (AR) (50) by AHR acting as an E3 ubiquitin ligase. The type of activation is suggested to be dependent on the availability of ARNT (203). When ARNT is available, AHR acts through the canonical signaling pathway, and when it is occupied, by AHRR or hypoxia-inducible factor 1 subunit alpha (HIF1-α), AHR functions as an E3 ligase (203). Forced overexpression of AHRR in the MCF-7 cell line reduces the expression of ER target genes *in vitro* and reduces tumor size *in vivo* (203).

AHR also interacts with EGFR and several different transcription factors (*e.g.,* STAT3, nuclear factor kappa-light-chain enhancer of B cells (NF-κB), and nuclear factor erythroid 2p45-related factor (NRF-2)) (50). NRF-2 is a key transcription factor that protects against oxidative stress, and AHR and NRF-2 have overlapping antioxidant-responsive elements (99). Xenobiotic induction of oxidative stress can activate both NRF-2 and AHR when either is knocked out in breast cancer cell lines (4), suggesting that AHR is important for redox homeostasis. AHR also maintains endogenous antioxidants in their reduced form, reduces lipid peroxidation, and scavenges superoxide (99). Silencing of ARNT in the MCF-7 cell line exposed to RT increases treatment sensitivity, whereas forced overexpression increases treatment resistance (205).

The wide range of possible mechanisms of AHR has yielded studies reporting either cancer-promoting or cancer inhibiting activities (99,197,198,203). In addition, the activation and response of AHR are highly specific to the ligand and breast cancer cell line involved, and the signaling pathways can differ between ER^+ and ER^- cell lines (197,198,203), as well as between tissues (204). Studies assessing breast cancer tissue by IHC have reported the presence of AHR^{cyt} and AHR^{nuc} at different levels in tumors (204,206,207) and in intratumoral non-epithelial tissues such as tumor infiltrating lymphocytes (204). Activation in other cancers such as lung, esophageal, bladder, colorectal, and head and neck cancer (HNC) have also been reported (208).

Generally, high levels of AHR^{cyt} are related to high levels of AHR^{nuc} (204,206-208), but different combinations occur (206). In a study on 920 patients from the BC-Blood cohort, AHR^{nuc} positivity was present in almost 32% of patients, and high levels of AHR^{cyt} were present in 80%. That study also reported different tumor characteristics in relation to AHR localization, where high levels of AHR^{cyt} were associated with ER^+, PR^+, lower histological grade, and lower frequency of TNBC tumors. Furthermore, AHR^{nuc} was associated with fewer PR^+ tumors (206).

The prognostic impact of AHR in breast cancer is not clear. Relapse-free survival time indicated improved OS, particularly for lymph node positive patients, when

assessing messenger ribonucleic acid (mRNA) *AHR* expression in the Gene Expression Omnibus database (207). A different study using *AHR* mRNA data from 439 tumors did not indicate any association between high and low *AHR* regarding metastasis-free survival, while high expression of *AHRR* mRNA improved metastasis-free survival (204). That study reported an association between high *AHR* mRNA and *AHRR* mRNA levels in only ER^- patients.

Furthermore, high *AHR* mRNA levels were related to high levels or *BRCA1* mRNA and *IL6* in ER^+ and ER^- breast cancers (204). In the BC-Blood study, patients with AHR^{cyt} levels had the most favorable clinical outcome regarding recurrence-free survival and distant metastasis-free survival, while patients with high AHR^{nuc} levels only had improved outcome during the first five years (206). Another study of IHC involving 302 patients with breast cancer reported worse OS for patients with nuclear AHR activity (207).

Head and neck cancer

Epidemiology and risk factors for head and neck cancer

HNCs involve tumors that arise in different subsites of the head and neck region: the nasopharynx, nasal cavity/sinuses, major salivary glands, lips, oral cavity, oropharynx, hypopharynx, larynx, and salivary glands, as well as head and neck carcinoma of unknown primary location (HNCUP). Tumors that arise in the mucosal epithelial lining are jointly referred to as head and neck squamous cell carcinomas (HNSCCs) and account for more than 90% of all HNC cases (209). Salivary-gland cancers and other types of cancers may also arise in the head and neck region and include malignant melanoma, lymphoma, and sarcoma (124).

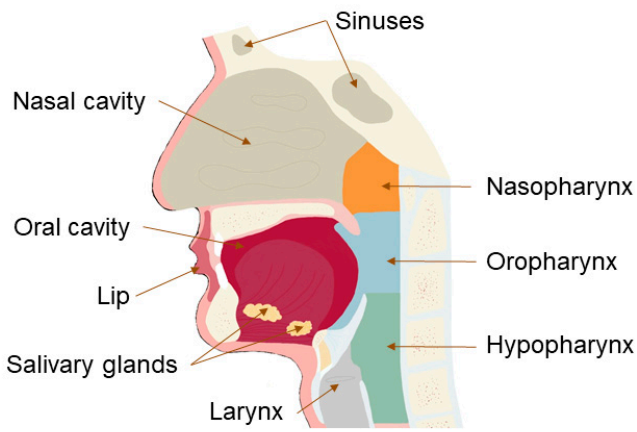

Figure 8. Schematic picture of tumor subsites of head and neck cancer

The global incidence of HNC is increasing, which is related to exposure to risk factors that vary with culture and global region (209). The latest report from the International Agency for Research on Cancer (IARC) presents the different tumor subsites are individually. If combined, HNC would represent the sixth most common type of cancer with more than 890,000 cases and would have the seventh highest mortality rate with more than 455,000 deaths in 2022 (2). Globally, cancers in the lip and oral cavity have the highest incidence, followed by tumors in the larynx, nasopharynx, and oropharynx (2). In Sweden, HNC constitutes 2.3% of all cancer diagnoses, and in 2022, 1064 men and 670 women were diagnosed (210). Tumors in the oral cavity and oropharynx are the most common tumor subsites and constitute about 60 % of all HNC in Sweden (Figure 9) (211).

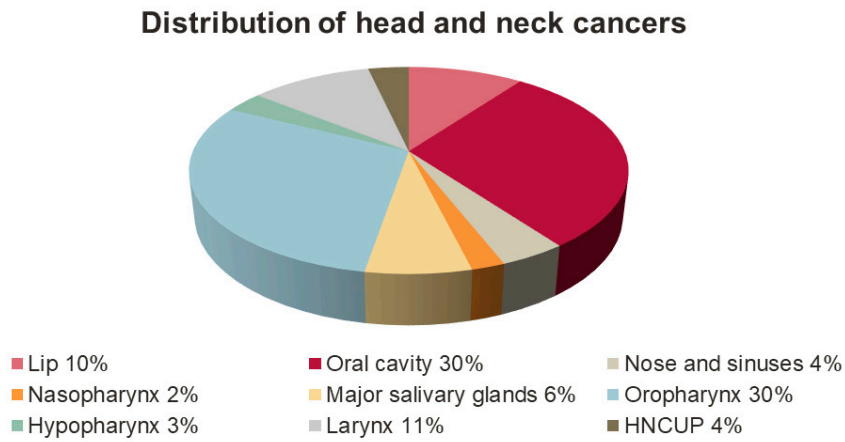

Figure 9. Approximate distribution of head and neck cancer cases in Sweden according to the Swedish head and neck cancer registry report for 2024 (211).

In Sweden, along with Western Europe and North America, the observed increase in HNSCC is strongly related to Human Papillomavirus (HPV) infections and an increasing incidence of oropharyngeal cancer (209,212,213). The incidence of oral cancer has also increased in Sweden in the last decades, while other HNC subgroups of tumor sites, such as laryngeal cancer and hypopharyngeal cancer, have decreased (214). In other regions, such as Southeast Asia, Australia, and India, consumption of products containing carcinogens, such as betel, increase the prevalence of HNSCC, particularly in the oral cavity (209).

The majority of patients with HNSCC are men, but the incidence pattern differs between different tumor subsites. In the case of oropharyngeal cancer, 72% of diagnosed patients are men in Sweden, while for oral cavity cancer and cancer of the major salivary glands, the sex difference is about 3% (210). The mean age at diagnosis also differs between the different tumor sites. Generally, patients with

HPV^+ oropharyngeal HNSCC are younger at diagnosis compared to patients with HPV^- (215), but a shift towards older age has been observed (213). In Sweden, the mean age at diagnosis for oropharyngeal cancer is 63 years, whereas patients with hypopharynx, larynx, and oral-cavity cancer have a median age of around 68 years (210).

Smoking and alcohol consumption

Smoking (70,215) and alcohol consumption (5,71,216) are well-known risk factors for HNSCC and have synergistic effects (70,73,217). Tobacco smoke contains more than 5300 compounds, including known carcinogens such as nitrosamines from the tobacco plant and polycyclic aromatic hydrocarbons that form when tobacco is burned (73). The reactive metabolites of tobacco smoke form covalent DNA adducts that can induce mutations (70) in genes such as the tumor suppressor gene *p53* (217). A Swedish report stated that smoking increases the risk for cancer in the lips, oral cavity, and pharynx more than fourfold and cancer in the larynx or hypopharynx 16-fold compared to non-smokers (48).

The metabolism of alcohol produces acetaldehyde, which is a reactive compound that has DNA-damaging abilities (70,216). The intensity and duration of alcohol consumption influence the risk of tumor development, which varies somewhat depending on the tumor subsites in the head and neck region (216). For heavy drinkers ($\geq$ 4 drinks per day or 50 g alcohol), increased relative risks are reported to range between 2.62 and 5.40 for cancers in the oral cavity, pharynx, or larynx (5).

Greater alcohol consumption is common among individuals that smoke (217-219). In combination with smoking, alcohol can function as a solvent for carcinogens from smoking and increase the permeability of the oral mucosa (73), leading to super-multiplicative increased risk (up to 14-fold increased risk for upper digestive and respiratory tract neoplasms among heavy smokers/heavy drinkers) (5). The synergistic effect may also increase acetaldehyde production by resident oral bacteria (73).

HPV infections

Infections with HPV are common sexually transmitted viral infections in men and women (220,221), and the majority of sexually active people become infected at some point during life. Most commonly, the immune system resolves the infection within two years, but in some cases, the infection persists and can lead to cellular changes (221,222). HPV is an established risk factor for oropharyngeal, cervical, anal, vaginal, and penile cancer (221,222). The presence of HPV in HNSCC was detected in the mid-1980s (221), but its carcinogenic role was not established until the early 2000s (213,223).

There are currently more than 200 known different strains of HPV, of which 12 types are classified as carcinogenic, and one is considered probably carcinogenic,

which are also included in testing for HPV in Sweden (222,224). HPV 16 and 18 are the most prevalent HPV types in cancer (224), including oropharyngeal cancer (212,213). Vaccination against HPV has been part of the national vaccination program in Sweden since 2012 (220,222,225) and currently covers nine different HPV strains, including HPV 16, 18, and 45, which have high oncogenic properties.

The vaccine was initially given to girls, but since 2020, it has also been offered to boys (226). A catch-up program for unvaccinated women is in place, and it has been recommended that it should also be initiated for men up to the age of 26 years (222). In 2023, the vaccination coverage with a single dose was 91% for girls and 87% for boys in Sweden (222), and a continuous decline has been reported for HPV 16/18 in cervical screening tests from birth cohorts of women offered school-based vaccination (220).

Additional risk factors

In certain parts of Asia, endemic infection by the Epstein–Barr virus is the main cause of nasopharyngeal cancer (209). Exposure to fine particles such as wood dust increase the risk for sinonasal cancer. Higher doses of ionizing radiation increase the risk for cancer in the major salivary glands, and sun exposure increases the risk for cancer of the lips (226). Poor oral hygiene (215) and the presence of premalignant leucoplakia (227) increase the risk of oral cancer. The incidence of oral cancer in younger populations without traditional risk factors has increased in the last decades, and women are more often affected than men (227,228). A family history of early onset of cancer has been proposed as a potential factor for this patient population (229).

Prognosis

The prognosis for HNC is strongly related to the etiology and tumor localization. Generally, survival rates have improved modestly in the last decades (209). Improved outcome is primarily observed in HPV-positive oropharyngeal cancer (230), for which the 5-year relative survival was 71% in Sweden in 2008–2017 (210). The corresponding 5-year relative survival rates for oral cavity, larynx, and hypopharyngeal cancers in Sweden are 62%, 67%, and 25%, respectively (210).

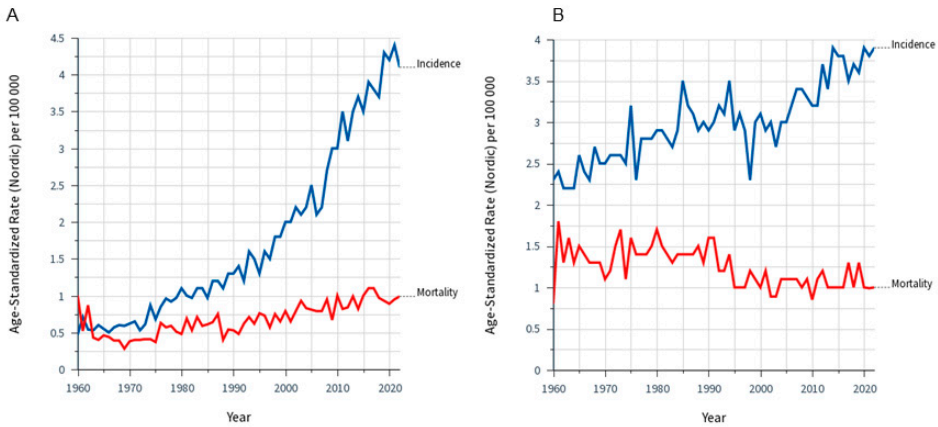

Figure 10. Age-standardized incidence and mortality rate of (A) oropharyngeal cancer and (B) oral cancer in Sweden between 1960 and 2022. © NORDCAN database, provided by the International Agency for Research and Cancer.

Head and neck carcinogenesis

Mucosal epithelial cells line the oral cavity, pharynx, larynx, and sinonasal tract and are continuously renewing. Exposure to carcinogens and viruses can transform normal adult cells and progenitor cells into cancer cells, potentially including cancer stem cells that harbor pluripotency and self-renewing properties. A complex interplay with surrounding cells, tissue, and the immune system can eventually lead to tumor development. In HNSCC, the tissue usually follows orderly histological changes from normal mucosa to hyperplasia, dysplasia, carcinoma in situ, and, eventually, invasive carcinoma (209).

HPV^- tumors often display a higher mutational burden than HPV^+, and HPV-driven carcinogenesis yields distinct tumor characteristics (7,231,232). The properties of the tonsillar tissue in the oropharynx enable virus particles to access the basal cell layer as a site for infection (213). Upon infection, the viral DNA may reside within the cell as episomes. When the basal cells start to differentiate, viral activity can be triggered in persistently infected cells. Thus, HPV increases the production of the viral oncoproteins E6 and E7 (213), and the increase of E6 and E7 can be accelerated by interruption of the *E2* gene, which is a natural suppressor for the *E6* and *E7* genes (213).

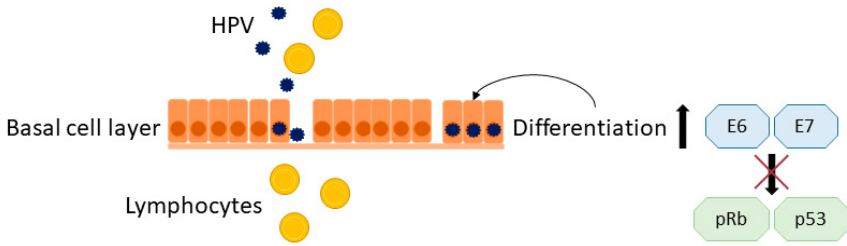

Figure 11. Schematic picture of HPV-induced infection, carcinogenesis, and increases in E6 and E7. Tonsillar cells at the basal level infected with HPV increase protein levels of E6 and E7 upon differentiation, which in turn inactivate the protein Rb in the retinoblastoma pathway and decrease p53 levels. Abbreviations: HPV: Human papillomavirus, pRb: retinoblastoma protein

In HPV-positive cancers, viral E6 and E7 inactivate the tumor suppressor p53 and the retinoblastoma (Rb) pathway (213,221). Inactivation of the Rb protein (pRb) removes the suppression of the transcription factor E2F, allowing tumor cells to bypass the pRb-dependent cell-cycle arrest, which results in high levels of p16 in cells (209). Immune infiltration levels differ between HPV^+ and HPV^- cancers (7,233). In the TCGA database, HPV^+-positive tumors have higher levels of cytotoxic T lymphocytes (T-cells) expressing cluster of differentiation (CD) 8 surface markers ($CD8^+$ T-cells) and lower rates of T-regulatory T-cells than HPV^- tumors (7).

In the study by van der Heijden e*t al.,* high levels of $CD8^+$ T-cells were only associated with good prognoses in patients with HPV^+ tumors (233). Additionally, high expression of immune-suppressive cytotoxic T-lymphocyte antigen 4 (CTLA-4) was observed for HPV^+ tumors, whereas expression of programmed cell death receptor 1 (PD-1) and PD-L1 and high expression of mature cytotoxic natural killer (NK) cells ($CD56^{dim}$) were similar between tumor types (7). An analysis using a smoking mutational signature demonstrated lower levels of T-cell immune infiltration with lower levels of T-cells and interferon gamma (IFN-γ) signaling independent of HPV status in smokers (7).

Clinical aspects

Diagnostics

The symptoms of HNSCC vary according to the anatomical site. Common symptoms are a palpable lump in the neck, difficulties in swallowing, hoarseness, and pain. The symptoms are often subtle, which can cause delay before patients seek health care (234). The diagnosis is confirmed by biopsy and imaging using CT or positron emission tomography and magnetic resonance imaging (MRI) (235). The majority of HNSCCs have metastases in regional lymph nodes at diagnosis, whereas distant metastases are less common (209).

HPV testing is recommended for oropharyngeal tumors (213), and the detection of HPV-DNA and HPV-mRNA is referred to as the gold standard (232,236). Overexpression of p16 can be assessed by IHC (236) and is recommended as a surrogate marker for HPV positivity in the TNM staging manual by the UICC (116). The cutoff for IHC p16-positivity usually requires >70% of strong staining present in the nucleus and cytoplasm, whereas weaker positivity requires additional testing (212,213,231,236).

Prognostic and predictive factors in HNSCC

HNSCC is classified according to the TNM classification and further categorized using subgroups of clinical stage. Given the distinct characteristics and favorable prognosis of HPV^+ oropharyngeal cancer, the 8th version of the TNM classification distinguishes between HPV^+ and HPV^- oropharyngeal cancer (116). Additionally, invasion depth in oral cancer and the presence of extracapsular extension nodal involvement have been added in the 8th edition (116).

Table 2. Clinical stage for p16-positive cancer in the oropharynx according to the UICC TNM classification of malignant tumors (clinical), 8th edition.

	T1	T2	T3	T4
N0	I	I	II	III
N1	I	I	II	III
N2	II	II	II	III
N3	III	III	III	III
M1	IV	IV	IV	IV

Abbreviations: T: primary tumor, N: regional lymph nodes, M: distant metastasis

For patients treated with surgery, the resection margin is an essential prognostic factor (116). Patient-related factors such as age, comorbidities (237), and performance status influence treatment tolerance and the choice of treatment (226). The Eastern Cooperative Oncology Group performance status (ECOG-PS) was developed specifically for oncological patients and yields an overall estimate of the condition of the patient (226). The ECOG-PS score can guide treatment choice (226) and influence clinical outcome (238,239).

Patients with HPV^+ oropharyngeal cancers generally display less comorbidity than patients with HPV^- cancer (237). In contrast, patients reporting high alcohol consumption and current smoking have more comorbidities (237). Additionally, smoking influences the efficacy of RT (116,240), and heavy past or ongoing alcohol consumption has been reported to decrease locoregional control following chemo- and radiation therapy (chemo-RT) (233).

The overall treatment time (OTT) is important in HNSCC (241) and reflects an increased capability for the repopulation of clonogenic cells during treatment triggered by RT (242). Higher levels of EGFR have been associated with worse

clinical outcome (233,243). Tumor hypoxia is common, particularly in larger tumors (233), and is associated with enhanced treatment resistance for RT and chemo-RT (233,244). Nimorazole is a drug that mimics the effect of oxygen in hypoxic cells and has been used as a radio sensitizer in Denmark following studies in the 1990s, where the combined treatment improved loco-regional control (245). However, in a recent phase III trial including older patients unfit for combined chemo-RT, the addition of nimorazole to RT did not significantly improve locoregional control or OS (236).

Established treatments for HNSCC with focus on RT

Treatment options for patients treated with curative intent include surgery, RT, and chemo-RT (226,246). The HNC region contains several critical structures for basal functions that can influence the possibility for radical surgery and preserved functionality. Therefore, the tumor location and resectability with acceptable side effects, together with patient tolerance, influence treatment choice. Conventional RT with curative intent is most commonly delivered with 2 Gy/day and 5 fractions per week to a total dose of 68−70 Gy to the tumor areas. Lymph nodes with risk of metastatic spread are usually treated with prophylactic RT doses (46−50 Gy) (226).

Meta-analyses of randomized trials report improved OS of 6.5% at five years for chemo-RT compared to RT alone (247), as well as an additional gain in clinical outcome for hyperfractionated chemo-RT (248). Altered fractionations are being explored to find the maximum gain in tumor control with the least risk of normal tissue complications (249-252). Besides total dose (which influences both early and late tissue complications), fractionation size and OTT influence early tissue complications, while for late tissue complications, fractionation size is the dominant factor (250,253,254).

Hyperfractionated RT with smaller doses per fractions at two or more fractions per day is beneficial in regard to late tissue complications (253). Thus, the total treatment dose can be increased to improve tumor control (251,255). Hyperfractionated chemo-RT is rarely used for HNSCC in Sweden outside clinical trials. This is mainly due to practical reasons since the treatment requires a break of at least six hours between sessions. Accelerated fractionation reduces the OTT by either reducing non-treatment days or using hyperfractionation, but it has been associated with increased treatment toxicity (249,252,254). A moderate acceleration with an increase of one fraction per week adopted from the DAHANCA 6/7 trial (250) is used at some centers in Sweden and to a large extent in Denmark and Norway.

Disease recurrence of HNSCC is often locoregional (255-257), and patients with HNSCC are predisposed to developing a new cancer in the head and neck area (240), as well as second primary tumors at other sites, especially in the lungs (240). Several vital organs reside in the head and neck region, so the total RT dose can be limited by constraints of critical organs at risk (226), such as the spinal cord, optical nerves,

and brain (252). Higher dose conformity and lower doses to normal tissues can be accomplished due to the development of more advanced treatment techniques in recent years, such as intensity-modulated RT (IMRT), volumetric modulated arc therapy (VMAT), and tomotherapy (226,258).

Treatment toxicity and adverse events

Treatment-induced side effects of RT influence treatment adherence (252) and substantially impact patients' quality of life (230,246,259). Treatment toxicity and side effects differ according to the tumor location, dose to adjacent normal tissue, fractionation schedule, concomitant or adjuvant treatment, and patient-related factors (226,246,252,254,260). Dysphagia and mucositis are common acute toxicities (235,246,254). Symptoms such as pain, xerostomia, and taste and smell alterations often arise during treatment (235,254), the ability for food intake can be severely influenced (261), and weight loss is common (259). Therefore, during and after treatment, surveillance and nutritional support are important to maintain patient weight and to prevent muscle loss, which is a hallmark of cachexia (261).

For patients with inadequate oral intake, enteral nutrition is necessary (235,246,257,261), but it is still important to encourage patients to maintain their swallowing function (261). In a single-center study with patients included in the ARTSCAN III trial, enteral nutrition at the end of treatment was more common among patients treated with cisplatin + RT compared to those treated with cetuximab + RT. However, no difference between the treatment groups was observed at the one-year follow-up (259). Another common side effect is radiation-induced dermatitis (235), and different interventions are used to alleviate the inflammatory reaction of the skin (262). Many patients also express high levels of fatigue during treatment, but within a year, the levels often return to their baseline (263). Late treatment toxicities include xerostomia, pain, trismus (235), fibrosis, and osteoradionecrosis (226,249).

Cisplatin

Cisplatin is the established first-line choice in chemotherapy for HNSCC (230,235,247). In a meta-analysis by Lacas *et al*., patients treated with concomitant platinum-containing chemotherapies had the lowest hazard ratios (HRs) for event-free survival (HR 0.74; 95% CI 0.67−0.82) (247). Cisplatin is an alkylating agent that forms adducts and crosslinks with DNA, resulting in cell-cycle arrest and cell death unless the damage is repaired (264). Additionally, cisplatin induces a mitochondrial-dependent ROS response that enhances the cytotoxic effect (98).

Cisplatin in combination with RT has traditionally been administered intravenously every third week (days 1, 22, and 43) at a dose of 100 mg/m^2 (230) with the aim of a cumulative dose of at least 200 mg/m^2 (265,266). A lower cumulative dose has been associated with reduced locoregional control (233). Due to the pronounced side effects, weekly administrations of lower cisplatin doses during RT have been

introduced, which have lower levels of toxicity and higher treatment adherence (235,265,266). Dose-limiting toxicities include nephrotoxicity, neurotoxicity, hearing impairment, myelosuppression (265,267), and oral and gastrointestinal toxicity (230). The high toxicity profile prevents patients with severe comorbidities from being treated with cisplatin, and dose reductions for treated patients are common (235,260).

Cetuximab

Cetuximab is a monoclonal antibody that is directed at EGFR (268), which is commonly upregulated in HNSCC (269). The effectiveness of cetuximab as a monotherapy is low (270), whereas its combination with RT was reported by Bonner *et al.* to improve OS by 9% compared to RT alone (271,272). Given the high toxicity burden of chemo-RT, several studies have been initiated to compare the addition of cetuximab to RT versus chemo-RT (230,235). Disappointingly, studies including mainly patients with HPV-positive oropharyngeal cancer have shown inferior results of cetuximab + RT compared to cisplatin + RT (230,235,257,273). In the NRG/RTOG 0522 study, including HNSCC of the oropharynx, larynx, or hypopharynx, OS rates at 10 years differed by 3.4% in favor of cisplatin + RT compared to cetuximab + cisplatin + RT (257).

Currently, cetuximab is less commonly used for patients with HNSCC in Sweden, but some sites and studies report use for patients who are not eligible for cisplatin treatment (223,274) and in a palliative setting (226). In combination with RT, cetuximab is administered with a loading dose of 400 mg/m^2 one week before RT, followed by weekly doses of 250 mg/m^2 during RT treatment (235,271). Common side effects include mucositis and acneiform rashes (235,273).

In addition to blocking EGFR, cetuximab acts through ADCC (268,275), which is mediated by the constant region (FcγIII) of cetuximab, which interacts with the CD16 receptor on monocytes and NK-cells (268,276). Studies are currently investigating the potential of combining cetuximab with immunotherapies (277,278), such as immune checkpoint inhibitors (279).

Immune checkpoint inhibitors

Immunotherapy has attracted increasing interest in oncology (277,280), and PD-1/PD-L1 inhibitors can be used as monotherapy or in combination with chemotherapy for patients with unresectable, recurrent, or metastatic HNSCC (281,282). In the KEYNOTE-048 trial, pembrolizumab (a PD-1 inhibitor) combined with chemotherapy showed improved outcomes compared to cetuximab combined with chemotherapy in the metastatic setting (282). For patients treated with curative intent, durvalumab (a PD-L1 inhibitor) was reported to be inferior to cetuximab for patients who were ineligible for cisplatin in the NRG-HN004 trial (274), and the addition of pembrolizumab to chemo-RT did not improve event-free survival in the recent KEYNOTE-412 study (283).

Sarcopenia and low skeletal muscle mass

The process of muscle wasting begins in early adulthood and increases with age (284-286). The term sarcopenia is defined as a combination of reduced muscle strength and muscle mass (16,287), which are often closely related symptoms (284). Age-related sarcopenia is denoted as primary sarcopenia, whereas secondary sarcopenia indicates that other causes influence the muscle-wasting process, such as organ failure, chronic disease, lack of physical activity, or inadequate nutrient intake (16). Studies have also reported sarcopenia in individuals with obesity, which is called sarcopenic obesity (16), where fat infiltration into muscles decreases muscle quality. This infiltration is increased with age and changes of fat deposits with lower levels of subcutaneous fat and higher levels of visceral fat (288).

In general, muscle mass and strength are higher in men than women (284,289), while longitudinal studies on older populations report earlier decline for muscle strength and muscle loss for men than women (284,289). Besides the primary function of aiding in body movements, muscles have important roles in regulating the immune system (290) and metabolic functions, and sarcopenic patients have increased risk of developing metabolic syndrome (288). Malnutrition, cachexia, and frailty are all differential diagnoses of sarcopenia and can each manifest as low muscle mass (16), so overlap between these syndromes is common (87). Figure 12 presents an example of how these syndromes were assessed in the population-based UK biobank study. The study reported an incidence of sarcopenia of 5.8% of included individuals, and all patients with sarcopenia in that study were defined as frail (87). In a systematic review of meta-analyses, the incidence of sarcopenia was 10–16% in elderly populations, with higher incidence in patients with different comorbidities (291).

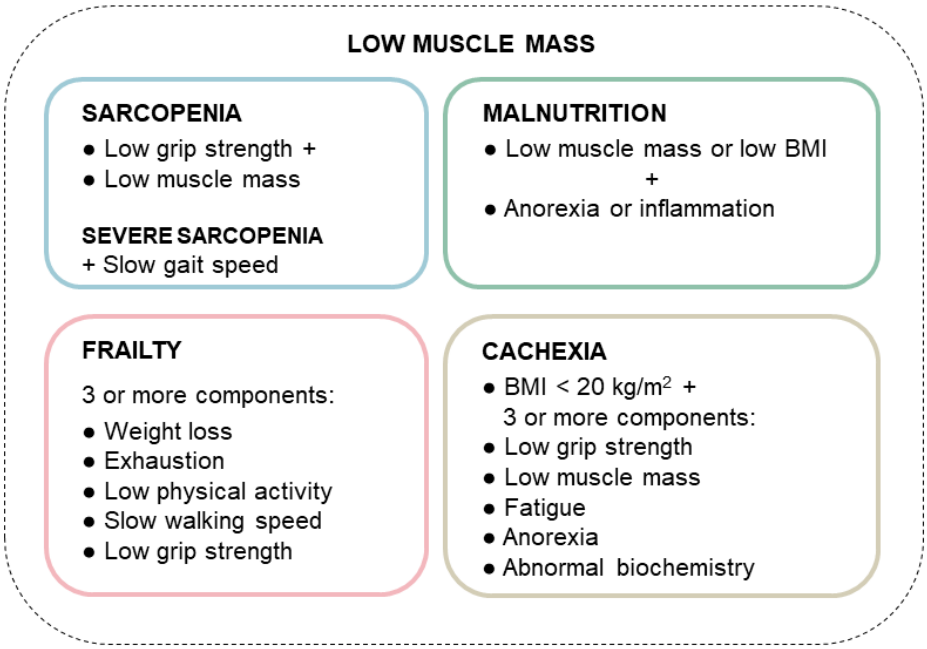

Figure 12. Sarcopenia, malnutrition, cachexia, and frailty often overlap and can all manifest as low muscle mass. The figure gives an example of commonly used definitions that were utilized by Petermann-Rocha et al. when investigating the cluster of these syndromes in the UK Biobank (87).

Estimates of low muscle mass can be used as an indicator of sarcopenia (17,285,286,292-294), and some studies still use the term sarcopenia for muscle-mass assessment (17), although this is not consistent with current definitions (291). In the geriatric population, the combined information of muscle mass and function yields stronger associations with common adverse events such as falls, self-reported mobility limitations, hip fractures, and death (287). In an oncological setting, where muscle-mass decline can be accentuated by disease conditions, shorter survival has been reported for patients with low muscle mass and different cancer types, including HNSCC (17,291-295). For patients with HNSCC, the tumor location increases the risk for involuntary weight loss, and reduced nutritional intake can be manifested as reduced muscle mass at diagnosis (9,296). This suggests that both primary and secondary sarcopenia can influence muscle loss in these patients.

With muscle loss that has already manifested, these patients may be more susceptible for treatment-related toxicities and more often require treatment interruptions or dose reductions (297,298). Few studies have investigated the prognostic role of low muscle mass in the context of a clinical trial with curative intent. Thus, there is a gap in knowledge about whether reduced muscle mass influences treatment adherence and clinical outcome, particularly in subsets of patients with less comorbidity.

Aims

Overall aims

The overall aim of this thesis was to investigate associations between patient and tumor characteristics in relation to outcome in patients with breast and head and neck squamous cell carcinoma.

Specific aims

Paper I

- To investigate how the tumor-specific level of pSTAT3^{Y705} is associated with patient and tumor characteristics and clinical outcomes in patients with primary breast cancer following different treatments in a Swedish breast cancer cohort

Paper II

- To investigate the association between pre- and postoperative antioxidant and multivitamin use, clinicopathological characteristics, and clinical outcome in different adjuvant treatment groups, with special focus on chemotherapy and radiation therapy, as well as the potential influence of antioxidants such as vitamin C on AHR activation

Paper III

- To evaluate the prognostic value of sarcopenia (defined as low skeletal muscle mass) in the ARTSCAN III patient population and whether sarcopenic patients have poorer treatment adherence

Materials and methods

The Breast Cancer and Blood cohort

The Breast Cancer and Blood (BC-Blood) cohort is a population-based prospective cohort of women diagnosed with primary breast cancer, used in studies I and II. The primary aim of the BC-Blood cohort is to investigate associations between host factors, lifestyle, and tumor characteristics in relation to prognosis. The BC-Blood cohort was initiated in 2002, and patients were enrolled at Skåne University Hospital in Lund (2002–2019) and Helsingborg Hospital (2006–2011). Women diagnosed with different cancer within the last 10 years or prior breast cancer at any time were not included in the study. A lack of research nurses or an unknown diagnosis were the main reasons for not being included in the study. Tumor tissue microarrays (TMAs) were constructed for patients included in Lund in 2002–2012 (n = 1018). Information from TMAs was used in study I (pSTAT3^{Y705}) and study II (AHR).

Table 3. Time period for inclusion, end of follow up, and median follow-up for patients included in studies I and II

	Time period for inclusion	End of follow-up	Median follow-up for patients still at risk, years, IQR
Study I	Oct 2002 – June 2012	June^{30th} 2016	5.6 (4.2–9.1)
Study II Lund	Oct 2002 – June 2018	June^{30th} 2019	4.8 (2.7–8.7)
Study II Helsingborg	Oct 2006 – June 2011	June^{30th} 2019	9.0 (8.0–11.1)

Upon inclusion in the study, prior to surgery, patients filled out a three-page questionnaire with questions concerning reproductive history, current medications, and several lifestyle factors such as exogenous hormone use, supplement use, smoking, and alcohol consumption. There were no questions about physical activity in the questionnaire. At this first visit, trained research nurses took anthropometric measurements, including height, weight, waist and hip circumference, and breast volume. The breast volume was measured using plastic cups, which is a method that was used by plastic surgeons at the time when the BC-Blood study was initiated (299).

Participants in the BC-Blood study were invited to follow-up visits with anthropometric measurements and filled out a one-page questionnaire at three to six months after inclusion. Patients treated with both adjuvant chemotherapy and RT were invited to an additional follow-up visit at seven to nine months after inclusion. All patients were later invited to an annual follow-up visit up to three years after inclusion. A questionnaire was then sent to the included patients biannually as of the fifth year following study inclusion (Figure 13).

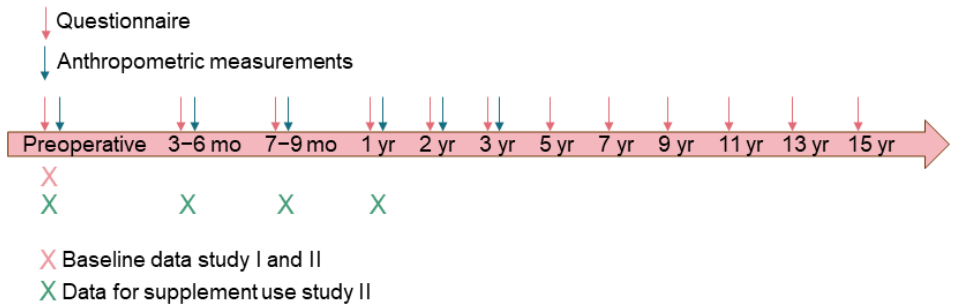

Figure 13. Timeline for data collection in the BC-Blood study. In studies I and II, baseline information from the preoperative visit was used. In study II, information on supplement use was collected from the preoperative visit and at the 3–6-month, 7–9-month, and 1-year visits.

Tumor information was gathered from pathology reports and included information on TNM classification, histological grade, and hormone-receptor status. Routine testing for HER2 was introduced into clinical routine in November 2005 for patients younger than 70 years with node-positive tumors. The HER2 information was supplemented with dual gene protein staining on TMA for patients lacking HER2 information (300).

Information on Ki-67 status was incorporated in clinical routine in March 2009. Since Ki-67 information was unavailable for the entire cohort, it was not included in the studies' statistical models. Adjuvant treatments were retrieved from patient charts and questionnaires. Date of death was collected from the Swedish Population Registry. Adjuvant treatments were considered until the first recorded breast cancer event, last follow-up, or death in case of no event.

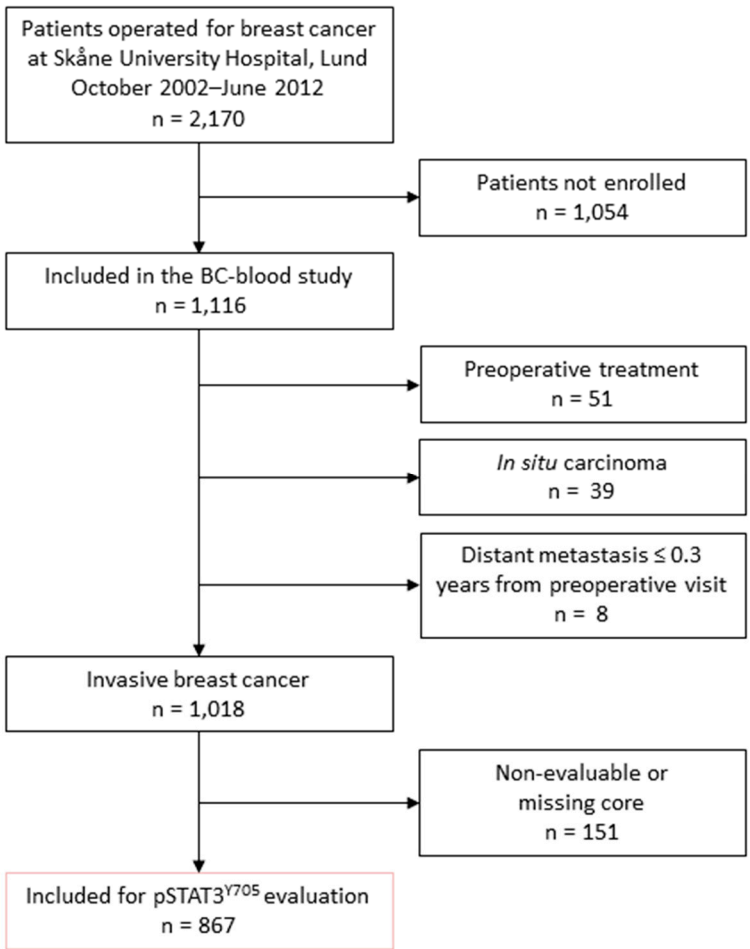

Figure 14. Flowchart of included and excluded patients in study I evaluating activation pSTAT3^{Y705} in the BC-Blood study.

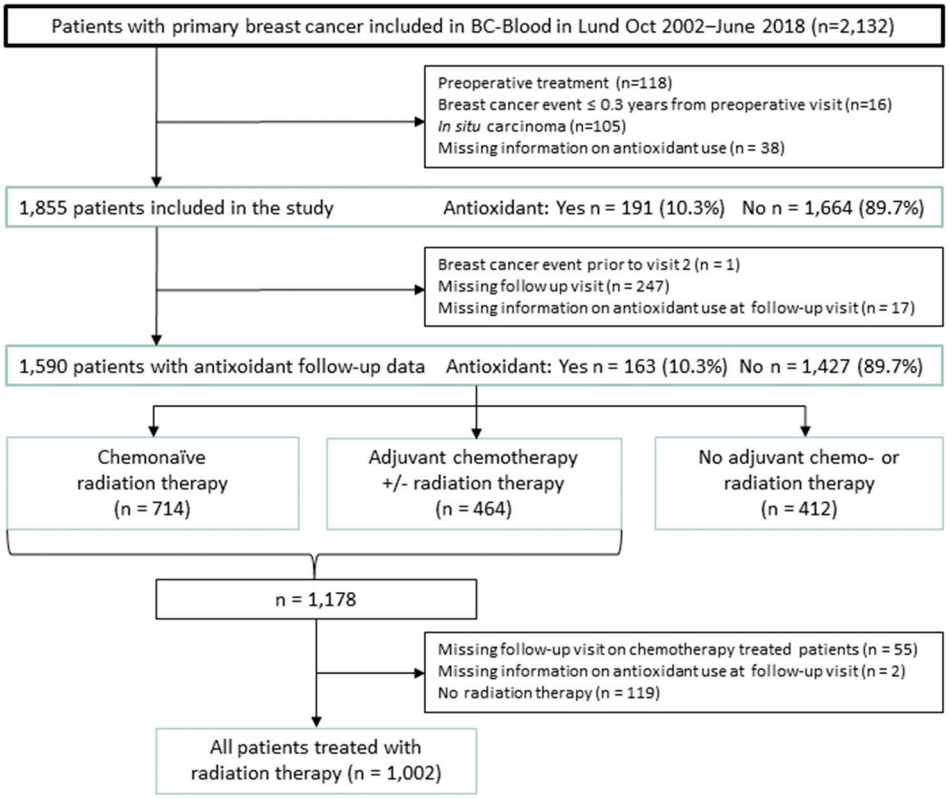

Figure 15. Flowchart of included and excluded patients in Lund in study II.

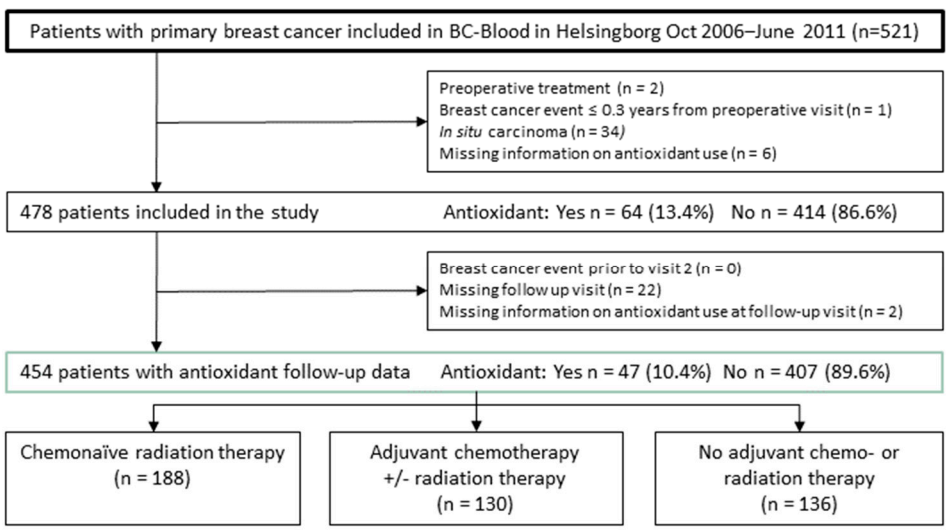

Figure 16. Flowchart of included and excluded patients in Helsingborg in study II.

Tumor tissue microarrays and immunohistochemistry

TMA is a commonly used technique to analyze markers in tumor tissue. The TMA is created from archival tumor tissue blocks by extracting cylindrical formalin-fixed paraffin-embedded tissue cores (1 mm) from representative tumor regions. The cores are then re-embedded in a single block with coordinates for each sample. One block can consist of many hundreds of patient samples, and each patient has duplicate tissue cores (301). The TMA in the BC-Blood study was constructed using a semi-automated tissue-array device (Beecher Instrument, Sun Prairie, WI, USA).

In study I, IHC was performed on TMA for pSTAT3^{Y705}. The pSTAT^{Y705} antibody is activated by STAT3α and STAT3β. The validation of homogenous distribution of pSTAT3^{Y705} has been reported previously (117), suggesting that staining of TMA would be representative for whole tumor tissue. Prior to IHC staining, the TMA block was sectioned into thin 4-µm sections, deparaffinized, and pretreated using an automatic PT-link system (DAKO, Glostrup, Denmark). The pSTAT3^{Y705} antibody (rabbit anti-STAT3 phosphor Y705, ab76315 Abcam, Cambridge, UK) has been validated in multi-tissue micro TMA (302) and HeLa cells by western blot. Staining was performed with the pSTAT3^{Y705} diluted to 1:100 for 30 min at pH 9 and an EnVision FLEX high-pH kit (10 min development time) using Autostainer Plus (DAKO). There was no staining for pSTAT3^{705} in tumor-adjacent normal ducts while the placenta (with a high rate of proliferation) was positive for the pSTAT3^{Y705} antibody.

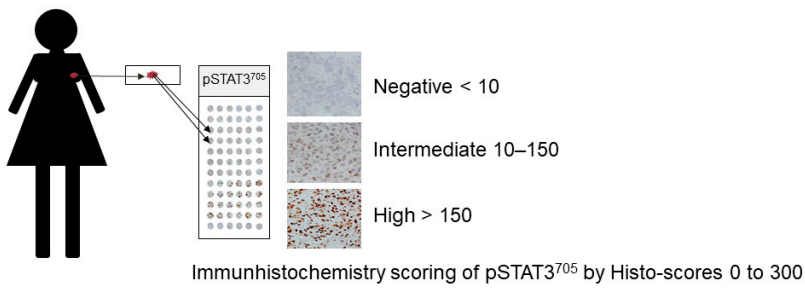

Figure 17. Immunohistochemistry using tissue microarray for pSTAT3^{Y705} with duplicate cores for each tumor. Scoring was performed using Histo-scores of 0 to 300, taking both intensity and fraction into account. Abbreviations: pSTAT3^{Y705}: phosphorylated signal transducer and activator of transcript 3 at residue tyrosine 705

Nuclear pSTAT3^{Y705} was scored independently by two observers (L.N. and E.S.), with support from a senior pathologist (K.J.), who were all blinded to other data. Duplicate cores were evaluated jointly across both cores. Since the pSTAT3^{Y705} staining was highly heterogenic, scoring was performed using the Histo-score (H-score), which takes both intensity (0–3) and fraction (%) into account (303). The fraction was scored in steps of 10 and calculated as follows:

$$0 \times (\%\ \text{cells intensity } 0) + 1 \times (\%\ \text{cells intensity } 1+) + 2 \times (\%\ \text{cells intensity } 2+) + 3 \times (\%\ \text{cells intensity } 3+)$$

All cases with scoring discrepancy were re-evaluated until consensus was reached. Statistical analyses were not performed to calculate the rate of discrepancy, which are otherwise a common approach to confirm the reliability of the scoring (300,303). Instead, the staining heterogeneity and scoring difficulties were taken into account when cutoff values were decided using the entire H-score scale despite having an uneven distribution. Although not quantified, stromal fibroblast showed both positive and negative staining of $pSTAT3^{Y705}$, indicating that $pSTAT3^{Y705}$ is present in both the tumor and stroma.

The sectioning, transfer, and staining procedures during the IHC staining of the TMA can result in loss of cores (301). Furthermore, a TMA that has been sectioned many times may have no remaining tumor tissue left, particularly when tumors are small. In study I, we considered cores with low numbers of tumor cells (<50 cells), cores with staining artefacts, and missing cores as non-evaluable. In total, 151 patients had non-evaluable or missing cores and were not included in the evaluation of $pSTAT3^{Y705}$. Since the missing data can lead to over- or underestimation of the tumor marker, it is important to compare evaluated and non-evaluated cases (301). In study I, women without evaluable cores had fewer children than women with evaluable cores.

In study II, levels of AHR reported by Tryggadottir *et al.* were used (206). In that study, AHR^{cyt} intensity scoring was categorized as either negative, weak, moderate, or strong. To obtain a dichotomized variable for AHR^{cyt}, negative and weak scores were combined and compared against the combination of moderate and strong scores. AHR^{nuc} was scored as positive or negative (206).

Storage of tissue samples can result in loss of antigen immunoreactivity (301). In study I, there was a significant correlation of time between surgery and staining for $pSTAT3^{Y705}$. A similar correlation was also reported for AHR levels used in study II (206). Analyses using these markers were adjusted for time between surgery and staining. Importantly, adjustment for time between surgery and staining will also adjust for other factors that have changed over time (*e.g.,* treatment protocols (124) and BMI (304)) in subsequent multivariate models.

At the time of study I and currently, there has been no consensus regarding cutoff levels of $pSTAT3^{Y705}$. We used three cutoff levels based on the calculated H-score: negative (0−9), intermediate (10−150), and high (160−300). In comparison to others (305-307), we allowed few weakly stained cells to be included in the negative group as well. Some studies use three (307) to four (306) different categories, and the median (117) and z-scores have also been applied (180).

In vitro studies

In study II, protein extraction and quantification by western blotting were performed by S.K. to investigate the AHR response to vitamin C at different concentrations and at different time points in two breast cancer cell lines: MCF-7 and MDA-MB-231. MCF-7 is a line of human breast cancer cells that are often used to represent ER-positive breast cancer since it has similar characteristics to a primary breast cancer. The MCF-7 cells were donated by Sister Catherine Frances, a 69-year-old nun who was initially diagnosed with a benign tumor in her right breast, followed by a malignant tumor in her left breast four years later. Despite radical mastectomy, adjuvant RT, and hormonal therapy (probably the high dose of the estrogen diethylstilbestrol), local recurrence was noted on the chest wall, which spread to the lymph nodes and lungs within three years.

The cell line was generated from malignant cells found in pleural effusion (45). The MCF-7 cells are used for their resemblance to luminal A tumors (43). When grown in cell culture, MCF-7 cells produce catechol estrogens (20), and estradiol induces upregulation of CYP1BI (308). The chromosome number has remained relatively stable over the years and is currently 82 (range 66 to 87), despite many years in culture. The high chromosomal number reflects the heterogeneity, adaptive abilities, and survival properties of MCF-7 (45).
The MDA-MB-231 is a highly aggressive human breast cancer cell line that was established in 1976 and is used to represent triple negative breast cancer lacking ER, PR, and HER2 positivity, as well as late-stage breast cancer. MDA-MB-231 was derived from pleural effusion from a 51-year-old woman with metastatic breast cancer (45). MDA-MB-231 has high expression of markers associated with epithelial-mesenchymal transition stem-cell-like properties ($CD44^{+}/CD24^{-}$) (309), and stemness (aldehyde dehydrogenase 1 family, member A1 (ALDH1)) (43). The MDA-MB-231 cell line also displays higher intrinsic levels of $pSTAT3^{705}$ (171) and AHR (198) than MCF-7.

The MCF-7 and MDA-MB-231 cell lines were purchased in 2019 from the American Type Culture Collection (ATCC, Manassas, VA, USA). The authentication of cell lines was performed by short tandem repeat. Since many passages of cell lines can introduce changes, such as mutations or phenotypical changes, the cell lines were never cultured for more than 20 passages. The cells were cultured at 37˚C in a humidified incubator containing 5% CO_2. The media was cell line specific Roswell Park Memorial Institute (RPMI) 1640 for MCF-7 and Dulbecco's Modified Eagle Medium (DMEM) for MDA-MB-231 (Gibco, Gaithersburg, MD, USA) with the addition of 10% fetal bovine serum (FBS) (Sigma Aldrich, Saint Louis, MO, USA) and penicillin–streptomycin (1%) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA).

Vitamin C addition and protein extraction

Upon vitamin C addition, cells were cultured in a volume of 25 cm^2 at a density of 5 × 105 cells/mL for 24 hours. The vitamin C (50 or 100 μM; Sigma Aldrich) was added at 1, 4, or 24 hours before cells were harvested. The concentrations of vitamin C were based on physiological plasma levels that are achievable by oral intake (310). Harvested cells were washed with cold phosphate buffered saline (PBS) (Gibco) and centrifuged for 5 minutes at 3000 revolutions per minute (rpm) at 4˚C. Protein extraction was performed by resuspending the cells in radioimmunoprecipitation assay (RIPA) lysis buffer (50mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate (Sigma Aldrich), and a protease and phosphatase inhibitor cocktail (Roche, Basel, Switzerland).

The lysates were placed on ice for 20 minutes to prevent protein degradation, and then the lysates were centrifuged at 13,000 rpm and 4˚C for 20 minutes. To determine the protein concentration, a Pierce BCA Protein Assay Kit (Pierce Biotechnology, Waltham, MA, USA) was used at 540 nm with a FLUOstar Omega microplate reader (BMG Labtech, Offenburg, Germany). Equal amounts of protein were separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (4–12% Bis-Tris-Protean gel, Invitrogen, Waltham, MA, USA).

Western blot

The extracted protein was transferred to a nitrocellulose membrane (Trans-Blot Turbo mini-Nitrocellulose Transfer Packs) (Bio-Rad, Hercules, CA, USA). To prevent non-specific binding of antibodies, membranes were blocked in Tris-buffered saline buffer containing 0.1% Tween-20 (TBS/T) with 5% bovine serum albumin (BSA) (Sigma Aldrich). The membranes were incubated with three different antibodies: monoclonal anti-aryl-hydrocarbon receptor antibody (ab190797, Abcam Cambridge), anti-CYP1B1 antibody (PA523139, Invitrogen), and the anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (MAB374, Sigma Aldrich, St Louis, MO, USA), which was used as control.

All antibodies had validated specificity by western blot. Protein visualization was performed using a secondary antibody conjugated horseradish peroxidase (Sigma Aldrich). Protein band detection was performed using SuperSignal West Dura Extended Duration Substrate (Thermofisher Scientific) with the Odessey imaging system (LI-COR Biosciences, Lincoln, NE, USA). The software Image Studio version 5.2 was used for densitometry analysis. The results were normalized by the density of GAPDH protein, which is a commonly used method to enable comparisons of protein levels between samples.

Antioxidant use

In paper II, antioxidant use in relation to prognosis was explored in the BC-Blood cohort. Information was gathered from preoperative and follow-up questionnaires asking what medications including supplements had been used in the last week. The constituents of the supplements were collected from retailers' web pages. The antioxidant variable combined the use of supplements, including vitamin A, C, E, carotenoids, and Q10. This antioxidant combination was previously used in an American study reporting a borderline 41% increase in incidence for recurrence of breast cancer if antioxidants were used both prior to and during chemotherapy, although no association was found for antioxidants included in multivitamins (186). Therefore, multivitamin use was recorded as any use of a supplement containing at least nine or more vitamins or minerals (182) without containing herbs, drugs, hormones, enzymes, or animal derivates.

The majority of the multivitamin supplements contained vitamin levels in the range of recommended daily intake. Patients reporting a supplement denoted as a "multivitamin" are also classified as taking a multivitamin supplement. The third variable, "non-MV antioxidant" (non-multivitamin antioxidant), excluded patients who only used multivitamins but contained some patients who used both "non-MV antioxidants" and multivitamins during the last week. To consider habitual use, patients were classified according to use prior to surgery (yes (Y) or no (N)) and use at the follow-up visit three to six months later, resulting in four different categories: NN, NY, YN, and YY. For patients from Lund, reported antioxidant use was evaluated for patients treated with adjuvant chemotherapy followed by RT based on data from the follow-up visits at the end of chemotherapy (three to six months after surgery) and a third visit at end of RT (seven to nine months after surgery).

Studies reporting antioxidant use in breast cancer have used different classifications for antioxidants (188). The majority of dietary supplements have antioxidant properties, doses, and combinations of constituents that influence the antioxidant capacity (311). Notably, among all patients included in the BC-Blood cohort at the time of supplement coding, about 430 different types of supplements, natural remedies, or traditional plant-based medicines were reported. Supplements could include between one and 40 constituents or more. In total, 365 different constituents were annotated. Only three brands of multivitamins included beta-carotene.

The ARTSCAN III trial

The ARTSCAN III study was a phase III (NCT01969877) multicenter randomized controlled trial comparing concomitant cisplatin plus RT to concomitant cetuximab plus RT for locoregionally advanced HNSCC. The study inclusion was prematurely closed after an unplanned interim analysis in 2018. In total, 298 patients were

included between 2013–2018. The five-year follow-up showed that patients treated with cetuximab had 11% lower survival rates than those treated with cisplatin (312).

Patients included in the ARTSCAN III trial were diagnosed with stage III–IV squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx according to the UICC TNM classification (7^{th} edition) (313) without distant metastases and were eligible for treatment with curative intent. All patients were aged 18 years or older. Patients were required to have no previous malignancies, pre-existing hearing loss, pre-existing neuropathy, or severe cardiac illness. Adequate renal, bone marrow, and liver functions were also required. Only patients with performance status of 0–2 were included in the study. Prior to treatment, a biopsy and a CT scan were performed to verify the diagnosis. The p16 marker was used as a proxy for HPV^{+} disease (235).

Included patients were randomized 1:1. Cisplatin was administered weekly with a dose of 40 mg/m^2. Treatment with cetuximab was administered as an induction dose (400 mg/m^2) one week prior to RT, followed by weekly doses of 250 mg/m^2 (271). The primary tumor and lymph-node metastases were treated with 68.0 Gy in 34 fractions, and elective lymph nodes were treated with 54.4 Gy in 34 fractions.

A second randomization was performed for patients with T3–T4 tumors between using 68.0 Gy and 73.1 Gy on the primary tumor volume. Treatments were given with five fractions per week. In cases of treatment interruptions (planned or unplanned), four sessions of hyperfractionation (with at least 6 hours apart between treatments) were allowed in order to stay within the predefined OTT of 49 days. RT was given with either VMAT, IMRT, or helical tomotherapy (235). Patients were invited for follow-up visits every three months in the first two years and then every six months until five years after treatment completion (235).

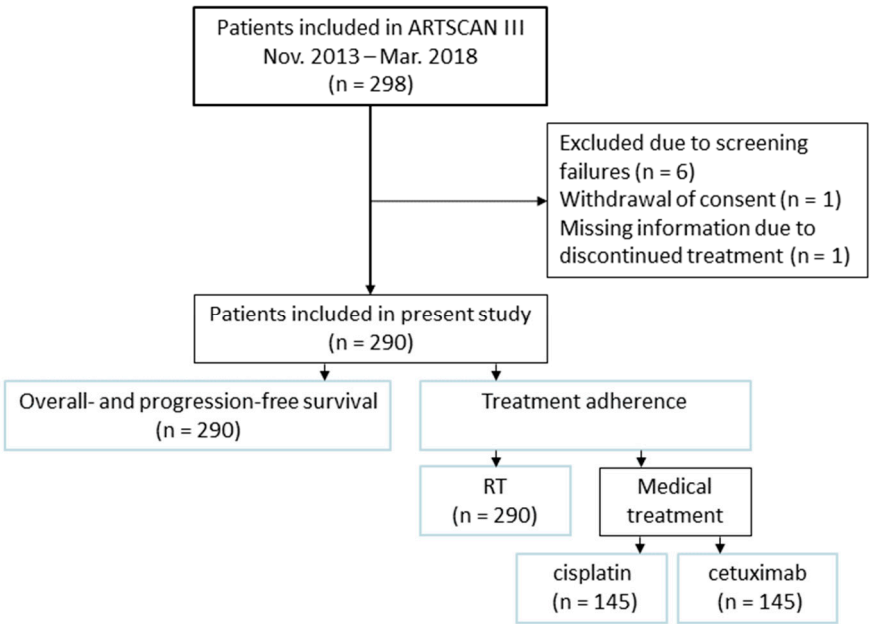

Figure 18. Flowchart of included and excluded patients in study III.

Skeletal muscle mass assessment

Methods to assess muscle mass include imaging techniques (dual X-ray absorptiometry, CT, MRI), bioelectrical impedance analysis, anthropometric measurements, and biochemical markers (17,293). Shen *et al*. first presented the method of using cross-sectional skeletal muscle area (CSA) at the level of lumbar vertebra 3 (L3) as a representative value for whole-body muscle mass for healthy adults (314) and by Mourtzakis *et al.* for cancer patients (315). The method has grown in popularity in research because it allows for retrospective evaluation of muscle mass in relation to clinical outcome (9,17,293,316-318).

Since many patients with cancer do not regularly undergo CT scans of the L3 region, various levels have been explored as alternative sites (318-320). In 2016, Swartz *et al*. reported a correlation between skeletal muscle mass at L3 and that of cervical vertebra 3 (C3) (319), which has been confirmed in subsequent studies (318,321) (322). Swartz *et al*. also suggested a prediction model for calculating the CSA of skeletal muscles at L3 by using that of the C3 level for HNSCC patients:

$$\text{CSA at L3 } (cm^2) = 27.304 + 1.363 \text{ x CSA at C3 } (cm^2) - 0.671 \text{ x age (years)} + 0.640 \text{ x weight (kg)} + 26.442 \text{ x sex (1 for female and 2 for male)}(319)$$

The prediction model was based on a cohort of HNSCC patients: 40 men and 12 women with a median age of 61.9 years and a median BMI of 24.3, and the majority (73%) of patients had oropharyngeal cancer (319). Validation studies report strong correlations between the measured and estimated volume at L3 (322,323), but there are also reports of both underestimations (322) and overestimations (323). To obtain a CSA value that is independent of height, the CSA is most commonly normalized by the height squared and denoted as the skeletal muscle index (SMI cm^2/m^2) (318,322,323). An alternative method is to normalize using BMI (16). It has also been suggested that normalization using height may be more appropriate for taller populations (324), which may become more important if younger populations are evaluated in regard to muscle mass.

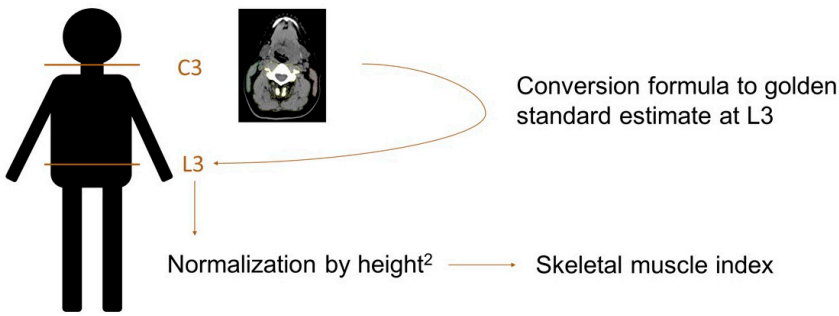

Figure 18. Delineation of paravertebral and sternocleidomastoid muscles was performed at the level of cervical vertebra 3 (C3) and converted to muscle mass at the level of lumbar vertebra 3 (L3), which is considered the gold standard level for cross-sectional muscle mass assessment. The muscle mass estimate is normalized by the patient height squared to remove the association between muscle mass and height, yielding the skeletal muscle index.

The proposed method by Swartz *et al*. has been applied to examine the clinical outcomes of patients with HNSCC (10,238,297,298,325) and was used in study III. The skeletal muscle areas of C3 were acquired using Eclipse version 16.1 (Varian Medical Systems, Palo Alto, CA) and CT scans obtained for treatment planning. Muscle delineation was done using the first slice scrolled from the caudal to the cranial direction of C3 and showed the entire vertebral arc and the transverse and spinous processes. The sternocleidomastoid muscles and paravertebral muscles were identified using a Hounsfield unit (HU) range of -29 to +150 and delineated separately.

Delineation was primarily performed automatically using the Image Thresholding function, followed by manual correction in cases of delineation of non-muscle tissue. The procedure was performed by L.N. and controlled by M.G.-M. In 37

patients, the sternocleidomastoid muscles were affected by tumor infiltration. For these patients, the volume of the affected side was substituted with the volume of the unaffected contralateral sternocleidomastoid muscle (319,322,323). The volumes were exported from Eclipse by a developed Python script and corrected by the thickness of the CT slice to obtain the CSA. A sample of volumes from 20 patients was cross-checked using the Raystation treatment-planning system (Version 12A SP1, Raysearch Laboratories, Stockholm, Sweden).

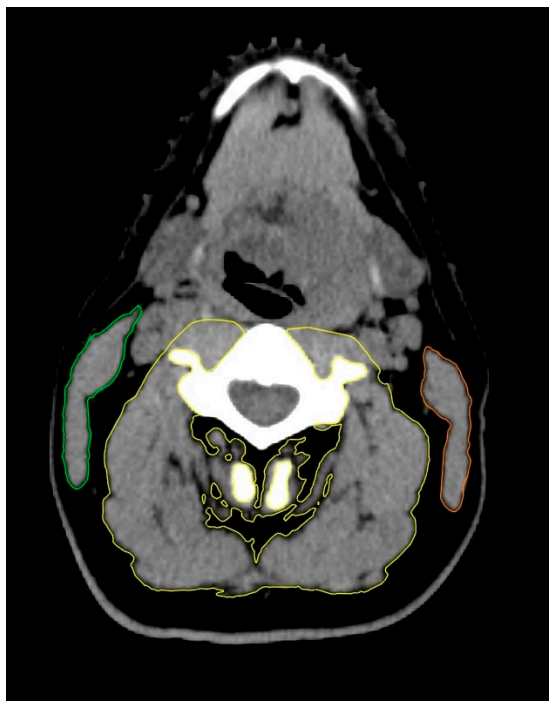

Figure 19. Delineation of paravertebral and sternocleidomastoid muscles at the level of cervical vertebra 3.

Cutoffs to define low skeletal muscle mass (SMI_{low}) in cases of HNSCC differ between studies. In study III, we used sex-specific quartiles to define SMI_{low} (<45.7 cm^2/m^2 in men and <33.0 cm^2/m^2 in women). Associations were reported between OS and PFS for SMI_{low} and SMI as a continuous variable. Two common approaches for cutoffs in cancer cohorts are those suggested by Martin *et al*. (295) or Prado *et al.* (326). Martin *et al.* included patients with gastrointestinal (GI) or respiratory tract cancer (n = 1473), while Prado *et al.* specifically focused on obese patients with GI, respiratory, or colorectal cancers (n = 250). These studies both used optimum stratification for OS do define SMI_{low}. See Table 4 for comparisons between different cutoffs used in different studies.

A general recommendation from the European Working Group on Sarcopenia in Older People is to define sarcopenia as two standard deviations below the population mean (16,320). Using the 5th percentile has also been suggested, and reference values assessed from a population of healthy kidney donors in Europe have been published (286). Table 4 presents a summary of cutoffs and cutoff approaches in different studies using the CT-derived cross-section SMI referred to in this thesis.

Table 4. Summary of cutoffs applied in different cohorts to define low skeletal muscle mass using computed tomography scans at the level of L3.

Study	Cohort	SMI_{low} cm^2/m^2 men	SMI_{low} cm^2/m^2 women	Comment / studies using suggested cut-off
Study III [a]	HNSCC	< 45.7	< 33.0	Lowest sex specific quartile
Van der Werf *et al.* 2018 (286)	Kidney donors (Europe)	< 41.6	< 32.0	5th percentile (age 20−82)
Derstine *et al.* 2018 (320)	Kidney donors (USA)	< 45.4	< 34.4	Two standard deviations below mean
Prado *et al.* 2008 (326)	GI, respiratory or colorectal cancer	< 52.4	< 38.5	Optimum stratification for OS in patients with obesity Applied in HNSCC studies: Grossberg *et al*. 2016 (317) Fattouh *et al*. 2018 (327)
Martin *et al.* 2013 (295)	GI or respiratory cancer	< 43.0 / < 53.0	< 41.0	Optimum stratification for OS, specific cut-off for men with BMI > 25 Applied in HNSCC studies: Findlay *et al*. 2021 (9) Ganju *et al*. 2019 [a] (297) Vangelo *et al*. 2022 [a] (323) Ufuk *et al*. 2019 (318)
Rijn-Dekker et al. 2020 [a] (238)	HNSCC	< 42.4	< 30.6	Lowest sex specific quartile
Huiskamp *et al.* 2020 [a] (325)	HNSCC	< 45.2	< 45.2	Log-likelihood for dose limiting toxicity of cetuximab
Wendrich *et al.* 2017 [a] (298)	HNSCC	< 43.2	< 43.2	Optimum stratification for chemotherapy dose limiting toxicity
Karavolia *et al.* 2022 [a] (10)	HNSCC	< 42.0	< 31.2	Lowest sex specific quartile
Zwart *et al.* 2022 [a] (328)	HNSCC	< 46.49	< 37.9	Occurrence of adverse events, all toxicities
Caan *et al.* 2018 (316)	Breast cancer	-	< 40.0	Optimum stratification for OS

[a] Measurements at the C3 level are used and converted to the L3 level using the prediction model suggested by Swartz *et al.* (319). Abbreviations: SMI_{low}: low skeletal muscle-mass index, HNSCC: head and neck squamous cell carcinoma, GI: gastrointestinal, OS: overall survival, BMI: body mass index

Variables and cutoffs

A research study includes not only the exposure and outcome variable, but also several other variables that need consideration. In the following section, some of the variables used in the different studies are discussed.

Alcohol consumption

At the preoperative visit in studies I and II, participants reported how often they consumed alcohol (never, ≤ 1 time/month, 2−4 times/month, 2−3 times/week, and more than 4 times/week) and the number of drinks consumed during the last week (none, 1−3 drinks, 4−9 drinks, 10−19 drinks, and more than 20 drinks). Simonson *et al*. evaluated alcohol use in the BC-Blood study, and the frequency of alcohol consumption showed a strong correlation with the reported number of drinks had per week. Therefore, patients were reclassified into four groups using the reported weekly alcohol consumption: none, low (1−3 drinks), moderate (4−9 drinks), and high (10+ drinks) (329). In studies I and II, alcohol consumption was dichotomized as "no consumption" or "any consumption" using the final categorization by Simonsson *et al.* (329).

The questions regarding alcohol use were derived from the AUDIT (Alcohol Use Disorders Identification Test) questionnaire (330). A shorter version of the AUDIT-C with the initial three questions is frequently used and has been validated (330). The alcohol variable in studies I−II lacks information about duration of use. It is also possible that patients who cautiously abstained from alcohol prior to surgery were misclassified. Interestingly, some patients recruited early in the study cohort report that they were recommended to drink a glass of wine to ease their worry while awaiting surgery. The recommendations regarding alcohol use prior to surgery have thus changed substantially during the course of the study.

Information on alcohol consumption was not included in the ARTSCAN III trial due to the unreliability of questions regarding alcohol consumption. A recent population-based study from Sweden confirmed that an AUDIT-C score of 0 can be used to exclude current excessive drinking. Among patients with moderate or excessive alcohol consummation, the AUDIT-C questionnaire underestimated or overestimated alcohol consumption in comparison to PEth (phosphatidylethanol) levels in serum (218).

Body mass index and waist-to-hip ratio

The increase of overweight and obesity in the general population (331) is reflected in cancer patients (296,304,327,332). The BMI was developed as a measure of excess body weight and was used in studies I−III. In papers I and II, the waist-to-

hip ratio was also used to assess central obesity and was measured by trained research nurses. The World Health Organization's classifications for overweight (≥25 kg/m^2) (333) and central obesity (waist-to-hip ratio > 0.85) were applied to these measures (334).

BMI is an easy and accessible measure (333), but it does not distinguish between fat tissue and other tissues such as bone and muscle tissues (316,335) and displays ethnic and geographical variations (291,335). A higher BMI is associated with worse clinical outcome in breast cancer (304,336,337). Additionally, underweight at diagnosis has been associated with increased incidence of death for female patients with breast cancer (337). A study including more than 3000 women with breast cancer in stages II and III proposed that CT-derived cross-sectional measures of total adipose tissue and skeletal muscle mass may associate more strongly with OS than BMI (316). In HNSCC, BMI at treatment initiation is often influenced by the severity of the disease and the nutritional status of the patient (9,338), as well as prior weight loss (296).

Exogenous hormone use

In studies I and II, information on the use of oral contraceptives and estrogen-containing MHT in relation to the exposure variable was assessed. Subgroups of oral contraceptives or MHTs were not defined. Use was defined as ever use, regardless of current/previous of use and its duration. Regarding MHT, approximately 40% of patients did not remember what type of MHT had been used (339). Intrauterine devices with progestin were not classified as MHT (339).

Smoking status

In studies I and II, smoking status was based on the categorization performed in a previous BC-Blood study (340). The categories were based on whether patients defined themselves as non-smokers, smokers, or occasional smokers. Patients reporting any cigarette use and occasional smokers were also defined as smokers (340). The proportion of patients who were current smokers at the time of inclusion in the BC-Blood study decreased between 2002 and 2016 (304).

In study III, the categories for smoking status were "never smoker," "smoker," and "previous smoker." Dichotomization of the smoking variable combined smokers and other/previous smokers in the multivariable model. Smoking status was also assessed in relation to low skeletal muscle mass to explore the combination of never smokers and previous smokers compared to smokers. Measures of cumulative use, such as pack–years, can provide more information regarding smoking status (215,341) and facilitate comparisons of studies (341). Another approach is to use molecular smoking signatures in tumor tissue (342). A study using the TCGA database suggested that this approach may be more accurate than collecting clinical

smoking history from HNSCC patients (7), which may reflect the difficulty of collecting representative data for smoking history.

Statistical methods

The choice of statistical methods depends on the characteristics of the data and how the data can be presented in a clear and meaningful way. To improve consistency in research reporting, recommendations and checklists have been published for research publications, such as the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for observational studies (343), and Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) for studies on tumor markers (344). Different statistical methods were used in the papers included in this thesis (Table 5). The application SPSS (versions 24, 26.1, and 28) was used in studies I and II, and the application RStudio (R Core Team 2023) was used in study III. Additionally, PS Power Sample Size Calculation Program version 3.1.2 was used for power calculation in study I. In study II, GraphPad version 8 (San Diego, CA, USA) was used for calculation and graphical illustration of *in vitro* data.

Table 5. Overview of statistical methods used in study I–III

Study	I	II	III	Comment
Descriptive statistics	x	x	x	
Shapiro-Wilks test			x	Test for normality
Group comparison tests				
Mann-Whitney *U-test*	x			Continuous vs 2 groups, non-parametric
Kruskal-Wallis test		x		2 or more groups, non-parametric
Chi-square		x		Categorical non-parametric
Fisher exact test			x	Categorical non-parametric
Student´s *t*-test		x		Continuous, parametric
Pairwise Fisher test			x	Post hoc, pairwise differences
Benjamini-Hochberger adjustment			x	Multiple testing
Spearman correlation	x		x	Non-parametric
Jonckheere Terpstra trend test	x			Continuous, non-parametric
Chi-square test for linear trend	x			Categorical, non-parametric
Receiver Operating Characteristic curve			x	Plot of test for specificity and sensitivity
Area Under the ROC Curve			x	Discriminative ability of test
Youden's index			x	Optimal cut-off identification
Logistic regression		x	x	Odds ratio
Kaplan-Meier	x	x	x	Univariate survival analysis
Log-rank test	x	x	x	Survival difference
Cox proportional hazards model	x	x	x	Multivariable survival analysis
Multiplicative interaction	x	x	x	Interaction calculation
Power calculation	x			Statistical power

Descriptive and inferential statistics

The purpose of descriptive statistics is to summarize information about included variables in an easy and comprehensible way for the reader to provide an overview of the data. By giving detailed information about study participants, before addressing possible associations, the reader can gain an understanding of how the study participants may differ from the population to which the results may be generalized and study groups being compared (343,345-347). In contrast to descriptive statistics, the aim of inferential statistics is to estimate characteristics and test research hypotheses about an underlying population based on a study sample.

To determine evidence of differences or relations between variables, hypothesis testing is performed. The null hypothesis states that there is no evidence of difference or relation between the variables included in the test. To quantify the evidence against the null hypothesis, a *P*-value (probability value) is calculated. The *P*-value is defined as the probability of observing a difference or relation that is as strong as or stronger than that observed when assuming that the null hypothesis is true. A general limit for statistical significance is 5% ($P < 0.05$), as was used in all three studies. A lower *P*-value indicates stronger statistical evidence against the null hypothesis, suggesting that the observed effect is unlikely to be due to chance alone.

It is important to evaluate *P*-values within their context, preferably together with a confidence interval that indicates the uncertainty of the result (345). Rejecting a null hypothesis that is true is called type I error, whereas failing to reject the null hypothesis if it is false is called type II error (Table 6). If multiple hypotheses are tested simultaneously, the false discovery rate and the risk to for type I errors increase. To decrease the false discovery rate, methods for multiple testing can be used. In study III, Benjamini–Hochberger adjustment for post-hoc tests was performed. The Benjamini–Hochberger method is considered less conservative than Bonferroni correction and was chosen to balance the risk of introducing type II error.

Table 6. Type I and type II errors

	H0 false	**H0 true**
Reject H0	Yes	Type I error (false positive)
Do not reject H0	Type II error (false negative)	Yes

All three studies included in this thesis investigated associations between patient characteristics, tumor characteristics, and variables of interest, so testing was performed on what would otherwise have been considered baseline characteristics. If possible and meaningful, descriptive statistics and measures of associations can be presented in a joint table (347), as was performed in all three studies. An association is a relation between two variables, while a correlation describes how

the variables change in relation to each other and can be both positive and negative (348). The characteristics of the data determine what statistical method should be used (Table 5). Data visualization with histograms or plots can be applied, as well as tests for normality.

Non-parametric tests are often used for small datasets and variables that are not normally distributed and were used in all three studies. Non-parametric tests are based on the difference in the median or rank, which makes them more robust when outliers are present. Non-parametric tests are not as powerful as parametric tests but can be used for normally distributed data, which can limit the number of different types of tests in a table to yield a uniform comparison between variables. In cases where there is doubt about whether the assumptions for a parametric test are fulfilled, non-parametric methods should be used. One approach to manage variables that are not normally distributed is to transform the variable, such as by log transformation.

In a correlation analysis, there is no dependent or independent variable. Regression analysis estimates the effect on a dependent variable by one or more independent variables. In a linear regression model, the dependent variable is continuous, whereas in logistic regression, the dependent variable is binary, while the independent variables can be continuous or categorical in both cases. The effect for each variable on the dependent variable is estimated in linear regression by estimating the regression coefficients for each variable using the least square method. In logistic regression, the coefficients represent the log-odds of an event occurring. For easier interpretation, log-odds are often converted to odds ratios using Euler's number (studies II and III).

Interaction analyses

Interaction analyses are performed to examine different effects of an exposure on a dependent variable in subgroups of other variables. Effect modification and interaction are two types of differential effects. Effect modification denotes that the causal effect of one exposure varies across different strata of another exposure, whereas interaction refers to the combined effect of two exposures together (349). Interaction analysis indicates that if data should be stratified and analyzed separately, an interaction term can be added to the model. If there are interactions in a model, it will be incorrect since the variables are treated as independent predictors.

In all three studies, multiplicative interaction calculations were performed before stratification. Interaction analyses can become significant by chance (as with all statistical tests). Therefore, it is important to evaluate whether the proposed differential effect is plausible. For well-defined groups, stratification without interaction analyses is possible. One example is hormone-receptor-positive breast cancers in a dataset of patients with breast cancer.

Collinearity and multicollinearity

Collinearity and multicollinearity indicate that two or more independent variables partly measure the same thing. In such cases, one or more of the variables should be excluded from the model. Multicollinearity can be estimated using tests such as tolerance and the variance of inflation factor (VIF). In study I, we experienced a problem with multicollinearity in the multivariate model based on clinically relevant variables. Creation of directed acyclic graphs (DAGs) (350) for visualization of causal associations between variables (351) and VIF analysis might have helped to create a better model.

Survival analyses

Endpoints

Different time-to-event endpoints were used in the studies of this thesis and are presented in Table 7 together with the definitions of the endpoints. To establish uniformity in time-to-event definitions, the Definition for the Assessment of Time-to-Event Endpoints in CANcer trials (DATECAN) has published guidelines for endpoints in RCTs for several types of cancer. For breast cancer, the guidelines were published in 2015 (352), and the definitions were used in both studies I and II. Currently, the results for the HNSCC population and the DATECAN definitions have not been published. A recent publication from the Head and Neck Cancer International Group highlights the different definitions in RCTs for HNSCC (353). This study also suggests how censoring should be handled (353), which is an issue that was not defined in DATECAN (352).

Table 7. Different time-to-event endpoints with definitions in studies I–III

Study	I	II	III
Breast cancer free interval, Lund cohort; *Time from inclusion until any breast cancer event, (locoregional, contralateral or distant) censored at last follow-up, emigration or death before June 30th 2016 (I) and 2019 (II)*	x	x	
Breast cancer-free interval, Helsingborg cohort; *Time from inclusion until any breast cancer event, (locoregional, contralateral or distant) censored at June 30th 2019 and of emigration or death by June 30th 2019*		x	
Distant metastasis-free interval; *Time from inclusion until first distant metastasis, censored at last follow-up or death before June 30th 2016*	x		
Progression-free survival; *Time from randomization to the first event (locoregional, distant, recurrence or death from any cause, censored at last follow-up*			x
Overall survival; *Time from inclusion until last follow up or death*	x		x

OS is often considered the primary time-to-event endpoint in clinical trials and is required for drug approval in Europe and United States (354). A practical problem with OS is that it requires long follow-up with patients who have good prognosis. Therefore, surrogate endpoints have been developed with shorter follow-up time, such as progression-free survival (PFS) (354,355), which was used together with OS in study III. In Europe, PFS and OS can be used as primary endpoints for drug approval in cases where OS is included as a secondary endpoint (354). In observational studies, standardization of surrogate endpoints does not provide the same quality as an RCT, so the results are more difficult to compare between studies (354).

In study I, three different time-to-event endpoints were used. Since this study aimed to determine the prognostic role of a tumor marker, one can argue that different endpoints are justified. OS was included as a complement to the breast cancer-free interval (BCFI). The distant metastasis-free interval (DMFI) was added since the prognosis of a patient that experiences local or loco-regional recurrence is more favorable than that of a patient with distant metastases. The use of several survival outcomes is an advantage for observational studies (354), but a downside is that it comes with more statistical tests and thus increased risk for chance findings.

In study II, BCFI was selected as an endpoint since we wanted to assess the risk of breast cancer events among patients taking antioxidant supplements. In hindsight, OS could have been a good complement to BCFI. Using BCFI means that patients who die without a previous breast cancer event from other causes are censored. Since many patients with cancer die from other causes than breast cancer, this could have biased the results. A different approach could have been to use the competing-risks method with the BCFI endpoint.

In study III, OS and PFS were selected as time-to-event endpoints. The combination of events and deaths increases the total amounts of events in a small study population and has been shown to be valid in populations with HNSCC and p16-positive cancers (355). Since the majority of patients included in the ARTSCAN III trial had p16-positive oropharyngeal cancers, the PFS variable seemed reasonable to use as a complement to OS.

Censoring

Censoring in survival analysis depends on the chosen endpoint. Patients whose follow-up times are censored are either lost to follow-up, have no event of interest when the follow-up ends, or have a competing risk. For instance, death from a different disease or older age can be a competing risk that may influence the survival analysis (356). Therefore, it is important to report the reasons for censoring and how it was handled in the analysis (343).

In study II, the patient cohort from Lund and the validation cohort from Helsingborg had different follow-up approaches, so they were censored differently. This could

have introduced bias when comparing the different cohorts since study participants that were censored at the last follow-up in Lund might have had a different risk profile from patients that were censored at the end of the study period. In case censored patients had worse clinical outcome, patients left in the study appear to have a better prognosis than they actually have (informative censoring) and the true negative effect of the exposure is underestimated in that subgroup.

Kaplan–Meier and log-rank test

The Kaplan–Meier (KM) estimate is commonly used in univariate analysis of survival and was developed to estimate the probability of survival as a function of follow-up time. The KM function estimates the probability that an event will occur at a specific time point among patients that have not already experienced an event (357). A KM estimate is often presented with the log-rank test. The null hypothesis for the log-rank test is that there is no difference in survival between the compared groups.

In all three studies, KM estimates and log-rank tests were used to visualize the survival and quantify the evidence against the null hypothesis of equal groups of patients. The KM estimate assumes non-informative censoring, meaning that those who are censored should have the same risk as those who remain in the study. When censoring is performed in the presence of competing risks, there is a risk of overestimation using the KM estimate (357), so it is important to consider the possible impact of a competing risk when choosing both the endpoint and type of survival analysis (356). A common approach to handle competing risks is to use a cumulative incidence function that estimates the time to more than one event (357). In study II, patients classified as YY users (antioxidant use at both time points) in Lund were older than other user groups, so their overall risk of death was increased, which can be only partly adjusted for in a Cox regression model.

Cox proportional hazards model

The Cox proportional hazards model is a commonly used regression model for time-to-event analysis for both uni- and multivariable analyses. The Cox regression yields HRs which are relative effects of included variables on the incidence of the chosen outcome. An important assumption for the Cox regression is proportional hazards, meaning that HRs need ratios to be at least approximately constant over time. The proportional hazards assumption can be verified using different tests, such as Shoenfeld residuals or time-variant hazards modeling. Restricted mean survival time is a method that has recently been used more to handle survival data that violate the hazard assumption. This method was considered but not used in study III.

A multivariable model is used for prediction and to adjust for potential confounders. In all three studies, variables for the multivariate analysis were based on clinical experience. To avoid overfitting a model, a general rule is to have at least 15 events for each variable included in the model. An alternative is to reduce the number of

variables in the model or to use stepwise regression models. Results from stepwise regression models are sometimes more difficult to replicate in different settings since the regression model is adapted to a specific set of data. Therefore, the Cox proportional hazards model was used in all three studies, although some models could be regarded as overfitted according to the general rule described above.

The Cox proportional hazards model does not take competing risks into account. If competing risks are present, a cumulative-incidence competing-risk method should be used, such as the Fine and Gray method. The use of competing-risk models has gained more attention for cancer populations with high levels of comorbidities like HNSCC (235) compared to breast cancer. Some data suggest that the adjustment for competing risks could improve risk modeling in some cases (356,358,359). In study II, we considered using the Fine–Gray method, and in retrospect, it would have been interesting to see whether death was a competing risk.

Ethical considerations

The Declaration of Helsinki emphasizes that all research should be conducted with respect for human dignity, and the benefit of research must always surpass the risks. All participants must provide informed consent and have the right to withdraw from the study at any time. All participants included in studies I−III provided written informed consent to participate in the study, and all studies are approved by ethics committees. In studies I−II, ethical approval was obtained from the Lund University Ethics Committee (Dnr75-02, Dnr37-08, and Dnr658-09 with amendments). In study III, ethical approval for the ARTSCAN III trial was obtained from the regional ethical committee in Lund (2013/110), and an amendment for the current study was approved by the Swedish Ethical Review Authority (2023-0709402).

The aim of the research conducted for this thesis was to individualize cancer treatment to benefit patients, which is justified from a societal perspective. The patients included in the studies included in this thesis were exposed to a minimal degree of risk since the information was based on registered and previously collected data. Some question topics included in the questionnaires, such as reproductive factors, smoking, and alcohol use, can be perceived as personal and raise feelings of guilt if these factors are also associated with an increased risk for the cancer disease. Body measurements could also raise feelings of guilt for some individuals. To preserve the trust of the study patients, it is important that no individuals can be identified involuntarily in the presented results and that all data are kept confidential. For patients included in the BC-Blood cohort, additional follow-up visits with a trained breast cancer nurse can be regarded as a patient benefit.

The use of TMA for IHC does not require any modified or additional procedures for patients. The TMA is constructed from archival tumor tissue after routine clinical use and necessary storage of tumor tissue for future clinical need. Therefore, smaller tumors are underrepresented in the TMA since small tumor volumes lack sufficient tissue for research purposes. The TMA method utilizes smaller volumes of the tumor material than whole-slide IHC, so more analyses can be performed. These analyzes can lead to identification of new prognostic or treatment predictive biomarkers. The patient identification of the TMA is coded, and the readers of the TMA were blinded to clinical data and outcome when annotating the TMA.

Experiments with cell lines do not require ethical approval, but there are still ethical considerations to take into account. The MCF-7 cell line was voluntarily donated by Sister Catharine Frances, and newspaper articles with pictures were written about the donation at the time. The community that Sister Catharine Frances was a part of still celebrates the success of the established cell line. There is no information suggesting that the MDA-MB-231 cell line was not voluntarily donated, but ethical guidelines regarding cell-line establishments have changed over time. One can argue that it would be impossible for the donor at the time to envision all of the information that could be obtained from cell lines today. The goal of experiments performed was only to evaluate the activation of signaling pathways by a common vitamin, not to re-identify the donor or related family members.

All evaluated treatment-planning CT scans were pseudo-anonymized. The retrospective nature of the study does not increase the radiation dose to patients. CT scans are necessary for planning RT. Utilization of the images for additional information can spare patients additional examinations or tests, in addition to being cost-effective.

Results and discussion

Results and discussion according to the specific aims

Study I

The main results of the study were that a higher level of pSTAT3^{Y705} assessed by IHC was associated with younger age, higher age at first child birth, any alcohol consumption, lower BMI, and lower waist-to-hip ratio. Regarding tumor characteristics, higher levels of pSTAT3^{Y705} were associated with smaller tumors, lower histological grade, lymph node negativity, PR positivity, and HER2 negativity. Patients with high pSTAT3^{Y705} levels had improved BCFI and distant metastasis-free intervals in univariable models, but the associations did not remain statistically significant in multivariable models. Chemonaïve patients with ER^{+}/STAT3^{Y705}-negative tumors treated with tamoxifen had a better BCFI than patients who were treated with AI or did not receive tamoxifen.

Discussion

In study I, we report that several patient characteristics are associated with levels of pSTAT3^{Y705}, of which few have been addressed by others. The association between higher pSTAT3^{Y705} levels and younger age is not consistent between different cohorts (64,117,181,305) and may be influenced by different cutoffs. Radenkovic *et al.* reported that higher levels of breast density are associated with pSTAT3^{Y705}, while there was no association between pSTAT3^{Y705} and being younger or older than 50 years (64). This suggests that genetic, hormonal, and lifestyle factors can all influence pSTAT3^{Y705}.

In the present study, we did find a weak association between any alcohol consumption with high levels of pSTAT3^{Y705}. Alcohol consumption was reported to influence breast density in non-overweight women (63), and ethanol can increase pSTAT3^{S727} levels in breast cancer cell lines (177), which may influence the levels of pSTAT3^{Y705} (360). However, additional studies are needed to investigate the possible causal effect of alcohol consumption and STAT3 activation in breast cancer.

Increased BMI is often associated with higher age and higher levels of IL-6 (65). Since IL-6 is a known activator of pSTAT3^{Y705} (171), it was unexpected that tumors in older patients presented lower levels of pSTAT3^{Y705}. Despite intrinsic low levels

of STAT3 in hormone-responsive cancer cells (171), cell cultures with enriched medium containing estrogen, epidermal growth factor, and TNF-α respond with an initial increase of pSTAT3^{S727}, followed by higher levels of pSTAT3^{Y705} 24 h hours later. This suggests that factors in the tumor microenvironment influence activation and that pSTAT3^{S727} and pSTAT3^{Y705} display different kinetics (44).

In light of pSTAT3^{Y705} being an important regulator of cancer stem cells, loss or inactivity of pSTAT3^{Y705} could render tumors more aggressive (44) and alter immune-suppressive functions (171). Deficiency of STAT3 isoforms due to mutations or inherent genomic alterations could possibly alter the STAT3^{Y705}/STAT3^{S727} balance and increase susceptibility to inflammatory stimulation. Different haplotypes of *STAT3* with geographical differences (361) and susceptibility for breast cancer risk (362) have been reported. In Europe, two haplotypes of *STAT3* dominate, but it has not been explored whether this would influence the results in our cohort.

The associations between pSTAT3^{Y705} and tumor characteristics indicate that a high level of pSTAT3^{Y705} is a marker for early-stage breast cancer. Others have reported the associations with smaller tumors (15,181,305,306,360), lower histological grade (360), absence of lymph-node involvement (360), and HER2 negativity (181) in high-pSTAT3^{Y705} tumors using IHC. Luminal A tumors present higher levels of pSTAT3^{Y705} than luminal B tumors (117,305). Intrinsic subtyping of tumors with high levels of pSTAT3^{Y705} show that upstream activation of pSTAT3^{Y705} differs between intrinsic subtypes (180,363).

In a study by Tell *et al.,* the high pSTAT3^{Y705} basal-like subtype had a distinct gene signature with upstream signaling that is also related to pSTAT3^{S727} activation (363). This association was recently confirmed in an IHC study of TNBC, and interestingly, an association with smaller tumors and lower histological grade in cases of high pSTAT3^{S727} was only present with coexisting high levels of pSTAT3^{Y705} (360). However, pSTAT3^{S727} activation was not associated with fewer lymph-node involvements (360). This study also indicated a moderate correlation between the two phosphorylation sites and that joint activation of pSTAT3^{S727} and pSTAT3^{Y705} yielded a higher H-score when assessing pSTAT3^{Y705} levels (360). Taken together, results from these studies indicate that when all breast cancer subtypes are analyzed jointly, as in the present study, some pSTAT3^{Y705}-positive tumors may be basal-like cancers, which could explain why there was no clear association between high pSTAT3^{Y705} and ER^{+}, as reported by others (117).

Studies report associations with high levels of pSTAT3^{Y705} and improved outcome (117,181). In the present study, statistical associations with improved BCFI and DMFI did not remain in the multivariate models. A recent study including 5694 patients with breast cancer enrolled in the ALLTO trial assessed targeted HER2 treatments. The study concluded that pSTAT3^{Y705} is also a favorable prognostic factor in ER^{+}/PR^{+}/HER2^{+} breast cancers (181). However, pSTAT3^{Y705} was not

associated with prognosis in hormone-receptor-negative patients (181), in contrast to data collected from the METABRIC dataset using STAT3 mRNA expression (117).

A possible explanation is that STAT3 is also expressed in stromal compartments (360). A meta-analysis performed in 2018 involving 12 studies and 4513 patients concluded that pSTAT3 (phosphorylation residues not reported) was associated with a 32% improvement in breast cancer-specific survival and a 28% improvement in OS. Total STAT3 was not associated with OS and instead pointed to increased risk of death, which supports the observations that not all types of STAT3 activation are beneficial (364). Importantly, the authors of the meta-analysis could not rule out publication bias of small negative studies (364).

In the present study, a subgroup analysis of chemonaïve hormone-receptor-positive breast cancers suggested an effect modification for $pSTAT3^{Y705}$-negative tumors and tamoxifen treatment for BCFI and DMFI. Patients treated with tamoxifen had longer BCFI and DMFI if their tumors were negative for $pSTAT3^{Y705}$. The non-activity of $pSTAT3^{Y705}$ suggests that other signaling pathways are activated. For instance, activation of p65 can compensate for the regulatory role of cancer stem cells in the absence of $pSTAT3^{Y705}$, and activated p65 is associated with treatment resistance to tamoxifen (365).

Interestingly, STAT3 reaction to tamoxifen and letrozole have opposing directions in proposed mechanisms of acquired resistance (163,366). Mechanistic studies suggest that cell lines exposed to tamoxifen show increase $pSTAT3^{Y705}$ together with an increase of EGFR (366), while letrozole decreases $STAT3^{Y705}$ accompanied by a nuclear increase of BRCA1 (163). Hypothetically, this could imply that treatment with AI maintains a low level of STAT3 with continued activity of other compensatory pathways, while $pSTAT3^{Y705}$-negative tumors treated with tamoxifen will require an adaption to STAT3 activation before treatment resistance is acquired by this pathway. Future studies are needed to validate these findings.

The introduction of STAT3 inhibitors in clinical trials in a metastatic setting have not been successful so far, despite the rationale that blocking the IL-6/JAK-STAT pathway could render tumors more sensitive to treatments (14,307). In a phase II study including patients with aromatase-inhibitor-resistant advanced breast cancer, the results showed minimal effect for patients treated with ruxolitinib (JAK1/2 inhibitor) in combination with exemestane. This suggests the activation of other inflammatory pathways. Interestingly, this study also reported a high frequency of patients carrying a high-risk IL-6 polymorphism that influenced lack of treatment response (14). The favorable outcome for patients with high levels of $pSTAT3^{Y705}$ in the early setting in the present study implies that activation of STAT3 has different roles, and studies need to assess what other regulatory pathways are activated when the cancer stem-cell-regulating function of STAT3 is blocked (44).

Study II

The main results from study II showed that antioxidant use was somewhat more common among patients from Helsingborg (13.4%) than patients from Lund (10.3%). Patient and tumor characteristics differed between users and non-users, and associations were not consistent between patients from Lund and Helsingborg except for YY use being associated with smaller tumors. In Lund, where data on AHR levels were available for 915 patients, AHR^{nuc} was twice as common in patients reporting use prior to surgery. Supplement use with multivitamins was not associated with outcome in either patient group.

In Lund, antioxidant use was borderline associated with outcome in the subgroup of non-MV antioxidant users, while in Helsingborg, antioxidant use and non-antioxidant use were associated with outcome. Among patients from both cities, the influence of antioxidant use on outcome differed with the pattern of use, where YY users had worse clinical outcomes, and NY users had favorable clinical outcomes. We could not identify that antioxidant use would interfere with oncological treatments in treatment subgroups.

Preoperative data revealed an interaction effect between AHR^{nuc} negativity and preoperative antioxidant use on BCFI. The combination of AHR^{nuc} negativity and preoperative antioxidant use was associated with a worse prognosis. In the hormone responsive cell line MCF-7, the addition of vitamin C increased the protein level of AHR and CYP1B1, whereas in the triple negative cell line MDA-MB-231, CYP1B1 levels increased without a significant increase in AHR.

Discussion

In study II, we used a previously published definition for the antioxidant variable, which took both pre- and post-treatment use into account (186). Despite other cohorts presenting high frequencies of multivitamin (91,182) or antioxidant use (188,190), low frequencies of antioxidant users in the present cohort yielded small user groups, which introduced uncertainty to the results. Antioxidant users from both cities reported more frequent MHT use than non-users, which has also been reported by others (190). Other patient and tumor characteristics were not consistent between patients from Lund and Helsingborg, and previous reported associations with a normal BMI (188-190) were only apparent in patients from Lund. This suggests that the antioxidant users constitute a heterogeneous group and not solely patients traditionally referred to as users of complementary alternative medicine (191,193,367).

Few studies have reported histological type in relation to antioxidants. The association between antioxidant use and lobular type among patients from Lund was not verified in Helsingborg and may therefore be due to chance. In a Danish study of dietary micronutrient intake, reduced risk for lobular breast cancer was reported for high levels of dietary beta-carotene, whereas no association was apparent for use

of beta-carotene supplements (368). The association with fewer tumors of histological grade III in patients from Lund could also not be confirmed in patients from Helsingborg, nor in the study by Jung *et al.* (190).

In contrast, Campbell *et al.* reported an association between higher tissue levels of ascorbate and histological grade I tumors (105). In addition, high ascorbate levels were associated with low activation of the HIF-1 pathway and a lower degree of necrosis in breast tumors, suggesting a protective role of vitamin C in the tumor. A gene analysis using a predicted vitamin C index reported that a high index was associated with lower tumor mutational burden and upregulation of genes involved in estrogen response, DNA repair, ROS regulation, and lipid and bile acid metabolism (104). The characteristics of tumors with a high vitamin C index could render these tumors less sensitive to adjuvant treatments and may possibly be underestimated (104).

In the present study, AHR activation was twice as common among patients using antioxidants prior to surgery compared to non-users. Whether this represents an effect of antioxidants used in the study or other factors that can activate AHR is unknown. The mechanistic study showed that vitamin C can activate the AHR signaling pathway with subsequent elevation of CYP1B1 levels. A lower increase of AHR despite CYP1B1 elevation in MDA-MB-231 can most likely be explained by the inherent high level of AHR in the MDA-MB-231 (369). Constitutive AHR activation with subsequent CYP1B1 elevation is linked to estrogen metabolism (49). Therefore, it could be hypothesized that the antioxidant capacity of vitamin C compensates for the increase of ROS due to CYP1B1 estrogen metabolism and thus keeps the mutational burden low.

Interestingly, associations between high levels of mRNA *AHR* and *CYP1B1* were only present in ER^- breast cancers in the study by Vacher *et al*. (204). This suggests that the AHR-CYP1B1 pathway may not typically be activated in ER^+ breast cancers. Collectively, these results suggest that the use of vitamin C (which at least 80% of patients in the present study used if using antioxidants) can influence tumor characteristics that may in turn influence treatment outcome (104,105) and treatment resistance.

Associations with clinical outcome among antioxidant users were largely attributed to patients using "non-MV antioxidants," and in line with other studies, multivitamin use was not associated with clinical outcome in the present study (186,189,196). In the study by Ambrosone *et al.,* 44% of participants reported regular use of multivitamins during chemotherapy in a randomized clinical trial, and there was no association with clinical outcome (186). A study from Germany combined multivitamins with the use of vitamins A, C, and E and zinc. The study reported increased risk for breast cancer recurrence and OS when these supplements were used during chemotherapy or RT (190).

In the present study, associations of outcome with combining multivitamins and antioxidant supplements were only apparent in patients from Helsingborg. "Non-MV antioxidant" users had worse clinical outcome among YY users in Lund and Helsingborg. These results are in line with those reported by Ambrosone *et al.,* who used the same antioxidant variable and adjusted for multivitamin use (186). This suggests that more regular use of "non-MV antioxidants" influences treatment resistance, although associations were weak among patients from Lund.

When adjuvant treatment groups were analyzed separately, no associations with outcome were apparent. Few patients used antioxidants during chemotherapy in the present study when analyzed separately, so the results of evaluating chemotherapy-treated patients should be interpreted with caution. Although we lack information on discontinued use of supplements, it is probably a result of information from healthcare personnel.

In a study by Zirpoli *et al.,* half of the included patients reported that no information was given regarding supplement use (195). In that study, patients that did receive information to discontinue use, the majority followed the recommendations, and prior use of vitamins C and E declined during treatment (195). Previous non-users that did receive information to use vitamins and supplements during treatment were more likely to use vitamins D, B6, B12, C, and E (195). Whether use of specific vitamins was recommended or a result of the patients' own initiative could not be determined in the study by Zirpoli *et al.* (195).

In the present study, NY users had an improved clinical outcomes. In a study by Ambrosone *et al.,* reduced hazards for NY users were non-significant and further attenuated in adjusted models. Similarly, in a study by Jung *et al*., reduced hazards for non-concurrent use among chemotherapy-treated patients did not reach statistical significance (190). Nechuta *et al.* included 4,433 women treated between 2002 and 2006 and reported fewer recurrences and reduced mortality for patients who used multivitamins, vitamin E, or vitamin C and were treated with chemotherapy without RT. However, patients treated with RT (8% of the study population) had non-significantly worse clinical outcomes (189).

Greenlee *et al.* also reported improved prognosis for vitamin E and C users among 2,264 women treated between 1997 and 2000. However, this study did report that patients using beta-carotene had an increased risk of breast cancer mortality and all-cause mortality (188). Nevertheless, a meta-analysis including eight studies and 17,062 patients with breast cancer could not find any association with OS when vitamins A, C, and E were combined, but vitamin C use improved OS by 16% (370). This meta-analysis also concluded that more recent studies report worse OS for antioxidant use during chemotherapy (370) than older studies (189), which may reflect changes in chemotherapy regimens over time (141,142). An earlier meta-analysis emphasized that improvements in outcome in relation to vitamin C intake were higher for dietary vitamin C intake than the use of vitamin C supplements

(371). Collectively, these findings suggests that there are currently no data supporting the association between NY use and favorable outcomes in the present study and that confounding factors and changes in oncological treatments may have influenced the results.

The interaction calculation with AHR indicated that outcomes in relation to antioxidants cannot solely be determined by reported use. In the present study, outcomes differed depending on the *AHR* activation and the timing of use in relation to adjuvant treatment. High levels of mRNA *AHR* activation have been associated with genes related to inflammation, metabolism, invasion, and IGF signaling, particularly in ER^- breast cancers (204). Furthermore, triple negative cell lines have higher metastatic capacity when AHR is activated (369). Future studies need to validate whether antioxidant use prior to treatment influences these associations in a positive way, such as by not activating the HIF-1 pathway or by reducing estrogen levels.

Interestingly, although the subgroups were small, applying the four user groups to the AHR status indicated that antioxidant users with activated AHR had the most favorable outcomes if use was discontinued. This suggests a possibility that protective roles in the tumor that could have introduced treatment resistance were not maintained during treatment. AHR acts as an E3 ubiquitin ligase that enhances degradation of the ER in cases where ARNT is occupied (203), and this mechanism could influence tumor characteristics and treatment outcomes.

Plant-derived substances such as carotenoids and curcumin can bind to AHR and attenuate receptor transformation by preventing the phosphorylation of the AHR/ARNT complex, thereby preventing nuclear translocation (372,373). In the diet, these substances are regarded as having protective effects against cancer (82). There have not been investigations of whether the same mechanism can be applied to dietary supplements of carotenoids containing higher doses compared to a regular diet (82). The interaction with AHR suggested that antioxidant use without AHR activation was associated with worse clinical outcome.

An association with increased mortality has been observed among patients with breast cancer using beta-carotene supplements (188), and pro- and anti-oxidative effects have been reported in studies on lung cancer (374,375). These findings imply that excessive blockage could have negative consequences. Since the report of increased risk for lung cancer among patients prophylactically administered beta-carotene supplements (107), few regular multivitamin brands have contained beta-carotene.

Study III

The main results in study III were that patients with SMI_{low} were older, had a lower BMI, and were more often current smokers. The tumors in patients with SMI_{low} were

more often located in hypopharynx than oropharynx. No association was found with treatment adherence. For RT, the median OTT differed by one day between patients with normal SMI and SMI_{low}. Having SMI_{low} was associated with increased hazard ratios for OS and PFS in univariable models. Separation by sex and evaluation of SMI as a continuous variable revealed associations with treatment outcome for men, while no associations were found for women. For men, SMI_{low} was an independent predictor in the multivariable model for OS and borderline for PFS. An interaction between SMI_{low} and randomized treatment in favor of cisplatin was identified and needs further validation.

Discussion

In this study based on a previously performed randomized controlled trial, we found that SMI_{low} provides prognostic information that can be attained from pre-treatment planning CT scans. The association of SMI_{low} with older age and BMI in this study is in line with other studies that used skeletal muscle mass to identify sarcopenia (285) and other measures of muscle strength, such as grip strength and gait speed (287,291). In the HNSCC population, age-related sarcopenia may be accentuated by decreased nutrient intake and weight loss related to the disease (296). Interestingly, the associations with older age were more pronounced in men than women. This discrepancy could be a result of weaker associations arising from fewer women having been included in the study or other sex-related factors that have been reported to be related to muscle loss in relation to age, such as BMI (286).

Smoking has previously been associated with the presence of SMI_{low} in other patient populations (291) and in HNSCC (238), which is in line with our results. Smoking may contribute to development of sarcopenia (341). For instance, mice exposed to long-term smoke show relatively low skeletal muscle mass and a reduced activation of the IGF-1 mTOR pathway in skeletal muscles, which could be abrogated by therapeutic exercise (376). However, it is difficult to evaluate the influence of smoking as an isolated factor, and different methodologies, definitions, and other contributing factors associated with smoking habits yield uncertain results (341).

The positive correlation found for oropharyngeal cancer with muscle mass (measured by bioelectrical impedance analysis) was also reported in a Swedish prospective cohort, although in that study, patients with oral cancers presented the lowest muscle mass according to combined values of men and women (296). Patients diagnosed with $p16^{+}$ oropharyngeal cancers are generally younger, have healthier lifestyle habits, and have fewer comorbidities (215,237,377), which may explain the relatively high level of muscle mass in this patient group. This patient group represents the majority of patients included in the present study, and few patients with high performance status were included in the ARTSCAN III trial. These factors may explain why there was no association between SMI_{low} and higher performance status in the present study compared to previous studies (9,238).

In contrast to previous observational studies (297,298), SMI_{low} was not associated with treatment adherence in the present study. The differing results may be influenced by the different study populations (*i.e.*, patients were not included in a randomized trial and received treatments) (297,298,325). Interestingly, studies have used non-sex-specific optimum stratification to determine SMI_{low} for low adherence to cisplatin (298) or cetuximab (325), and in those studies' patients with SMI_{low} were overrepresented by women. Despite low adherence, SMI_{low} was not associated with outcome in these studies (298,325) compared to other studies on HNSCC populations (238,292,297,317,318,327).

The cutoffs used in the present study were somewhat higher for men and women compared to the study by Rijn-Dekker *et al.* They used the same methodology and reported SMI_{low} to be an independent prognostic marker in a retrospective cohort study of HNSCC patients treated with curative intent (238). In contrast, it was much lower for women compared to the study by Ganju *et al.,* which evaluated patients treated with chemo-RT (297). These large discrepancies could partly be due to ethnic differences (378) in muscle mass but may also represent different levels of fat infiltration in muscles, which reduces muscle quality (379).

One study has reported a difference in the discriminative ability of different muscle groups according to sex, where the paravertebral muscles at C3 showed the best predictability for men, whereas the sternocleidomastoids had the best predictability value for women (318). Since the total volume of the paravertebral muscles is larger than that of the sternocleidomastoids, it could be argued that a combined value of all muscles at the C3 level may be a better predictor for men than women. Additionally, Olson *et al.* aimed to establish cutoff values for SMI at the level of C3 and thereby omit using the conversion formula. However, they were only able to confirm the prognostic value of C3 muscle mass for men in the validation cohort (321). It is also possible that the generalizability of this method to other cohorts was influenced by the small number of women included in the study by Swartz *et al.* (319) to generate the conversion formula.

Few studies have evaluated muscle mass as a continuous variable for HNSCC (293) and have reported either no association (380) or improved outcome (296) for larger muscle mass when men and women were combined. Since men generally have larger muscle mass than women (286), non-sex-specific cutoffs can generate skewed distributions where women are overrepresented as having low muscle mass (267,298,325). Interestingly, some studies on the general population have reported that compared to men, women are more often considered as having low muscle mass (285) and more often frail (all sarcopenic individuals were considered frail) (87). Different associations between SMI_{low} and overall mortality between men and women have also been described with an initially linear association that reaches a plateau for men, while the association for women displays a u-shaped association (294). To improve the prognostic implication for SMI, sex-specific nomograms of

SMI for different populations are needed, as recently highlighted in a systematic review of cancer in general by Vicker *et al.*(17*)*.

There have not been previous reports of the interaction between SMI_{low} and randomized treatment in favor of cisplatin for PFS. Although not powered for subgroup analysis, the study by Bonner *et al.* reported improved benefit of cetuximab for male patients younger than 65 years (272). Younger age and fewer comorbidities render patients with HPV-positive oropharyngeal cancers less likely to suffer from sarcopenia, and high survival rates have been reported for low-risk patients (230). Interestingly, a retrospective study of patients with metastatic colorectal cancer reported worse survival for patients with low BMI who were treated with cetuximab, suggesting that body constitution may influence the response (381).

The decline of muscle function in aging influences the immune system (290) and reduces levels of interleukin 15 (IL-15), which is essential for immune regulation and activation of NK-cells (382) and has been reported in patients with sarcopenia (383). Thus, it could be hypothesized that these changes could influence the ADCC activity of cetuximab, thereby reducing the inherent effect. Physical activity increases systemic levels of IL-15 and has the potential to trigger immune responses (384). However, for some patients, physical activity is not an option, therefore medical treatments are also needed to improve immune functions for patients with cancer (268,385). The NCT06239220 study is an ongoing phase II study involving patients with advanced HNSCC that is evaluating a super agonist of IL-15 in combination with modified NK-cells and cetuximab. This study may provide more knowledge about the influence of IL-15 and NK-cells in cetuximab treatment (280).

Methodological considerations

"Repeated observations of white swans did not prove that all swans are white, but the observation of a single black swan sufficed to falsify that general statement"

Karl Popper, 1976

Study design

This thesis is based on two cohort studies (studies I and II) and one retrospective analysis from an RCT. Although RCTs are considered advantageous in medical research, they also have some drawbacks. For instance, well conducted RCTs are often expensive and time consuming, and some research questions might not be ethically justified or possible to conduct within the randomized research setting (345). To accomplish a strict setting for the study, RCTs often exclude patients that are older with severe comorbidities and worse performance status (346).

In this sense, observational studies can provide a complimentary method to RCTs. In observational studies, there is no intervention, and patients are followed over time. This enables inclusion of more heterogeneous patient populations with less rigorous treatment protocols (354). Well-managed registries are good sources of observational data. Since observational studies have a higher risk of random errors than RCTs, they need to be considered and handled during all phases of the study (345).

In a retrospective analysis of an RCT, the assumption of equally distributed variables is lost, and the higher risk of random errors is equal to that of an observational study. One advantage of a retrospective analysis of an RCT is that the treatment setting is controlled, and many variables that might influence the outcome of interest are well defined and can be balanced. In all three studies included in this thesis, data collection was performed to add additional information to the existing database in order to be able to answer the research questions.

Errors in a study that eventually lead to incorrect results can be random or systematic. Random errors in the data can be quantified using different statistical methods (345) and are more likely to influence the results when the studied

population is small. The systematic errors (also called bias) have three main components: selection bias, information bias, and confounding (345).

Selection bias refers to when the study population does not represent the population of interest (345). Perfect selection of a study population does not exist, but it is important to reflect upon how the differences might influence the results of the study. Information bias refers to bias that occurs during data collection (345), which can come both from the study participants, as in cases of recall bias and self-representational bias, and from the researcher (observer bias). Confounding is a major challenge in observational studies and is discussed below.

Confounding

Confounding occurs when an observed association between a variable and an outcome is distorted by the influence of a third variable (the confounder). The confounder is related to both the variable of interest and the outcome, and this makes it difficult and often erroneous to draw conclusions from the association of interest. If confounders are well known and measured, they can be controlled for by different statistical methods, such as multivariable regression models and stratification (345).

There are also confounders that are more difficult to handle, which have been proposed as explanations for studies where results from randomized trials and cohort studies do not agree. The "healthy user effect" in observational studies is a bias related to patient behaviors, such as higher levels of physical activity and avoidance of alcohol (345,386). In study I, patients with high pSTAT3^{Y750} levels had several patient characteristics that could be addressed as "healthy user" characteristics and could have influenced the favorable prognosis in the univariable model.

In study II, this possible confounding is an important aspect since supplement users have been reported to have healthier lifestyle habits than non-users. It may also have influenced the results of study III, where current smoking was more common in patients with a low skeletal muscle mass. More information about lifestyle habits, such as dietary habits (studies I–III), physical activity level (studies I and II), and alcohol consumption (study III) could have helped support or reject "healthy user" confounders.

Similar to the "healthy user" the "healthy adherer" relates to the observation that patients that adhere to a prescribed treatment are more likely to engage in other healthy behaviors. For example, one RCT showed that patients who were adherent to a placebo had lower rates of mortality than non-adherent patients who were also assigned to the placebo (386). In study II, adherence to endocrine therapy was investigated in relation to supplement use since some studies had suggested that supplement users might be less adherent to the prescribed treatment. In contrast to

the suggestion of non-adherence, there was no difference in endocrine treatment adherence between supplement users and non-users in study II.

A rigid treatment protocol in an RCT may reduce the adaptability of the treatment and thereby increase the risk of non-adherence that would otherwise be accounted for in a clinical setting (345). "Selective prescribing" is a possible confounding factor in observational studies and refers to adjustments in prescribed treatment, such as adjustments made to account for comorbidities or frailty (386). This type of confounding is difficult to adjust for in observational studies unless the given treatment is recorded in detail in the research database.

Internal validity and reliability

Internal validity is defined as the extent to which a concept is accurately measured (387). Biases are common threats to internal validity that hinder conclusions between a studied variable and an outcome (345). Reliability refers to the consistency of the measure, and high reliability means that the same result will be obtained if the measures are repeated. If the validity is low, there is no point in having high reliability since a different effect is measured (387).

In studies I and II, questionnaires were used to collect lifestyle-related information from patients. Questions about events or habits from the past might be at risk of introducing recall bias, whereas questions about lifestyle habits may introduce self-representation bias. Anthropometric measurements were conducted by trained research nurses (299,340), which reduces the risk of self-representational bias and increases the reliability of the measurements. In study III, smoking status might have introduced a risk of self-representation bias. In studies I, II, and III, dual assessments of the exposure variables were used to improve reliability. The laboratory experiments in study II were performed at least five times for each exposure to ensure consistency in the results and to increase validity and reliability.

Generalizability

Generalizability (external validity) refers to what degree to which the findings are applicable to other populations. For research results to be clinically useful, they need to be relevant for the intended population, not just the study sample. If treatment efficacy is modified by different attributes of the patient population, this can result in different effects from what was observed in an initial RCT (345). In general, patients not included in RCTs more often have mental health disorders, more comorbidities, and in some cases, limited functionality and lower socioeconomic status. In oncology studies, patients not included in RCTs are more often older, more likely to be female, have a higher performance status ($\geq$ 2), and a have worse prognosis (346).

An advantage of observational studies is that the participants often show closer resemblance to the general population than patients included in clinical trials. Studies I and II are based on the BC-Blood study, which is considered population-based and representative of female patients with breast cancer treated in Lund and Helsingborg at the time of the study. Although not investigated thoroughly, the majority of included patients were of Swedish or European decent, so generalizations to other patient populations with different ethnicities and cultures might not be applicable. In study III, generalizability may be restricted for those with HNSCC and good performance status who are eligible to receive treatment with curative intent.

Table 8. Strengths and limitations in studies I−III

	Study I	Study II	Study III
Strengths	Large population-based prospective cohort. Validated antibody. Rigorous information on patient and tumor characteristics.	Large population-based prospective cohort. Detailed information from supplement users. Two patient cohorts were investigated and displayed similar trends. Two different cell lines were evaluated. Experiments were performed with a minimum of five replications.	Randomized controlled trial with well-controlled data. No bias in treatment selection. Delineation according to validated method. Patients are representative of HNSCC patients with good performance status.
Limitations	Few patients with high levels of $pSTAT3^{Y705}$. Positive staining for $pSTAT3^{Y705}$ may represent different isoforms of STAT3 activation. Residual confounding.	Exact time period of supplement use, doses, and dietary intake could not be considered. Few antioxidant users yielded small groups for comparison. Nonspecific exposure variable. Residual confounding. Vitamin C is easily oxidized in a laboratory setting. The AHR is highly responsive to chemicals and materials that may influence the estimated activation.	Use of measurements at the level of C3 may over/underestimate the level of L3, which is the gold standard method. Few women limit conclusions for this group.

Abbreviations: $pSTAT3^{Y705}$: phosphorylated single transducer and activator of transcript 3 at residue 705, AHR: aryl hydrocarbon receptor, HNSCC: head and neck squamous cell carcinoma, C3: cervical vertebra 3, L3: lumbar vertebra 3

Causality

Causation means that an exposure causes a measured effect. Variables can have different relations and associations, but a relation does not imply causation (348). For example, Pathak *et al.* (388) aimed to identify risk factors for a loco-regional recurrence in breast cancer, and the variable of neoadjuvant treatment was associated with more frequent events of locoregional recurrence. Readers familiar with oncology, including the authors of the article, will conclude that the association can be explained by the reason why the patient was treated with neoadjuvant treatment.

When it comes to less well-known associations, the interpretations may not always be as easily concluded. All three studies included in this thesis report both known and previously unknown associations. In epidemiology, which seeks to find causations but primarily reports associations, nine viewpoints proposed by Sir Bradford Hill in 1965 can be used as a guide to draw conclusions (350,389). The viewpoints are sometimes referred to as Hill's criteria, although they were not intended to be regarded as rules for causation, but rather as a tool for reflection when interpreting results (Figure 20).

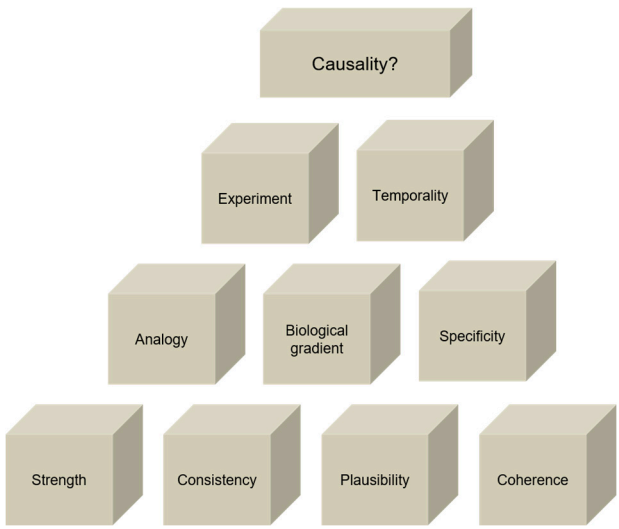

Figure 20. Hill's viewpoints for causality

In short, a *strong* association is more likely to have a causal component than a weak association, although exceptions always occur. *Consistency* of a reported association in different cohorts and contexts is more likely to represent a causal relationship. It is also important to take the s*pecificity* of the exposure, the variable, or the population into consideration when conclusions are drawn. Does the association imply a single causation or multi-causation, which is more common in medicine and biology?

For a causal effect to be present, the exposure must always precede the outcome (*temporality*), which is not always easy to prove in observational studies. If the condition can be altered by an *experiment*, and there is an *analogy* with similar causes and effects, this also indicates greater likelihood for causation. If a *biological gradient* can be identified, whether linear, non-linear, or dependent on a threshold, it may indicate causation.

When associations are detected, it is always important to consider the *plausibility* of the association to diminish the risk of incidental findings. Importantly, what is considered plausible depends on current knowledge. *Coherence* with current knowledge and biological mechanisms should also be considered (389). Since their first publication, Hill's viewpoints have been modernized and further developed (350). For instance, causal pie charts can illustrate the multifactorial nature of causations to help interpret causal mechanisms and interactions of causal components.

Another way to illustrate associations between variables and identify possible confounders is by using DAGs (351). The "Grading of Recommendations Assessment, Development, and Evaluation" (GRADE) system has been developed to assess the certainty of evidence and is often used in systematic reviews (390). Certainty is defined as the extent of the confidence that the estimates of an effect are correct (350) and includes validity, precision, consistency, and applicability of the result estimates (390).

Table 9: Main findings of the thesis in relation to the viewpoints for causality by Sir Bradford Hill

Viewpoint	Reflection of prognostic value on clinical outcome in studies I–III
Strength	**Study I:** The effect size for high levels of $pSTAT3^{Y705}$ was low (HR 0.17/no event) for BCFI, DMFI, and OS but not significant when adjusted for time between surgery and staining. **Study II:** Effect estimates for BCFI were strongest for non-MV antioxidants with differential effect regarding timing of use (Lund; NY 0.21 /YY 1.59, Helsingborg; NY no event/YY 3.76). An interaction between AHR activation and antioxidant use indicated improved BCFI for patients with positive AHR in combination with preoperative use (adjusted effect estimate 0.25). **Study III:** Effect estimates for SMI_{low} were 2.09 and 2.19 for OS and PFS in univariable models, respectively.
Consistency	**Study I:** High levels of $pSTAT3^{Y705}$ have been associated with improved prognosis in other studies, particularly ER^{+} breast cancer. **Study II:** The result for YY use is in line with a previous study using the same antioxidant variable and a recent study from Germany. Older studies report associations with improved prognosis for some antioxidants post diagnosis. **Study III:** SMI_{low} has been associated with worse prognosis in HNSCC, other cancer diagnoses, and geriatric populations.
Specificity	**Study I:** $pSTAT3^{Y705}$ activates two isoforms of STAT3 with differential effects that introduce uncertainties to the result. Oncological treatments can activate $pSTAT3^{705}$. **Study II:** The combined antioxidant variable cannot distinguish between different antioxidants with diverse properties, which may influence the result. Antioxidants from diet were not assessed and may influence total intake. **Study III:** A validated method for assessment of skeletal muscle mass was used. However, this method is a proxy for the gold standard measurement at L3 with reported reports of over- and underestimations.
Temporality	**Study I:** IHC was performed on pretreatment tumor tissue suggesting activation prior to assessment. **Study II:** Antioxidant use was assessed prior to initial adjuvant treatment and associations with tumor characteristics. Use at follow-up visit involved information of antioxidant use prior to that date. **Study III:** SMI measurements were collected prior to treatment initiation.
Experiment	**Study I:** STAT3 may decrease and increase treatment resistance in cell studies. Inhibition of STAT3 has not been successful in studies of metastatic breast cancer. **Study II:** Clinical trial reports worse OS for smoking patients with HNSCC using antioxidants during treatment. *In vitro* studies showed that vitamin C can activate AHR by increasing downstream CYP1B1 levels, suggesting that antioxidants can influence tumors besides scavenging of free radicals. **Study III:** *In vivo* studies report lower muscle mass in mice exposed to long-term smoking.
Analogy	**Study I:** High levels of $pSTAT3^{Y705}$ were associated with favorable patient and tumor characteristics known to have a positive influence on outcome. **Study II:** The diverse results of antioxidant use depending on timing are both supported and contradicted by previous studies. **Study III:** Worse prognoses in HNSCC have been reported for older patients and patients with higher levels of comorbidities, often associated with reduced muscle mass.
Biological gradient	**Study I:** A trend towards earlier recurrences for lower levels of $pSTAT3^{Y705}$ was observed along with less favorable tumor characteristics for lower levels. **Study II:** We found no associations for multivitamin supplements with daily recommended doses of nutrients, suggesting that associations are driven by higher doses or other non-evaluated factors related to these supplements or supplement use. **Study III:** In men, higher muscle mass evaluated as a continuous variable was associated with improved OS and PFS.
Plausibility	**Study I:** STAT3 isoforms appear to have differential effects *in vivo* and *in vitro*. Pathways for $pSTAT3^{Y705}$ activation differ between intrinsic subtypes. **Study II:** Higher mutational burden increases ROS levels in cells. Antioxidants protect normal cells and can increase cancer cells' ability to survive and resist oncological treatment. **Study III:** Muscle loss influences several important functions in the body, including immune regulation and metabolism, and can be an indication of malnutrition, frailty, and cachexia, which can all influence HNSCC prognosis.
Coherence	**Study I:** STAT3 has both pro-tumor and tumor-inhibiting properties. Most studies report associations between high levels of $pSTAT3^{Y705}$ and improved prognosis. **Study II:** Worse BCFI for YY use is in line with recent studies of continuous use. Recent studies report non-significant reduced risks for NY use, which may be related to residual confounding. **Study III:** Most studies report associations with worse prognosis for SMI_{low}. Women are underrepresented in HNSCC studies, and a larger samples is needed to evaluate the prognostic value in women.

Abbreviations: STAT3: signal transducer and activator of transcript 3, $pSTAT3^{705}$: phosphorylated STAT3 at tyrosine residue 705, BCFI: breast cancer-free interval, DMFI: distant metastasis-free interval, OS: overall survival, PFS: progression-free survival, non-MV antioxidant: non-multivitamin antioxidant, NY: no-yes user, YY: yes-yes user, SMI_{low}: low skeletal muscle mass index, HNSCC: head and neck squamous cell carcinoma, L3, lumbar vertebral, CYP1B1: cytochrome P450 family 1B1.

Conclusion

Study I

- High levels of pSTAT3^{Y705} in breast cancer were associated with patient characteristics such as younger age, smaller body constitution, and any alcohol consumption
- A high level of pSTAT3^{Y705} was associated with smaller tumors, lower histological grade, PR negativity, HER2 negativity and lymph-node negativity but not ER status
- pSTAT3^{Y705} was not an independent prognostic factor
- pSTAT3^{Y705} negativity may influence prognosis in tamoxifen-treated chemo-naïve patients with breast cancer

Study II

- Antioxidant users more often reported MHT use. Other characteristics differed between patients in Lund and Helsingborg regarding antioxidant use
- Associations between antioxidant use and preoperative tumor characteristics were not consistent between patients in Lund and Helsingborg and regular use (YY) was associated with smaller tumors
- Antioxidant use influenced clinical outcome differently depending on the type of antioxidant, timing of use, and activation of AHR
- Exposure to vitamin C increased levels of CYP1B1, indicating activation of the AHR pathway in the MCF-7 and MDA-MB-231 cell lines

Study III

- SMI_{low} was associated with older age, lower BMI, current smoking, and tumors more often located in the hypopharynx and less often in the oropharynx
- There was no association of either medical treatment adherence or RT adherence and SMI_{low}
- Patients with SMI_{low} had worse OS and PFS in univariable models. In multivariable models, associations with outcome were only significant for men when analyzed separately
- An interaction between SMI_{low} and randomized treatment for PFS in favor of cisplatin was identified and warrants further validation

Future perspectives

The results presented in this thesis exemplify ways in which lifestyle is associated with tumor characteristics and influences treatment outcome. The influence of lifestyle in an oncological setting is complex and difficult to decipher, particularly since lifestyles change with time and trends, and analysis often relies on patient-reported data (73,86). To further elucidate the prognostic role of activated STAT3 in breast cancer, it is necessary to determine the influence of different phosphorylation sites and STAT3 isoforms (391). Increased IL-6 levels prior to adjuvant treatments have been associated with increased distant metastasis risk (392). These changes may need to be taken into consideration if pSTAT3^{Y705} is to be used as a prognostic marker, given that patient characteristics (392) and different treatments can influence systemic IL-6 levels (393) and tumor pSTAT3$^{Y705/S727}$ levels (394).

An association between pSTAT3^{Y705} in basal-like breast cancer and miRNA-222 has been reported (363). MiRNA-222 has oncogenic properties (363) but may also influence neovascularization negatively in endothelial cells. Interestingly, increased levels have been reported in patients with breast cancer one month after RT (395). The ways in which pSTAT3^{Y705} and miRNA-222 influence each other in regard to oncogenic properties and atherosclerosis warrant further research.

The STAT3 and AHR pathways both appear to have different roles depending on estrogen status, so future studies may gain from analyzing these markers separately. The AHR pathway has multiple roles, including modulation of immune functions (396). By using multiplexed IHC, Kim *et al.* detected AHR positivity in tumor and immune cells in five different cancer types, which each present different characteristics of AHR positivity in immune cells (208). A similar evaluation in breast cancer could generate a comprehensive analysis of AHR positivity and the influence of antioxidant use, and it could complement studies reporting activation in both tumors and stroma in breast cancer (204).

Inhibitors of AHR aimed at immune-suppressive functions in the tumor microenvironment (396) are currently being tested in a metastatic setting of solid cancers (NCT04069026), and an initial positive response has been reported in a conference abstract (397). In contrast, dietary supplements are not specific, while influences such as those of the AHR pathway can be either agonistic or antagonistic, and the effects are difficult to predict (373). For instance, the impact of vitamin C

on the AHR pathway with possible influence of estrogen metabolism warrants validation, particularly since concentrations used in the experiments are achievable with dietary vitamin C intake. The influence of genetically predicted vitamin C on characteristics of tumors and the tumor microenvironment reported by Yuan *et al.* (104) is a topic of interest. It would be interesting if future studies could validate these findings in combination with the expression of vitamin C receptors in tumors and reported vitamin C intake.

The use of medical imaging performed for diagnostic (398) or treatment-planning purposes (238) has the potential to provide prognostic information, but it will be essential to establish relevant sex-specific cutoffs to define SMI_{low} for different ethnicities or geographical areas (286,320). In the HNSCC population, where women are underrepresented, collaborations to establish larger study samples are needed to address the influence of SMI_{low} on prognosis (321). With improved technology (379,399), the methodology has the potential to improve the prognostic value without increased work load. For instance, multiple levels of measurements could be used (379), including the quantification of muscle density as a proxy for muscle quality and levels of fat infiltration (400). These aspects should be explored further in the HNSCC population.

The underlying cause of reduced muscle mass influences the possibility for interventions prior to, during, and after treatment. For instance, the ongoing DAHANCA 31 randomized trial is evaluating resistance training for patients with HNSCC treated with chemo-RT (401). With improved technology also or newer treatment equipment, online images in radiation therapy will be able to provide accurate HU values for comparisons at different time points during the treatment period. Future studies on interventions and prognosis in HNSCC could include this information. Overall, host–tumor interactions are important to consider in oncological research and could provide information on how to improve personalized and precision medicine in the future.

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References

1. United Nations. Transforming our world: The 2030 agenda for sustainable development 2019, accessed 10-12-2024.
2. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-263. DOI: 10.3322/caac.21834.
3. The Swedish Board of Social Affairs and Health (Socialstyrelsen). Cancer i Siffror 2023, www.socialstyrelsen.se2023 (2023).
4. Kubli SP, Bassi C, Roux C, et al. AhR controls redox homeostasis and shapes the tumor microenvironment in BRCA1-associated breast cancer. Proc Natl Acad Sci U S A 2019;116:3604-3613. DOI: 10.1073/pnas.1815126116.
5. Scoccianti C, Cecchini M, Anderson AS, et al. European Code against Cancer 4th Edition: Alcohol drinking and cancer. Cancer Epidemiol 2016;45:181-188. DOI: 10.1016/j.canep.2016.09.011.
6. Heng YJ, Hankinson SE, Wang J, et al. The Association of Modifiable Breast Cancer Risk Factors and Somatic Genomic Alterations in Breast Tumors: The Cancer Genome Atlas Network. Cancer Epidemiol Biomarkers Prev 2020;29:599-605. DOI: 10.1158/1055-9965.Epi-19-1087.
7. Mandal R, Şenbabaoğlu Y, Desrichard A, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. JCI Insight 2016;1:e89829. DOI: 10.1172/jci.insight.89829.
8. Collaborative. Group on Hormonal Factors in Breast Cancer Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence. Lancet 2019;394:1159-1168. DOI: 10.1016/s0140-6736(19)31709-x.
9. Findlay M, White K, Brown C, et al. Nutritional status and skeletal muscle status in patients with head and neck cancer: Impact on outcomes. J Cachexia Sarcopenia Muscle 2021;12:2187-2198. DOI: 10.1002/jcsm.12829.
10. Karavolia E, van Rijn-Dekker MI, Van den Bosch L, et al. Impact of sarcopenia on acute radiation-induced toxicity in head and neck cancer patients. Radiother Oncol 2022;170:122-128. DOI: 10.1016/j.radonc.2022.03.009.
11. Meyer F, Bairati I, Fortin A, et al. Interaction between antioxidant vitamin supplementation and cigarette smoking during radiation therapy in relation to long-term effects on recurrence and mortality: a randomized trial among head and neck cancer patients. Int J Cancer 2008;122:1679-1683. DOI: 10.1002/ijc.23200.
12. Abell K, Bilancio A, Clarkson RW, et al. Stat3-induced apoptosis requires a molecular switch in PI(3)K subunit composition. Nat Cell Biol 2005;7:392-398. DOI: 10.1038/ncb1242.

13. Arnold KM, Opdenaker LM, Flynn NJ, et al. Radiation induces an inflammatory response that results in STAT3-dependent changes in cellular plasticity and radioresistance of breast cancer stem-like cells. Int J Radiat Biol 2020;96:434-447. DOI: 10.1080/09553002.2020.1705423.
14. Makhlin I, McAndrew NP, Wileyto EP, et al. Ruxolitinib and exemestane for estrogen receptor positive, aromatase inhibitor resistant advanced breast cancer. NPJ Breast Cancer 2022;8:122. DOI: 10.1038/s41523-022-00487-x.
15. He Z, Song B, Zhu M, et al. Comprehensive pan-cancer analysis of STAT3 as a prognostic and immunological biomarker. Scientific Reports 2023;13:5069. DOI: 10.1038/s41598-023-31226-2.
16. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2019;48:16-31. DOI: 10.1093/ageing/afy169.
17. Vickers AJ, McSweeney DM, Choudhury A, et al. The prognostic significance of sarcopenia in patients treated with definitive radiotherapy: A systematic review. Radiother Oncol 2025;203:110663. DOI: 10.1016/j.radonc.2024.110663.
18. Cohn BA, Cirillo PM, Terry MB. DDT and Breast Cancer: Prospective Study of Induction Time and Susceptibility Windows. J Natl Cancer Inst 2019;111:803-810. DOI: 10.1093/jnci/djy198.
19. Buchholz MB, Scheerman DI, Levato R, et al. Human breast tissue engineering in health and disease. EMBO Mol Med 2024;16:2299-2321. DOI: 10.1038/s44321-024-00112-3.
20. Russo J, Hu YF, Yang X, et al. Developmental, cellular, and molecular basis of human breast cancer. J Natl Cancer Inst Monogr 2000:17-37. DOI: 10.1093/oxfordjournals.jncimonographs.a024241.
21. Eckert-Lind C, Busch AS, Petersen JH, et al. Worldwide Secular Trends in Age at Pubertal Onset Assessed by Breast Development Among Girls: A Systematic Review and Meta-analysis. JAMA Pediatr 2020;174:e195881. DOI: 10.1001/jamapediatrics.2019.5881.
22. Paavolainen O, Peurla M, Koskinen LM, et al. Volumetric analysis of the terminal ductal lobular unit architecture and cell phenotypes in the human breast. Cell Rep 2024;43:114837. DOI: 10.1016/j.celrep.2024.114837.
23. Wunder J, Pemp D, Cecil A, et al. Influence of breast cancer risk factors on proliferation and DNA damage in human breast glandular tissues: role of intracellular estrogen levels, oxidative stress and estrogen biotransformation. Arch Toxicol 2022;96:673-687. DOI: 10.1007/s00204-021-03198-7.
24. Jindal S, Gao D, Bell P, et al. Postpartum breast involution reveals regression of secretory lobules mediated by tissue-remodeling. Breast Cancer Research 2014;16:R31. DOI: 10.1186/bcr3633.
25. Hanna M, Dumas I, Orain M, et al. Association between local inflammation and breast tissue age-related lobular involution among premenopausal and postmenopausal breast cancer patients. PLoS One 2017;12:e0183579. DOI: 10.1371/journal.pone.0183579.

26. Jindal S, Narasimhan J, Borges VF, et al. Characterization of weaning-induced breast involution in women: implications for young women's breast cancer. NPJ Breast Cancer 2020;6:55. DOI: 10.1038/s41523-020-00196-3.
27. Basree MM, Shinde N, Koivisto C, et al. Abrupt involution induces inflammation, estrogenic signaling, and hyperplasia linking lack of breastfeeding with increased risk of breast cancer. Breast Cancer Res 2019;21:80. DOI: 10.1186/s13058-019-1163-7.
28. Hughes K, Watson CJ. The Multifaceted Role of STAT3 in Mammary Gland Involution and Breast Cancer. Int J Mol Sci 2018;19. DOI: 10.3390/ijms19061695.
29. Martinson HA, Jindal S, Durand-Rougely C, et al. Wound healing-like immune program facilitates postpartum mammary gland involution and tumor progression. Int J Cancer 2015;136:1803-1813. DOI: 10.1002/ijc.29181.
30. Milanese TR, Hartmann LC, Sellers TA, et al. Age-related lobular involution and risk of breast cancer. J Natl Cancer Inst 2006;98:1600-1607. DOI: 10.1093/jnci/djj439.
31. Figueroa JD, Pfeiffer RM, Patel DA, et al. Terminal duct lobular unit involution of the normal breast: implications for breast cancer etiology. J Natl Cancer Inst 2014;106. DOI: 10.1093/jnci/dju286.
32. Gabrielson M, Hammarström M, Bäcklund M, et al. Effects of tamoxifen on normal breast tissue histological composition: Results from a randomised six-arm placebo-controlled trial in healthy women. Int J Cancer 2023;152:2362-2372. DOI: 10.1002/ijc.34430.
33. Stallings-Mann ML, Heinzen EP, Vierkant RA, et al. Postlactational involution biomarkers plasminogen and phospho-STAT3 are linked with active age-related lobular involution. Breast Cancer Res Treat 2017;166:133-143. DOI: 10.1007/s10549-017-4413-3.
34. Niehoff NM, Keil AP, Jones RR, et al. Outdoor air pollution and terminal duct lobular involution of the normal breast. Breast Cancer Res 2020;22:100. DOI: 10.1186/s13058-020-01339-x.
35. T G, Sw K, S S, et al. Ethnicity and breast cancer incidence in over 329 500 women in England in 2011-2019. Eur J Surg Oncol 2025:109585. DOI: 10.1016/j.ejso.2025.109585.
36. Seely JM, Ellison LF, Billette JM, et al. Incidence of Breast Cancer in Younger Women: A Canadian Trend Analysis. Can Assoc Radiol J 2024;75:847-854. DOI: 10.1177/08465371241246422.
37. Kehm RD, Daaboul JM, Tehranifar P, et al. Geographic differences in early-onset breast cancer incidence trends in the USA, 2001-2020, is it time for a geographic risk score? Cancer Causes Control 2025;36:707-717. DOI: 10.1007/s10552-025-01968-7.
38. Wojtyla C, Bertuccio P, Wojtyla A, et al. European trends in breast cancer mortality, 1980-2017 and predictions to 2025. Eur J Cancer 2021;152:4-17. DOI: 10.1016/j.ejca.2021.04.026.
39. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-752. DOI: 10.1038/35021093.

40. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-8423. DOI: 10.1073/pnas.0932692100.
41. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750-2767. DOI: 10.1172/jci45014.
42. Kumar B, Khatpe AS, Guanglong J, et al. Stromal heterogeneity may explain increased incidence of metaplastic breast cancer in women of African descent. Nat Commun 2023;14:5683. DOI: 10.1038/s41467-023-41473-6.
43. Li W, Ma H, Zhang J, et al. Unraveling the roles of CD44/CD24 and ALDH1 as cancer stem cell markers in tumorigenesis and metastasis. Sci Rep 2017;7:13856. DOI: 10.1038/s41598-017-14364-2.
44. Ben-Yaakov H, Meshel T, Pasmanik-Chor M, et al. A Tumor Microenvironment-Driven Network Regulated by STAT3 and p65 Negatively Controls the Enrichment of Cancer Stem Cells in Human HR+/HER2- Breast Cancer. Cancers (Basel) 2023;15. DOI: 10.3390/cancers15082255.
45. Lee AV, Oesterreich S, Davidson NE. MCF-7 cells--changing the course of breast cancer research and care for 45 years. J Natl Cancer Inst 2015;107. DOI: 10.1093/jnci/djv073.
46. Press DJ, Pharoah P. Risk factors for breast cancer: a reanalysis of two case-control studies from 1926 and 1931. Epidemiology 2010;21:566-572. DOI: 10.1097/EDE.0b013e3181e08eb3.
47. Tamimi RM, Spiegelman D, Smith-Warner SA, et al. Population Attributable Risk of Modifiable and Nonmodifiable Breast Cancer Risk Factors in Postmenopausal Breast Cancer. Am J Epidemiol 2016;184:884-893. DOI: 10.1093/aje/kww145.
48. Fridhammar A. HT, Persson S. Cancer i Sverige - Hur mycket beror på påverkbara riskfaktorer?, https://ihe.se/rapport/cancer-och-paverkbara-riskfaktorer/ 2020 accessed 11-08-2024.
49. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett 2005;227:115-124. DOI: 10.1016/j.canlet.2004.10.007.
50. Sondermann NC, Faßbender S, Hartung F, et al. Functions of the aryl hydrocarbon receptor (AHR) beyond the canonical AHR/ARNT signaling pathway. Biochem Pharmacol 2023;208:115371. DOI: 10.1016/j.bcp.2022.115371.
51. Andersson S, Sundberg M, Pristovsek N, et al. Insufficient antibody validation challenges oestrogen receptor beta research. Nat Commun 2017;8:15840. DOI: 10.1038/ncomms15840.
52. Nelson AW, Groen AJ, Miller JL, et al. Comprehensive assessment of estrogen receptor beta antibodies in cancer cell line models and tissue reveals critical limitations in reagent specificity. Mol Cell Endocrinol 2017;440:138-150. DOI: 10.1016/j.mce.2016.11.016.

53. Ruiz R, Herrero C, Strasser-Weippl K, et al. Epidemiology and pathophysiology of pregnancy-associated breast cancer: A review. Breast 2017;35:136-141. DOI: 10.1016/j.breast.2017.07.008.
54. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002;360:187-195. DOI: 10.1016/s0140-6736(02)09454-0.
55. Islami F, Liu Y, Jemal A, et al. Breastfeeding and breast cancer risk by receptor status--a systematic review and meta-analysis. Ann Oncol 2015;26:2398-2407. DOI: 10.1093/annonc/mdv379.
56. Anderson AS, Key TJ, Norat T, et al. European Code against Cancer 4th Edition: Obesity, body fatness and cancer. Cancer Epidemiol 2015;39 Suppl 1:S34-45. DOI: 10.1016/j.canep.2015.01.017.
57. Zhang GQ, Chen JL, Luo Y, et al. Menopausal hormone therapy and women's health: An umbrella review. PLoS Med 2021;18:e1003731. DOI: 10.1371/journal.pmed.1003731.
58. Mørch LS, Skovlund CW, Hannaford PC, et al. Contemporary Hormonal Contraception and the Risk of Breast Cancer. N Engl J Med 2017;377:2228-2239. DOI: 10.1056/NEJMoa1700732.
59. Cohn BA, La Merrill M, Krigbaum NY, et al. DDT Exposure in Utero and Breast Cancer. J Clin Endocrinol Metab 2015;100:2865-2872. DOI: 10.1210/jc.2015-1841.
60. Bodewes FTH, van Asselt AA, Dorrius MD, et al. Mammographic breast density and the risk of breast cancer: A systematic review and meta-analysis. Breast 2022;66:62-68. DOI: 10.1016/j.breast.2022.09.007.
61. Sturesdotter L, Sandsveden M, Johnson K, et al. Mammographic tumour appearance is related to clinicopathological factors and surrogate molecular breast cancer subtype. Sci Rep 2020;10:20814. DOI: 10.1038/s41598-020-77053-7.
62. Cuzick J, Warwick J, Pinney E, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. J Natl Cancer Inst 2011;103:744-752. DOI: 10.1093/jnci/djr079.
63. McBride RB, Fei K, Rothstein JH, et al. Alcohol and Tobacco Use in Relation to Mammographic Density in 23,456 Women. Cancer Epidemiol Biomarkers Prev 2020;29:1039-1048. DOI: 10.1158/1055-9965.Epi-19-0348.
64. Radenkovic S, Konjevic G, Gavrilovic D, et al. pSTAT3 expression associated with survival and mammographic density of breast cancer patients. Pathol Res Pract 2019;215:366-372. DOI: 10.1016/j.prp.2018.12.023.
65. Cho BA, Iyengar NM, Zhou XK, et al. Increased trunk fat is associated with altered gene expression in breast tissue of normal weight women. NPJ Breast Cancer 2022;8:15. DOI: 10.1038/s41523-021-00369-8.
66. Leitzmann M, Powers H, Anderson AS, et al. European Code against Cancer 4th Edition: Physical activity and cancer. Cancer Epidemiol 2015;39 Suppl 1:S46-55. DOI: 10.1016/j.canep.2015.03.009.

67. Bea JW, Ochs-Balcom HM, Valencia CI, et al. Abdominal visceral and subcutaneous adipose tissue associations with postmenopausal breast cancer incidence. JNCI Cancer Spectr 2025;9. DOI: 10.1093/jncics/pkaf007.
68. Löf M, Sandin S, Hilakivi-Clarke L, et al. Birth weight in relation to endometrial and breast cancer risks in Swedish women. British Journal of Cancer 2007;96:134-136. DOI: 10.1038/sj.bjc.6603504.
69. Sandvei MS, Opdahl S, Valla M, et al. The association of women's birth size with risk of molecular breast cancer subtypes: a cohort study. BMC Cancer 2021;21:299. DOI: 10.1186/s12885-021-08027-9.
70. International Agency for Research on Cancer. Personal habits and indoor combustions (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100E). World Health Organization 2012, accessed 09-01-2024.
71. Rumgay H, Shield K, Charvat H, et al. Global burden of cancer in 2020 attributable to alcohol consumption: a population-based study. Lancet Oncol 2021;22:1071-1080. DOI: 10.1016/s1470-2045(21)00279-5.
72. Cederfjäll J, Lidfeldt J, Nerbrand C, et al. Alcohol consumption among middle-aged women: a population-based study of Swedish women. The Women's Health in Lund Area (WHILA) study. Eur Addict Res 2004;10:15-21. DOI: 10.1159/000073722.
73. Leon ME, Peruga A, McNeill A, et al. European Code against Cancer, 4th Edition: Tobacco and cancer. Cancer Epidemiol 2015;39 Suppl 1:S20-33. DOI: 10.1016/j.canep.2015.06.001.
74. Scala M, Bosetti C, Bagnardi V, et al. Dose-response Relationships Between Cigarette Smoking and Breast Cancer Risk: A Systematic Review and Meta-analysis. J Epidemiol 2023;33:640-648. DOI: 10.2188/jea.JE20220206.
75. Possenti I, Scala M, Carreras G, et al. Exposure to second-hand smoke and breast cancer risk in non-smoking women: a comprehensive systematic review and meta-analysis. Br J Cancer 2024;131:1116-1125. DOI: 10.1038/s41416-024-02732-5.
76. Boraka Ö, Klintman M, Rosendahl AH. Physical Activity and Long-Term Risk of Breast Cancer, Associations with Time in Life and Body Composition in the Prospective Malmö Diet and Cancer Study. Cancers (Basel) 2022;14. DOI: 10.3390/cancers14081960.
77. Huerta JM, Molina AJ, Chirlaque MD, et al. Domain-specific patterns of physical activity and risk of breast cancer sub-types in the MCC-Spain study. Breast Cancer Res Treat 2019;177:749-760. DOI: 10.1007/s10549-019-05358-x.
78. Ma H, Xu X, Ursin G, et al. Reduced risk of breast cancer associated with recreational physical activity varies by HER2 status. Cancer Med 2015;4:1122-1135. DOI: 10.1002/cam4.465.
79. Bettariga F, Taaffe DR, Borsati A, et al. Effects of exercise on inflammation in female survivors of nonmetastatic breast cancer: a systematic review and meta-analysis. J Natl Cancer Inst 2025. DOI: 10.1093/jnci/djaf062.
80. Swain CTV, Drummond AE, Milne RL, et al. Linking Physical Activity to Breast Cancer Risk via Inflammation, Part 1: The Effect of Physical Activity on

Inflammation. Cancer Epidemiol Biomarkers Prev 2023;32:588-596. DOI: 10.1158/1055-9965.Epi-22-0928.
81. Le Guennec D, Hatte V, Farges M-C, et al. Modulation of inter-organ signalling in obese mice by spontaneous physical activity during mammary cancer development. Scientific Reports 2020;10:8794. DOI: 10.1038/s41598-020-65131-9.
82. Bouayed J, Bohn T. Exogenous antioxidants--Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxid Med Cell Longev 2010;3:228-237. DOI: 10.4161/oxim.3.4.12858.
83. Lewis LN, Hayhoe RPG, Mulligan AA, et al. Lower Dietary and Circulating Vitamin C in Middle- and Older-Aged Men and Women Are Associated with Lower Estimated Skeletal Muscle Mass. J Nutr 2020;150:2789-2798. DOI: 10.1093/jn/nxaa221.
84. Welch AA, Jennings A, Kelaiditi E, et al. Cross-Sectional Associations Between Dietary Antioxidant Vitamins C, E and Carotenoid Intakes and Sarcopenic Indices in Women Aged 18-79 Years. Calcif Tissue Int 2020;106:331-342. DOI: 10.1007/s00223-019-00641-x.
85. Mahasneh AA, Zhang Y, Zhao H, et al. Lifestyle predictors of oxidant and antioxidant enzyme activities and total antioxidant capacity in healthy women: a cross-sectional study. J Physiol Biochem 2016;72:745-762. DOI: 10.1007/s13105-016-0513-5.
86. Cadeau C, Fournier A, Mesrine S, et al. Vitamin C supplement intake and postmenopausal breast cancer risk: interaction with dietary vitamin C. Am J Clin Nutr 2016;104:228-234. DOI: 10.3945/ajcn.115.126326.
87. Petermann-Rocha F, Pell JP, Celis-Morales C, et al. Frailty, sarcopenia, cachexia and malnutrition as comorbid conditions and their associations with mortality: a prospective study from UK Biobank. J Public Health (Oxf) 2022;44:e172-e180. DOI: 10.1093/pubmed/fdaa226.
88. Mangione CM, Barry MJ, Nicholson WK, et al. Vitamin, Mineral, and Multivitamin Supplementation to Prevent Cardiovascular Disease and Cancer: US Preventive Services Task Force Recommendation Statement. Jama 2022;327:2326-2333. DOI: 10.1001/jama.2022.8970.
89. Thakkar S, Anklam E, Xu A, et al. Regulatory landscape of dietary supplements and herbal medicines from a global perspective. Regul Toxicol Pharmacol 2020;114:104647. DOI: 10.1016/j.yrtph.2020.104647.
90. Egenvård S. Egenvårdsmarknaden 2019, https://www.svenskegenvard.se/content/uploads/2020/03/forsaljningsstatistik-2019.pdf 2019, accessed 10-08-2021.
91. Amcoff E, Edberg A, Barbieri H, et al. Riksmaten-Adults 2010–11: Food, Nutrient Intake among Adults in Sweden. In: Livsmedelsverket ed. Uppsala, 2012.
92. Carlsen MH, Halvorsen BL, Holte K, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutr J 2010;9:3. DOI: 10.1186/1475-2891-9-3.

93. Dietz BM, Hajirahimkhan A, Dunlap TL, et al. Botanicals and Their Bioactive Phytochemicals for Women's Health. Pharmacol Rev 2016;68:1026-1073. DOI: 10.1124/pr.115.010843.
94. Beckman Sundh. HH, Nälsén C., Wallin S. Kosttillskott - Kunskapsöversikt, https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2017/2017-nr-16-del-2-kosttillskott-kunskapsoversikt.pdf 2017, accessed 01-20-2025.
95. Barnes K, Ball L, Desbrow B, et al. Consumption and reasons for use of dietary supplements in an Australian university population. Nutrition 2016;32:524-530. DOI: 10.1016/j.nut.2015.10.022.
96. Mullie P, Clarys P, Hulens M, et al. Socioeconomic, health, and dietary determinants of multivitamin supplements use in Belgium. Int J Public Health 2011;56:289-294. DOI: 10.1007/s00038-010-0210-z.
97. Kristoffersen AE, Stub T, Nilsen JV, et al. Exploring dietary changes and supplement use among cancer patients in Norway: prevalence, motivations, disclosure, information, and perceived risks and benefits: a cross sectional study. BMC Nutr 2024;10:65. DOI: 10.1186/s40795-024-00872-8.
98. Yang H, Villani RM, Wang H, et al. The role of cellular reactive oxygen species in cancer chemotherapy. J Exp Clin Cancer Res 2018;37:266. DOI: 10.1186/s13046-018-0909-x.
99. Dietrich C. Antioxidant Functions of the Aryl Hydrocarbon Receptor. Stem Cells Int 2016;2016:7943495. DOI: 10.1155/2016/7943495.
100. Bakker MF, Peeters PH, Klaasen VM, et al. Plasma carotenoids, vitamin C, tocopherols, and retinol and the risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition cohort. Am J Clin Nutr 2016;103:454-464. DOI: 10.3945/ajcn.114.101659.
101. Russnes KM, Möller E, Wilson KM, et al. Total antioxidant intake and prostate cancer in the Cancer of the Prostate in Sweden (CAPS) study. A case control study. BMC Cancer 2016;16:438. DOI: 10.1186/s12885-016-2486-8.
102. Wang T, Dong Y, Huang Z, et al. Antioxidants stimulate BACH1-dependent tumor angiogenesis. J Clin Invest 2023. DOI: 10.1172/jci169671.
103. Wiel C, Le Gal K, Ibrahim MX, et al. BACH1 Stabilization by Antioxidants Stimulates Lung Cancer Metastasis. Cell 2019;178:330-345.e322. DOI: 10.1016/j.cell.2019.06.005.
104. Yuan J, Zhang YH, Hua X, et al. Genetically predicted vitamin C levels significantly affect patient survival and immunotypes in multiple cancer types. Front Immunol 2023;14:1177580. DOI: 10.3389/fimmu.2023.1177580.
105. Campbell EJ, Dachs GU, Morrin HR, et al. Activation of the hypoxia pathway in breast cancer tissue and patient survival are inversely associated with tumor ascorbate levels. BMC Cancer 2019;19:307. DOI: 10.1186/s12885-019-5503-x.
106. Klein EA, Thompson IM, Jr., Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Jama 2011;306:1549-1556. DOI: 10.1001/jama.2011.1437.
107. The Alpha-Tocopherol BCCPSG. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 1994;330:1029-1035. DOI: 10.1056/nejm199404143301501.

108. Cadeau C, Farvid MS, Rosner BA, et al. Dietary and Supplemental Vitamin C Intake and Risk of Breast Cancer: Results from the Nurses' Health Studies. J Nutr 2022;152:835-843. DOI: 10.1093/jn/nxab407.
109. Curigliano G, Burstein HJ, Gnant M, et al. Understanding breast cancer complexity to improve patient outcomes: The St Gallen International Consensus Conference for the Primary Therapy of Individuals with Early Breast Cancer 2023. Ann Oncol 2023;34:970-986. DOI: 10.1016/j.annonc.2023.08.017.
110. Yoshimura A, Imoto I, Iwata H. Functions of Breast Cancer Predisposition Genes: Implications for Clinical Management. Int J Mol Sci 2022;23. DOI: 10.3390/ijms23137481.
111. Song YM, Qian XL, Xia XQ, et al. STAT3 and PD-L1 are negatively correlated with ATM and have impact on the prognosis of triple-negative breast cancer patients with low ATM expression. Breast Cancer Res Treat 2022;196:45-56. DOI: 10.1007/s10549-022-06679-0.
112. Socialstyrelsen. Framtidens screening Redovisning av regeringsuppdraget Framtidsinriktad omvärldsanalys om förändringar och utvecklingstendenser inom cancerscreening, https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/artikelkatalog/ovrigt/2019-4-13.pdf 2019, 11-02-2024.
113. Saghir H, Veerla S, Malmberg M, et al. How Reliable Are Gene Expression-Based and Immunohistochemical Biomarkers Assessed on a Core-Needle Biopsy? A Study of Paired Core-Needle Biopsies and Surgical Specimens in Early Breast Cancer. Cancers (Basel) 2022;14. DOI: 10.3390/cancers14164000.
114. Thennavan A, Beca F, Xia Y, et al. Molecular analysis of TCGA breast cancer histologic types. Cell Genom 2021;1. DOI: 10.1016/j.xgen.2021.100067.
115. Kaidar-Person O, Boersma LJ, Poortmans P, et al. Residual Glandular Breast Tissue After Mastectomy: A Systematic Review. Ann Surg Oncol 2020;27:2288-2296. DOI: 10.1245/s10434-020-08516-4.
116. Brierley J.D. GMK, Wittekin C. . UICC TNM Classification of malignant tumors Eight Edition. Chichester: Whiley, 2017.
117. Aleskandarany MA, Agarwal D, Negm OH, et al. The prognostic significance of STAT3 in invasive breast cancer: analysis of protein and mRNA expressions in large cohorts. Breast Cancer Res Treat 2016;156:9-20. DOI: 10.1007/s10549-016-3709-z.
118. Acs B, Hartman J, Sönmez D, et al. Real-world overall survival and characteristics of patients with ER-zero and ER-low HER2-negative breast cancer treated as triple-negative breast cancer: a Swedish population-based cohort study. Lancet Reg Health Eur 2024;40:100886. DOI: 10.1016/j.lanepe.2024.100886.
119. de Boniface J, Szulkin R, Johansson ALV. Survival After Breast Conservation vs Mastectomy Adjusted for Comorbidity and Socioeconomic Status: A Swedish National 6-Year Follow-up of 48 986 Women. JAMA Surg 2021;156:628-637. DOI: 10.1001/jamasurg.2021.1438.
120. Early Breast Cancer Trialists' Collaborative Groupe (EBCTCG). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. Lancet Oncol 2021;22:1139-1150. DOI: 10.1016/s1470-2045(21)00288-6.

121. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206-2223. DOI: 10.1093/annonc/mdt303.
122. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020;38:1346-1366. DOI: 10.1200/jco.19.02309.
123. Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 2021;113:808-819. DOI: 10.1093/jnci/djaa201.
124. Swedish Breast Cancer Group (SweBCG). Kirurgisk och onkologisk behandling av bröstcancer - SweBCGs behandlingsrekommendationer, http://www.swebcg.se/wp-content/uploads/2024/10/Kortversionen-NVP-Brostcancer-241001-1.pdf 2024, accessed 12-01-2025.
125. Holm-Rasmussen EV, Jensen MB, Balslev E, et al. Reduced risk of axillary lymphatic spread in triple-negative breast cancer. Breast Cancer Res Treat 2015;149:229-236. DOI: 10.1007/s10549-014-3225-y.
126. Wilke LG, Czechura T, Wang C, et al. Repeat surgery after breast conservation for the treatment of stage 0 to II breast carcinoma: a report from the National Cancer Data Base, 2004-2010. JAMA Surg 2014;149:1296-1305. DOI: 10.1001/jamasurg.2014.926.
127. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N Engl J Med 2002;347:1233-1241. DOI: 10.1056/NEJMoa022152.
128. Fisher B, Jeong JH, Anderson S, et al. Twenty-five-year follow-up of a randomized trial comparing radical mastectomy, total mastectomy, and total mastectomy followed by irradiation. N Engl J Med 2002;347:567-575. DOI: 10.1056/NEJMoa020128.
129. Darby S, McGale P, Correa C, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet 2011;378:1707-1716. DOI: 10.1016/s0140-6736(11)61629-2.
130. Litière S, Werutsky G, Fentiman IS, et al. Breast conserving therapy versus mastectomy for stage I-II breast cancer: 20 year follow-up of the EORTC 10801 phase 3 randomised trial. Lancet Oncol 2012;13:412-419. DOI: 10.1016/s1470-2045(12)70042-6.
131. Veronesi U, Cascinelli N, Mariani L, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med 2002;347:1227-1232. DOI: 10.1056/NEJMoa020989.
132. van Maaren MC, de Munck L, de Bock GH, et al. 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast

cancer in the Netherlands: a population-based study. Lancet Oncol 2016;17:1158-1170. DOI: 10.1016/s1470-2045(16)30067-5.
133. Gulis K, Ellbrant J, Bendahl PO, et al. Health-related quality of life by type of breast surgery in women with primary breast cancer: prospective longitudinal cohort study. BJS Open 2024;8. DOI: 10.1093/bjsopen/zrae042.
134. de Boniface J, Szulkin R, Johansson ALV. Medical and surgical postoperative complications after breast conservation versus mastectomy in older women with breast cancer: Swedish population-based register study of 34 139 women. Br J Surg 2023;110:344-352. DOI: 10.1093/bjs/znac411.
135. de Boniface J, Szulkin R, Johansson ALV. Major surgical postoperative complications and survival in breast cancer: Swedish population-based register study in 57 152 women. Br J Surg 2022;109:977-983. DOI: 10.1093/bjs/znac275.
136. Tvedskov TF, Szulkin R, Alkner S, et al. Axillary clearance and chemotherapy rates in ER+HER2- breast cancer: secondary analysis of the SENOMAC trial. Lancet Reg Health Eur 2024;47:101083. DOI: 10.1016/j.lanepe.2024.101083.
137. Appelgren M, Sackey H, Wengström Y, et al. Patient-reported outcomes one year after positive sentinel lymph node biopsy with or without axillary lymph node dissection in the randomized SENOMAC trial. Breast 2022;63:16-23. DOI: 10.1016/j.breast.2022.02.013.
138. Majid S, Bendahl PO, Huss L, et al. Validation of the Skåne University Hospital nomogram for the preoperative prediction of a disease-free axilla in patients with breast cancer. BJS Open 2021;5. DOI: 10.1093/bjsopen/zrab027.
139. Gentilini OD, Botteri E, Sangalli C, et al. Sentinel Lymph Node Biopsy vs No Axillary Surgery in Patients With Small Breast Cancer and Negative Results on Ultrasonography of Axillary Lymph Nodes: The SOUND Randomized Clinical Trial. JAMA Oncol 2023;9:1557-1564. DOI: 10.1001/jamaoncol.2023.3759.
140. Andersson Y, Bergkvist L, Frisell J, et al. Omitting completion axillary lymph node dissection after detection of sentinel node micrometastases in breast cancer: first results from the prospective SENOMIC trial. Br J Surg 2021;108:1105-1111. DOI: 10.1093/bjs/znab141.
141. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. Lancet 2023;401:1277-1292. DOI: 10.1016/s0140-6736(23)00285-4.
142. Bonadonna G, Moliterni A, Zambetti M, et al. 30 years' follow up of randomised studies of adjuvant CMF in operable breast cancer: cohort study. Bmj 2005;330:217. DOI: 10.1136/bmj.38314.622095.8F.
143. Hubbert L, Mallios P, Karlström P, et al. Long-term and real-life incidence of cancer therapy-related cardiovascular toxicity in patients with breast cancer: a Swedish cohort study. Front Oncol 2023;13:1095251. DOI: 10.3389/fonc.2023.1095251.
144. Thorsen LB, Offersen BV, Danø H, et al. DBCG-IMN: A Population-Based Cohort Study on the Effect of Internal Mammary Node Irradiation in Early Node-Positive Breast Cancer. J Clin Oncol 2016;34:314-320. DOI: 10.1200/jco.2015.63.6456.

145. Whelan TJ, Pignol JP, Levine MN, et al. Long-term results of hypofractionated radiation therapy for breast cancer. N Engl J Med 2010;362:513-520. DOI: 10.1056/NEJMoa0906260.
146. Haviland JS, Owen JR, Dewar JA, et al. The UK Standardisation of Breast Radiotherapy (START) trials of radiotherapy hypofractionation for treatment of early breast cancer: 10-year follow-up results of two randomised controlled trials. Lancet Oncol 2013;14:1086-1094. DOI: 10.1016/s1470-2045(13)70386-3.
147. Coles CE, Haviland JS, Kirby AM, et al. Dose-escalated simultaneous integrated boost radiotherapy in early breast cancer (IMPORT HIGH): a multicentre, phase 3, non-inferiority, open-label, randomised controlled trial. Lancet 2023;401:2124-2137. DOI: 10.1016/s0140-6736(23)00619-0.
148. Adra J, Lundstedt D, Killander F, et al. Distribution of Locoregional Breast Cancer Recurrence in Relation to Postoperative Radiation Fields and Biological Subtypes. Int J Radiat Oncol Biol Phys 2019;105:285-295. DOI: 10.1016/j.ijrobp.2019.06.013.
149. Bartelink H, Maingon P, Poortmans P, et al. Whole-breast irradiation with or without a boost for patients treated with breast-conserving surgery for early breast cancer: 20-year follow-up of a randomised phase 3 trial. Lancet Oncol 2015;16:47-56. DOI: 10.1016/s1470-2045(14)71156-8.
150. Murray Brunt A, Haviland JS, Wheatley DA, et al. Hypofractionated breast radiotherapy for 1 week versus 3 weeks (FAST-Forward): 5-year efficacy and late normal tissue effects results from a multicentre, non-inferiority, randomised, phase 3 trial. Lancet 2020;395:1613-1626. DOI: 10.1016/s0140-6736(20)30932-6.
151. Taylor C, Correa C, Duane FK, et al. Estimating the Risks of Breast Cancer Radiotherapy: Evidence From Modern Radiation Doses to the Lungs and Heart and From Previous Randomized Trials. J Clin Oncol 2017;35:1641-1649. DOI: 10.1200/jco.2016.72.0722.
152. Killander F, Wieslander E, Karlsson P, et al. No Increased Cardiac Mortality or Morbidity of Radiation Therapy in Breast Cancer Patients After Breast-Conserving Surgery: 20-Year Follow-up of the Randomized SweBCGRT Trial. Int J Radiat Oncol Biol Phys 2020;107:701-709. DOI: 10.1016/j.ijrobp.2020.04.003.
153. Milo MLH, Thorsen LBJ, Johnsen SP, et al. Risk of coronary artery disease after adjuvant radiotherapy in 29,662 early breast cancer patients: A population-based Danish Breast Cancer Group study. Radiother Oncol 2021;157:106-113. DOI: 10.1016/j.radonc.2021.01.010.
154. Thomsen MS, Alsner J, Lutz CM, et al. Breast induration and irradiated volume in the DBCG HYPO trial: The impact of age, smoking, and boost. Radiother Oncol 2024;201:110574. DOI: 10.1016/j.radonc.2024.110574.
155. Alkner S, de Boniface J, Lundstedt D, et al. Protocol for the T-REX-trial: tailored regional external beam radiotherapy in clinically node-negative breast cancer patients with 1-2 sentinel node macrometastases - an open, multicentre, randomised non-inferiority phase 3 trial. BMJ Open 2023;13:e075543. DOI: 10.1136/bmjopen-2023-075543.

156. Coles CE, Griffin CL, Kirby AM, et al. Partial-breast radiotherapy after breast conservation surgery for patients with early breast cancer (UK IMPORT LOW trial): 5-year results from a multicentre, randomised, controlled, phase 3, non-inferiority trial. Lancet 2017;390:1048-1060. DOI: 10.1016/s0140-6736(17)31145-5.
157. Kunkler IH, Williams LJ, Jack WJL, et al. Breast-Conserving Surgery with or without Irradiation in Early Breast Cancer. N Engl J Med 2023;388:585-594. DOI: 10.1056/NEJMoa2207586.
158. Sjöström M, Fyles A, Liu FF, et al. Development and Validation of a Genomic Profile for the Omission of Local Adjuvant Radiation in Breast Cancer. J Clin Oncol 2023;41:1533-1540. DOI: 10.1200/jco.22.00655.
159. Sjöstrom M, Lundstedt D, Hartman L, et al. Response to Radiotherapy After Breast-Conserving Surgery in Different Breast Cancer Subtypes in the Swedish Breast Cancer Group 91 Radiotherapy Randomized Clinical Trial. J Clin Oncol 2017;35:3222-3229. DOI: 10.1200/JCO.2017.72.7263.
160. Crewe HK, Notley LM, Wunsch RM, et al. Metabolism of tamoxifen by recombinant human cytochrome P450 enzymes: formation of the 4-hydroxy, 4'-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. Drug Metab Dispos 2002;30:869-874. DOI: 10.1124/dmd.30.8.869.
161. Davies C, Godwin J, Gray R, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011;378:771-784. DOI: 10.1016/s0140-6736(11)60993-8.
162. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015;386:1341-1352. DOI: 10.1016/s0140-6736(15)61074-1.
163. Sheweita SA, Ammar RG, Sabra SA, et al. Letrozole and zoledronic acid changed signalling pathways involved in the apoptosis of breast cancer cells. J Taibah Univ Med Sci 2021;16:112-120. DOI: 10.1016/j.jtumed.2020.10.017.
164. Pagani O, Walley BA, Fleming GF, et al. Adjuvant Exemestane With Ovarian Suppression in Premenopausal Breast Cancer: Long-Term Follow-Up of the Combined TEXT and SOFT Trials. J Clin Oncol 2023;41:1376-1382. DOI: 10.1200/jco.22.01064.
165. Francis PA, Pagani O, Fleming GF, et al. Tailoring Adjuvant Endocrine Therapy for Premenopausal Breast Cancer. N Engl J Med 2018;379:122-137. DOI: 10.1056/NEJMoa1803164.
166. Meattini I, Becherini C, Caini S, et al. International multidisciplinary consensus on the integration of radiotherapy with new systemic treatments for breast cancer: European Society for Radiotherapy and Oncology (ESTRO)-endorsed recommendations. Lancet Oncol 2024;25:e73-e83. DOI: 10.1016/s1470-2045(23)00534-x.
167. Tóth G, Szőőr Á, Simon L, et al. The combination of trastuzumab and pertuzumab administered at approved doses may delay development of

trastuzumab resistance by additively enhancing antibody-dependent cell-mediated cytotoxicity. MAbs 2016;8:1361-1370. DOI: 10.1080/19420862.2016.1204503.
168. Loibl S, Jassem J, Sonnenblick A, et al. Adjuvant Pertuzumab and Trastuzumab in Early Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer in the APHINITY Trial: Third Interim Overall Survival Analysis With Efficacy Update. J Clin Oncol 2024;42:3643-3651. DOI: 10.1200/jco.23.02505.
169. Hu Y, Dong Z, Liu K. Unraveling the complexity of STAT3 in cancer: molecular understanding and drug discovery. J Exp Clin Cancer Res 2024;43:23. DOI: 10.1186/s13046-024-02949-5.
170. Lu L, Dong J, Wang L, et al. Activation of STAT3 and Bcl-2 and reduction of reactive oxygen species (ROS) promote radioresistance in breast cancer and overcome of radioresistance with niclosamide. Oncogene 2018;37:5292-5304. DOI: 10.1038/s41388-018-0340-y.
171. Zerdes I, Wallerius M, Sifakis EG, et al. STAT3 Activity Promotes Programmed-Death Ligand 1 Expression and Suppresses Immune Responses in Breast Cancer. Cancers (Basel) 2019;11. DOI: 10.3390/cancers11101479.
172. Jhaveri K, Teplinsky E, Silvera D, et al. Hyperactivated mTOR and JAK2/STAT3 Pathways: Molecular Drivers and Potential Therapeutic Targets of Inflammatory and Invasive Ductal Breast Cancers After Neoadjuvant Chemotherapy. Clin Breast Cancer 2016;16:113-122 e111. DOI: 10.1016/j.clbc.2015.11.006.
173. Marino F, Orecchia V, Regis G, et al. STAT3β controls inflammatory responses and early tumor onset in skin and colon experimental cancer models. Am J Cancer Res 2014;4:484-494.
174. Wang X, Guo J, Che X, et al. PCBP1 inhibits the expression of oncogenic STAT3 isoform by targeting alternative splicing of STAT3 exon 23. Int J Biol Sci 2019;15:1177-1186. DOI: 10.7150/ijbs.33103.
175. Zhang HF, Chen Y, Wu C, et al. The Opposing Function of STAT3 as an Oncoprotein and Tumor Suppressor Is Dictated by the Expression Status of STAT3beta in Esophageal Squamous Cell Carcinoma. Clin Cancer Res 2016;22:691-703. DOI: 10.1158/1078-0432.CCR-15-1253.
176. Huynh J, Chand A, Gough D, et al. Therapeutically exploiting STAT3 activity in cancer - using tissue repair as a road map. Nat Rev Cancer 2019;19:82-96. DOI: 10.1038/s41568-018-0090-8.
177. Narayanan PD, Nandabalan SK, Baddireddi LS. Role of STAT3 Phosphorylation in Ethanol-Mediated Proliferation of Breast Cancer Cells. J Breast Cancer 2016;19:122-132. DOI: 10.4048/jbc.2016.19.2.122.
178. Kim JS, Kim HA, Seong MK, et al. STAT3-survivin signaling mediates a poor response to radiotherapy in HER2-positive breast cancers. Oncotarget 2016;7:7055-7065. DOI: 10.18632/oncotarget.6855.
179. Sonnenblick A, Uziely B, Nechushtan H, et al. Tumor STAT3 tyrosine phosphorylation status, as a predictor of benefit from adjuvant chemotherapy for breast cancer. Breast Cancer Res Treat 2013;138:407-413. DOI: 10.1007/s10549-013-2453-x.
180. Sonnenblick A, Brohee S, Fumagalli D, et al. Constitutive phosphorylated STAT3-associated gene signature is predictive for trastuzumab resistance in

primary HER2-positive breast cancer. BMC Med 2015;13:177. DOI: 10.1186/s12916-015-0416-2.
181. Sonnenblick A, Agbor-Tarh D, de Azambuja E, et al. STAT3 activation in HER2-positive breast cancers: Analysis of data from a large prospective trial. Int J Cancer 2021;148:1529-1535. DOI: 10.1002/ijc.33385.
182. Larsson SC, Åkesson A, Bergkvist L, et al. Multivitamin use and breast cancer incidence in a prospective cohort of Swedish women. Am J Clin Nutr 2010;91:1268-1272. DOI: 10.3945/ajcn.2009.28837.
183. Wode K, Henriksson R, Sharp L, et al. Cancer patients' use of complementary and alternative medicine in Sweden: a cross-sectional study. BMC Complement Altern Med 2019;19:62. DOI: 10.1186/s12906-019-2452-5.
184. O'Donovan E, Hennessy MA, O'Reilly S. Dietary supplement beliefs and use patterns among Irish patients with early-stage breast cancer. Ir J Med Sci 2024;193:1749-1753. DOI: 10.1007/s11845-024-03676-8.
185. Zaorsky NG, Churilla TM, Ruth K, et al. Men's health supplement use and outcomes in men receiving definitive intensity-modulated radiation therapy for localized prostate cancer. Am J Clin Nutr 2016;104:1583-1593. DOI: 10.3945/ajcn.115.119958.
186. Ambrosone CB, Zirpoli GR, Hutson AD, et al. Dietary Supplement Use During Chemotherapy and Survival Outcomes of Patients With Breast Cancer Enrolled in a Cooperative Group Clinical Trial (SWOG S0221). J Clin Oncol 2020;38:804-814. DOI: 10.1200/jco.19.01203.
187. Averbeck D, Candéias S, Chandna S, et al. Establishing mechanisms affecting the individual response to ionizing radiation. Int J Radiat Biol 2020;96:297-323. DOI: 10.1080/09553002.2019.1704908.
188. Greenlee H, Kwan ML, Kushi LH, et al. Antioxidant supplement use after breast cancer diagnosis and mortality in the Life After Cancer Epidemiology (LACE) cohort. Cancer 2012;118:2048-2058. DOI: 10.1002/cncr.26526.
189. Nechuta S, Lu W, Chen Z, et al. Vitamin supplement use during breast cancer treatment and survival: a prospective cohort study. Cancer Epidemiol Biomarkers Prev 2011;20:262-271. DOI: 10.1158/1055-9965.Epi-10-1072.
190. Jung AY, Cai X, Thoene K, et al. Antioxidant supplementation and breast cancer prognosis in postmenopausal women undergoing chemotherapy and radiation therapy. Am J Clin Nutr 2019;109:69-78. DOI: 10.1093/ajcn/nqy223.
191. Hammersen F, Pursche T, Fischer D, et al. Use of Complementary and Alternative Medicine among Young Patients with Breast Cancer. Breast Care (Basel) 2020;15:163-170. DOI: 10.1159/000501193.
192. Greenlee H, Kwan ML, Ergas IJ, et al. Changes in vitamin and mineral supplement use after breast cancer diagnosis in the Pathways Study: a prospective cohort study. BMC Cancer 2014;14:382. DOI: 10.1186/1471-2407-14-382.
193. Hietala M, Henningson M, Ingvar C, et al. Natural remedy use in a prospective cohort of breast cancer patients in southern Sweden. Acta Oncol 2011;50:134-143. DOI: 10.3109/0284186X.2010.484812.
194. Harrigan M, McGowan C, Hood A, et al. Dietary Supplement Use and Interactions with Tamoxifen and Aromatase Inhibitors in Breast Cancer Survivors

Enrolled in Lifestyle Interventions. Nutrients 2021;13. DOI: 10.3390/nu13113730.
195. Zirpoli GR, Brennan PM, Hong CC, et al. Supplement use during an intergroup clinical trial for breast cancer (S0221). Breast Cancer Res Treat 2013;137:903-913. DOI: 10.1007/s10549-012-2400-2.
196. Harris HR, Bergkvist L, Wolk A. Vitamin C intake and breast cancer mortality in a cohort of Swedish women. Br J Cancer 2013;109:257-264. DOI: 10.1038/bjc.2013.269.
197. Romagnolo DF, Papoutsis AJ, Laukaitis C, et al. Constitutive expression of AhR and BRCA-1 promoter CpG hypermethylation as biomarkers of ERα-negative breast tumorigenesis. BMC Cancer 2015;15:1026. DOI: 10.1186/s12885-015-2044-9.
198. Gearhart-Serna LM, Davis JB, Jolly MK, et al. A polycyclic aromatic hydrocarbon-enriched environmental chemical mixture enhances AhR, antiapoptotic signaling and a proliferative phenotype in breast cancer cells. Carcinogenesis 2020;41:1648-1659. DOI: 10.1093/carcin/bgaa047.
199. Murphy KA, Quadro L, White LA. The intersection between the aryl hydrocarbon receptor (AhR)- and retinoic acid-signaling pathways. Vitam Horm 2007;75:33-67. DOI: 10.1016/s0083-6729(06)75002-6.
200. Al-Dhfyan A, Alhoshani A, Korashy HM. Aryl hydrocarbon receptor/cytochrome P450 1A1 pathway mediates breast cancer stem cells expansion through PTEN inhibition and β-Catenin and Akt activation. Mol Cancer 2017;16:14. DOI: 10.1186/s12943-016-0570-y.
201. Baba T, Mimura J, Nakamura N, et al. Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. Mol Cell Biol 2005;25:10040-10051. DOI: 10.1128/mcb.25.22.10040-10051.2005.
202. Saito R, Miki Y, Hata S, et al. Aryl hydrocarbon receptor induced intratumoral aromatase in breast cancer. Breast Cancer Res Treat 2017;161:399-407. DOI: 10.1007/s10549-016-4063-x.
203. Luecke-Johansson S, Gralla M, Rundqvist H, et al. A Molecular Mechanism To Switch the Aryl Hydrocarbon Receptor from a Transcription Factor to an E3 Ubiquitin Ligase. Mol Cell Biol 2017;37. DOI: 10.1128/mcb.00630-16.
204. Vacher S, Castagnet P, Chemlali W, et al. High AHR expression in breast tumors correlates with expression of genes from several signaling pathways namely inflammation and endogenous tryptophan metabolism. PLoS One 2018;13:e0190619. DOI: 10.1371/journal.pone.0190619.
205. Mandl M, Lieberum M, Dunst J, et al. The expression level of the transcription factor Aryl hydrocarbon receptor nuclear translocator (ARNT) determines cellular survival after radiation treatment. Radiat Oncol 2015;10:229. DOI: 10.1186/s13014-015-0539-9.
206. Tryggvadottir H, Sandén E, Björner S, et al. The Prognostic Impact of Intratumoral Aryl Hydrocarbon Receptor in Primary Breast Cancer Depends on the Type of Endocrine Therapy: A Population-Based Cohort Study. Front Oncol 2021;11:642768. DOI: 10.3389/fonc.2021.642768.

207. Jeschke U, Zhang X, Kuhn C, et al. The Prognostic Impact of the Aryl Hydrocarbon Receptor (AhR) in Primary Breast Cancer Depends on the Lymph Node Status. Int J Mol Sci 2019;20. DOI: 10.3390/ijms20051016.
208. Kim DK, Lee CY, Han YJ, et al. Exploring aryl hydrocarbon receptor expression and distribution in the tumor microenvironment, with a focus on immune cells, in various solid cancer types. Front Immunol 2024;15:1330228. DOI: 10.3389/fimmu.2024.1330228.
209. Johnson DE, Burtness B, Leemans CR, et al. Head and neck squamous cell carcinoma. Nat Rev Dis Primers 2020;6:92. DOI: 10.1038/s41572-020-00224-3.
210. SweHNCR. The Swedish Head and Neck Cancer Register, https://cancercentrum.se/globalassets/cancerdiagnoser/huvud-och-hals/kvalitetsregister/presentation-in-english-swehncr.pdf 2018, accessed 10-02-2024.
211. Registry SHaNC. Nationellt kvalitetsregister för huvud-och halscancer (SweHNCR), https://statistik.incanet.se/Huvud-hals/ (2025, accessed 0509 2025).
212. Mehanna H, Taberna M, von Buchwald C, et al. Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational, individual patient data analysis. Lancet Oncol 2023;24:239-251. DOI: 10.1016/s1470-2045(23)00013-x.
213. Ferris RL, Westra W. Oropharyngeal Carcinoma with a Special Focus on HPV-Related Squamous Cell Carcinoma. Annu Rev Pathol 2023;18:515-535. DOI: 10.1146/annurev-pathmechdis-031521-041424.
214. IARC. NORDCAN Association of the Nordic Cancer Registries, https://nordcan.iarc.fr/en 2024,accessed 10-09-2024.
215. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008;100:407-420. DOI: 10.1093/jnci/djn025.
216. Di Credico G, Polesel J, Dal Maso L, et al. Alcohol drinking and head and neck cancer risk: the joint effect of intensity and duration. Br J Cancer 2020;123:1456-1463. DOI: 10.1038/s41416-020-01031-z.
217. Brennan JA, Boyle JO, Koch WM, et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. N Engl J Med 1995;332:712-717. DOI: 10.1056/nejm199503163321104.
218. Fredriksson M, Werner M. AUDIT C compared to PEth in middle-aged volunteers. Alcohol Alcohol 2024;59. DOI: 10.1093/alcalc/agae048.
219. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. PLoS Med 2005;2:e160. DOI: 10.1371/journal.pmed.0020160.
220. Gray P, Wang J, Nordqvist Kleppe S, et al. Population-Based Age-Period-Cohort Analysis of Declining Human Papillomavirus Prevalence. J Infect Dis 2025;231:e638-e649. DOI: 10.1093/infdis/jiaf032.
221. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2007;90.
222. Folkhälsomyndigheten. Barnvaccinationsprogrammet i Sverige 2023, https://www.folkhalsomyndigheten.se/contentassets/39e211b5dbc449de8574d81f

1a416a26/barnvaccinationsprogrammet-sverige-2023.pdf 2023, accessed 02-14-2025.
223. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Head Neck 2013;35:747-755. DOI: 10.1002/hed.22015.
224. Wei F, Georges D, Man I, et al. Causal attribution of human papillomavirus genotypes to invasive cervical cancer worldwide: a systematic analysis of the global literature. Lancet 2024;404:435-444. DOI: 10.1016/s0140-6736(24)01097-3.
225. Hu K, Barker MM, Herweijer E, et al. The role of mental illness and neurodevelopmental conditions in human papillomavirus vaccination uptake within the Swedish school-based vaccination programme: a population-based cohort study. Lancet Public Health 2024;9:e674-e683. DOI: 10.1016/s2468-2667(24)00182-8.
226. Cancercentrum R. Huvud- och halscancer Nationellt vårdprogram, https://kunskapsbanken.cancercentrum.se/diagnoser/huvud-och-halscancer/vardprogram/ 2024, accessed 02-02-2025.
227. Deneuve S, Guerlain J, Dupret-Bories A, et al. Oral tongue squamous cell carcinomas in young patients according to their smoking status: a GETTEC study. Eur Arch Otorhinolaryngol 2022;279:415-424. DOI: 10.1007/s00405-021-06793-7.
228. Farquhar DR, Tanner AM, Masood MM, et al. Oral tongue carcinoma among young patients: An analysis of risk factors and survival. Oral Oncol 2018;84:7-11. DOI: 10.1016/j.oraloncology.2018.06.014.
229. Toporcov TN, Znaor A, Zhang ZF, et al. Risk factors for head and neck cancer in young adults: a pooled analysis in the INHANCE consortium. Int J Epidemiol 2015;44:169-185. DOI: 10.1093/ije/dyu255.
230. Mehanna H, Robinson M, Hartley A, et al. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTE HPV): an open-label randomised controlled phase 3 trial. Lancet 2019;393:51-60. DOI: 10.1016/s0140-6736(18)32752-1.
231. Brooks JM, Zheng Y, Hunter K, et al. Digital Spatial Profiling identifies distinct patterns of immuno-oncology-related gene expression within oropharyngeal tumours in relation to HPV and p16 status. Front Oncol 2024;14:1428741. DOI: 10.3389/fonc.2024.1428741.
232. Castellsagué X, Alemany L, Quer M, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. J Natl Cancer Inst 2016;108:djv403. DOI: 10.1093/jnci/djv403.
233. van der Heijden M, Essers PBM, de Jong MC, et al. Biological Determinants of Chemo-Radiotherapy Response in HPV-Negative Head and Neck Cancer: A Multicentric External Validation. Front Oncol 2019;9:1470. DOI: 10.3389/fonc.2019.01470.
234. Allgar VL, Oliver SE, Chen H, et al. Time intervals from first symptom to diagnosis for head and neck cancers: An analysis of linked patient reports and

medical records from the UK. Cancer Epidemiol 2019;59:37-45. DOI: 10.1016/j.canep.2019.01.008.
235. Gebre-Medhin M, Brun E, Engström P, et al. ARTSCAN III: A Randomized Phase III Study Comparing Chemoradiotherapy With Cisplatin Versus Cetuximab in Patients With Locoregionally Advanced Head and Neck Squamous Cell Cancer. J Clin Oncol 2021;39:38-47. DOI: 10.1200/jco.20.02072.
236. Thomson DJ, Slevin NJ, Baines H, et al. Randomized Phase 3 Trial of the Hypoxia Modifier Nimorazole Added to Radiation Therapy With Benefit Assessed in Hypoxic Head and Neck Cancers Determined Using a Gene Signature (NIMRAD). Int J Radiat Oncol Biol Phys 2024;119:771-782. DOI: 10.1016/j.ijrobp.2023.11.055.
237. Schimansky S, Lang S, Beynon R, et al. Association between comorbidity and survival in head and neck cancer: Results from Head and Neck 5000. Head Neck 2019;41:1053-1062. DOI: 10.1002/hed.25543.
238. van Rijn-Dekker MI, van den Bosch L, van den Hoek JGM, et al. Impact of sarcopenia on survival and late toxicity in head and neck cancer patients treated with radiotherapy. Radiother Oncol 2020;147:103-110. DOI: 10.1016/j.radonc.2020.03.014.
239. Marschner SN, Maihöfer C, Späth R, et al. Adjuvant (chemo)radiotherapy for patients with head and neck cancer: can comorbidity risk scores predict outcome? Strahlenther Onkol 2024;200:1025-1037. DOI: 10.1007/s00066-024-02282-y.
240. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24-35. DOI: 10.1056/NEJMoa0912217.
241. Eriksen JG, Steiniche T, Overgaard J. The role of epidermal growth factor receptor and E-cadherin for the outcome of reduction in the overall treatment time of radiotherapy of supraglottic larynx squamous cell carcinoma. Acta Oncol 2005;44:50-58. DOI: 10.1080/02841860510007396.
242. Bese NS, Hendry J, Jeremic B. Effects of prolongation of overall treatment time due to unplanned interruptions during radiotherapy of different tumor sites and practical methods for compensation. Int J Radiat Oncol Biol Phys 2007;68:654-661. DOI: 10.1016/j.ijrobp.2007.03.010.
243. Alterio D, Marvaso G, Maffini F, et al. Role of EGFR as prognostic factor in head and neck cancer patients treated with surgery and postoperative radiotherapy: proposal of a new approach behind the EGFR overexpression. Med Oncol 2017;34:107. DOI: 10.1007/s12032-017-0965-7.
244. Zschaeck S, Löck S, Hofheinz F, et al. Individual patient data meta-analysis of FMISO and FAZA hypoxia PET scans from head and neck cancer patients undergoing definitive radio-chemotherapy. Radiother Oncol 2020;149:189-196. DOI: 10.1016/j.radonc.2020.05.022.
245. Overgaard J, Hansen HS, Overgaard M, et al. A randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85. Radiother Oncol 1998;46:135-146. DOI: 10.1016/s0167-8140(97)00220-x.

246. Baude J, Guigou C, Thibouw D, et al. Definitive radio(chemo)therapy versus upfront surgery in the treatment of HPV-related localized or locally advanced oropharyngeal squamous cell carcinoma. PLoS One 2024;19:e0307658. DOI: 10.1371/journal.pone.0307658.
247. Lacas B, Carmel A, Landais C, et al. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC Group. Radiother Oncol 2021;156:281-293. DOI: 10.1016/j.radonc.2021.01.013.
248. Petit C, Lacas B, Pignon JP, et al. Chemotherapy and radiotherapy in locally advanced head and neck cancer: an individual patient data network meta-analysis. Lancet Oncol 2021;22:727-736. DOI: 10.1016/s1470-2045(21)00076-0.
249. Saksø M, Jensen K, Andersen M, et al. DAHANCA 28: A phase I/II feasibility study of hyperfractionated, accelerated radiotherapy with concomitant cisplatin and nimorazole (HART-CN) for patients with locally advanced, HPV/p16-negative squamous cell carcinoma of the oropharynx, hypopharynx, larynx and oral cavity. Radiother Oncol 2020;148:65-72. DOI: 10.1016/j.radonc.2020.03.025.
250. Overgaard J, Hansen HS, Specht L, et al. Five compared with six fractions per week of conventional radiotherapy of squamous-cell carcinoma of head and neck: DAHANCA 6 and 7 randomised controlled trial. Lancet 2003;362:933-940. DOI: 10.1016/s0140-6736(03)14361-9.
251. Horiot JC, Le Fur R, N'Guyen T, et al. Hyperfractionation versus conventional fractionation in oropharyngeal carcinoma: final analysis of a randomized trial of the EORTC cooperative group of radiotherapy. Radiother Oncol 1992;25:231-241. DOI: 10.1016/0167-8140(92)90242-m.
252. Horiot JC, Bontemps P, van den Bogaert W, et al. Accelerated fractionation (AF) compared to conventional fractionation (CF) improves loco-regional control in the radiotherapy of advanced head and neck cancers: results of the EORTC 22851 randomized trial. Radiother Oncol 1997;44:111-121. DOI: 10.1016/s0167-8140(97)00079-0.
253. Dische S, Saunders M, Barrett A, et al. A randomised multicentre trial of CHART versus conventional radiotherapy in head and neck cancer. Radiother Oncol 1997;44:123-136. DOI: 10.1016/s0167-8140(97)00094-7.
254. Mortensen HR, Overgaard J, Specht L, et al. Prevalence and peak incidence of acute and late normal tissue morbidity in the DAHANCA 6&7 randomised trial with accelerated radiotherapy for head and neck cancer. Radiother Oncol 2012;103:69-75. DOI: 10.1016/j.radonc.2012.01.002.
255. Beitler JJ, Zhang Q, Fu KK, et al. Final results of local-regional control and late toxicity of RTOG 9003: a randomized trial of altered fractionation radiation for locally advanced head and neck cancer. Int J Radiat Oncol Biol Phys 2014;89:13-20. DOI: 10.1016/j.ijrobp.2013.12.027.
256. Kristensen MH, Holm AIS, Hansen CR, et al. High-dose loco-regional pattern of failure after primary radiotherapy in p16 positive and negative head and neck squamous cell carcinoma - A DAHANCA 19 study. Clin Transl Radiat Oncol 2024;46:100772. DOI: 10.1016/j.ctro.2024.100772.

257. Caudell JJ, Torres-Saavedra PA, Rosenthal DI, et al. Long-Term Update of NRG/RTOG 0522: A Randomized Phase 3 Trial of Concurrent Radiation and Cisplatin With or Without Cetuximab in Locoregionally Advanced Head and Neck Cancer. Int J Radiat Oncol Biol Phys 2023;116:533-543. DOI: 10.1016/j.ijrobp.2022.12.015.
258. Vergeer MR, Doornaert PA, Rietveld DH, et al. Intensity-modulated radiotherapy reduces radiation-induced morbidity and improves health-related quality of life: results of a nonrandomized prospective study using a standardized follow-up program. Int J Radiat Oncol Biol Phys 2009;74:1-8. DOI: 10.1016/j.ijrobp.2008.07.059.
259. Berg M, Hansson C, Silander E, et al. A randomized study comparing the nutritional effects of radiotherapy with cetuximab versus cisplatin in patients with advanced head and neck cancer. Head Neck 2024. DOI: 10.1002/hed.27619.
260. Trotti A, Pajak TF, Gwede CK, et al. TAME: development of a new method for summarising adverse events of cancer treatment by the Radiation Therapy Oncology Group. Lancet Oncol 2007;8:613-624. DOI: 10.1016/s1470-2045(07)70144-4.
261. Muscaritoli M, Arends J, Bachmann P, et al. ESPEN practical guideline: Clinical Nutrition in cancer. Clin Nutr 2021;40:2898-2913. DOI: 10.1016/j.clnu.2021.02.005.
262. Chan RJ, Blades R, Jones L, et al. A single-blind, randomised controlled trial of StrataXRT® - A silicone-based film-forming gel dressing for prophylaxis and management of radiation dermatitis in patients with head and neck cancer. Radiother Oncol 2019;139:72-78. DOI: 10.1016/j.radonc.2019.07.014.
263. Abel E, Silander E, Nordström F, et al. Fatigue in Patients With Head and Neck Cancer Treated With Radiation Therapy: A Prospective Study of Patient-Reported Outcomes and Their Association With Radiation Dose to the Cerebellum. Adv Radiat Oncol 2022;7:100960. DOI: 10.1016/j.adro.2022.100960.
264. Hu H, Li B, Wang J, et al. New advances into cisplatin resistance in head and neck squamous carcinoma: Mechanisms and therapeutic aspects. Biomed Pharmacother 2023;163:114778. DOI: 10.1016/j.biopha.2023.114778.
265. Szturz P, Wouters K, Kiyota N, et al. Weekly Low-Dose Versus Three-Weekly High-Dose Cisplatin for Concurrent Chemoradiation in Locoregionally Advanced Non-Nasopharyngeal Head and Neck Cancer: A Systematic Review and Meta-Analysis of Aggregate Data. Oncologist 2017;22:1056-1066. DOI: 10.1634/theoncologist.2017-0015.
266. Borel C, Sun XS, Coutte A, et al. Standard versus fractionated high-dose cisplatin plus radiation for locally advanced head and neck cancer: Results of the CisFRad (GORTEC 2015-02) randomized phase II trial. Radiother Oncol 2024;197:110329. DOI: 10.1016/j.radonc.2024.110329.
267. Chargi N, Molenaar-Kuijsten L, Huiskamp LFJ, et al. The association of cisplatin pharmacokinetics and skeletal muscle mass in patients with head and neck cancer: The prospective PLATISMA study. Eur J Cancer 2022;160:92-99. DOI: 10.1016/j.ejca.2021.10.010.

268. Jacobs MT, Wong P, Zhou AY, et al. Memory-like Differentiation, Tumor-Targeting mAbs, and Chimeric Antigen Receptors Enhance Natural Killer Cell Responses to Head and Neck Cancer. Clin Cancer Res 2023;29:4196-4208. DOI: 10.1158/1078-0432.Ccr-23-0156.
269. Eriksen JG, Steiniche T, Askaa J, et al. The prognostic value of epidermal growth factor receptor is related to tumor differentiation and the overall treatment time of radiotherapy in squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys 2004;58:561-566. DOI: 10.1016/j.ijrobp.2003.09.043.
270. Vermorken JB, Trigo J, Hitt R, et al. Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy. J Clin Oncol 2007;25:2171-2177. DOI: 10.1200/jco.2006.06.7447.
271. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 2006;354:567-578. DOI: 10.1056/NEJMoa053422.
272. Bonner JA, Harari PM, Giralt J, et al. Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 2010;11:21-28. DOI: 10.1016/s1470-2045(09)70311-0.
273. Gillison ML, Trotti AM, Harris J, et al. Radiotherapy plus cetuximab or cisplatin in human papillomavirus-positive oropharyngeal cancer (NRG Oncology RTOG 1016): a randomised, multicentre, non-inferiority trial. Lancet 2019;393:40-50. DOI: 10.1016/s0140-6736(18)32779-x.
274. Mell LK, Torres-Saavedra PA, Wong SJ, et al. Radiotherapy with cetuximab or durvalumab for locoregionally advanced head and neck cancer in patients with a contraindication to cisplatin (NRG-HN004): an open-label, multicentre, parallel-group, randomised, phase 2/3 trial. Lancet Oncol 2024;25:1576-1588. DOI: 10.1016/s1470-2045(24)00507-2.
275. Faden DL, Concha-Benavente F, Chakka AB, et al. Immunogenomic correlates of response to cetuximab monotherapy in head and neck squamous cell carcinoma. Head Neck 2019;41:2591-2601. DOI: 10.1002/hed.25726.
276. Miyauchi S, Kim SS, Pang J, et al. Immune Modulation of Head and Neck Squamous Cell Carcinoma and the Tumor Microenvironment by Conventional Therapeutics. Clin Cancer Res 2019;25:4211-4223. DOI: 10.1158/1078-0432.Ccr-18-0871.
277. Ferris RL, Lenz HJ, Trotta AM, et al. Rationale for combination of therapeutic antibodies targeting tumor cells and immune checkpoint receptors: Harnessing innate and adaptive immunity through IgG1 isotype immune effector stimulation. Cancer Treat Rev 2018;63:48-60. DOI: 10.1016/j.ctrv.2017.11.008.
278. Sacco AG, Chen R, Worden FP, et al. Pembrolizumab plus cetuximab in patients with recurrent or metastatic head and neck squamous cell carcinoma: an open-label, multi-arm, non-randomised, multicentre, phase 2 trial. Lancet Oncol 2021;22:883-892. DOI: 10.1016/s1470-2045(21)00136-4.

279. Burcher KM, Bloomer CH, Gavrila E, et al. Study protocol: phase II study to evaluate the effect of cetuximab monotherapy after immunotherapy with PD-1 inhibitors in patients with head and neck squamous cell cancer. Ther Adv Med Oncol 2024;16:17588359231217959. DOI: 10.1177/17588359231217959.
280. Trials.Gov C. PD-L1 T-haNK, N-803 IL-15sa and Cetuximab for Recurrent, Metastatic HNSCC NTC06239220. 2024. Available at: https://clinicaltrials.gov/study/NCT06239220?term=NCT06239220&rank=1 2024, accessed 04-05-2025.
281. Ferris RL, Blumenschein G, Jr., Fayette J, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med 2016;375:1856-1867. DOI: 10.1056/NEJMoa1602252.
282. Burtness B, Harrington KJ, Greil R, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet 2019;394:1915-1928. DOI: 10.1016/s0140-6736(19)32591-7.
283. Machiels JP, Tao Y, Licitra L, et al. Pembrolizumab plus concurrent chemoradiotherapy versus placebo plus concurrent chemoradiotherapy in patients with locally advanced squamous cell carcinoma of the head and neck (KEYNOTE-412): a randomised, double-blind, phase 3 trial. Lancet Oncol 2024;25:572-587. DOI: 10.1016/s1470-2045(24)00100-1.
284. Goodpaster BH, Park SW, Harris TB, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci 2006;61:1059-1064. DOI: 10.1093/gerona/61.10.1059.
285. Xie RZ, Li XS, Zha FD, et al. Relationship Between Body Mass Index and Low Skeletal Muscle Mass in Adults Based on NHANES 2011-2018. Sci Rep 2025;15:2596. DOI: 10.1038/s41598-025-87176-4.
286. van der Werf A, Langius JAE, de van der Schueren MAE, et al. Percentiles for skeletal muscle index, area and radiation attenuation based on computed tomography imaging in a healthy Caucasian population. Eur J Clin Nutr 2018;72:288-296. DOI: 10.1038/s41430-017-0034-5.
287. Cawthon PM, Manini T, Patel SM, et al. Putative Cut-Points in Sarcopenia Components and Incident Adverse Health Outcomes: An SDOC Analysis. J Am Geriatr Soc 2020;68:1429-1437. DOI: 10.1111/jgs.16517.
288. Wiedmer P, Jung T, Castro JP, et al. Sarcopenia - Molecular mechanisms and open questions. Ageing Res Rev 2021;65:101200. DOI: 10.1016/j.arr.2020.101200.
289. Kim KM, Lim S, Oh TJ, et al. Longitudinal Changes in Muscle Mass and Strength, and Bone Mass in Older Adults: Gender-Specific Associations Between Muscle and Bone Losses. J Gerontol A Biol Sci Med Sci 2018;73:1062-1069. DOI: 10.1093/gerona/glx188.
290. Al-Attar A, Presnell SR, Clasey JL, et al. Human Body Composition and Immunity: Visceral Adipose Tissue Produces IL-15 and Muscle Strength

Inversely Correlates with NK Cell Function in Elderly Humans. Front Immunol 2018;9:440. DOI: 10.3389/fimmu.2018.00440.
291. Yuan S, Larsson SC. Epidemiology of sarcopenia: Prevalence, risk factors, and consequences. Metabolism 2023;144:155533. DOI: 10.1016/j.metabol.2023.155533.
292. Surov A, Wienke A. Low skeletal muscle mass predicts relevant clinical outcomes in head and neck squamous cell carcinoma. A meta analysis. Ther Adv Med Oncol 2021;13:17588359211008844. DOI: 10.1177/17588359211008844.
293. Vickers AJ, McSweeney DM, Choudhury A, et al. The prognostic significance of sarcopenia in patients treated with definitive radiotherapy: A systematic review. Radiother Oncol 2024;203:110663. DOI: 10.1016/j.radonc.2024.110663.
294. Cheng Y, Li T, Huang G, et al. Low appendicular skeletal muscle mass is associated with the risk of mortality among adults in the United States. Sci Rep 2025;15:9908. DOI: 10.1038/s41598-025-94357-8.
295. Martin L, Birdsell L, Macdonald N, et al. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol 2013;31:1539-1547. DOI: 10.1200/jco.2012.45.2722.
296. Talani C, Astradsson T, Farnebo L, et al. Pretreatment fat-free mass index correlates with early death in patients with head and neck squamous cell carcinoma. Head Neck 2024. DOI: 10.1002/hed.27628.
297. Ganju RG, Morse R, Hoover A, et al. The impact of sarcopenia on tolerance of radiation and outcome in patients with head and neck cancer receiving chemoradiation. Radiother Oncol 2019;137:117-124. DOI: 10.1016/j.radonc.2019.04.023.
298. Wendrich AW, Swartz JE, Bril SI, et al. Low skeletal muscle mass is a predictive factor for chemotherapy dose-limiting toxicity in patients with locally advanced head and neck cancer. Oral Oncol 2017;71:26-33. DOI: 10.1016/j.oraloncology.2017.05.012.
299. Ringberg A, Bågeman E, Rose C, et al. Of cup and bra size: reply to a prospective study of breast size and premenopausal breast cancer incidence. Int J Cancer 2006;119:2242-2243; author reply 2244. DOI: 10.1002/ijc.22104.
300. Sandén E, Khazaei S, Tryggvadottir H, et al. Re-evaluation of HER2 status in 606 breast cancers-gene protein assay on tissue microarrays versus routine pathological assessment. Virchows Arch 2020. DOI: 10.1007/s00428-020-02768-x.
301. Voduc D, Kenney C, Nielsen TO. Tissue microarrays in clinical oncology. Semin Radiat Oncol 2008;18:89-97. DOI: 10.1016/j.semradonc.2007.10.006.
302. Krzyzanowska A, Don-Doncow N, Marginean FE, et al. Expression of tSTAT3, pSTAT3(727) , and pSTAT3 (705) in the epithelial cells of hormone-naive prostate cancer. Prostate 2019. DOI: 10.1002/pros.23787.
303. Engelberg JA, Retallack H, Balassanian R, et al. "Score the Core" Web-based pathologist training tool improves the accuracy of breast cancer IHC4 scoring. Hum Pathol 2015;46:1694-1704. DOI: 10.1016/j.humpath.2015.07.008.

304. Wisse A, Tryggvadottir H, Simonsson M, et al. Increasing preoperative body size in breast cancer patients between 2002 and 2016: implications for prognosis. Cancer Causes Control 2018;29:643-656. DOI: 10.1007/s10552-018-1042-z.
305. Sonnenblick A, Salgado R, Brohée S, et al. p-STAT3 in luminal breast cancer: Integrated RNA-protein pooled analysis and results from the BIG 2-98 phase III trial. Int J Oncol 2018;52:424-432. DOI: 10.3892/ijo.2017.4212.
306. Dolled-Filhart M, Camp RL, Kowalski DP, et al. Tissue microarray analysis of signal transducers and activators of transcription 3 (Stat3) and phospho-Stat3 (Tyr705) in node-negative breast cancer shows nuclear localization is associated with a better prognosis. Clin Cancer Res 2003;9:594-600.
307. Stover DG, Gil Del Alcazar CR, Brock J, et al. Phase II study of ruxolitinib, a selective JAK1/2 inhibitor, in patients with metastatic triple-negative breast cancer. NPJ Breast Cancer 2018;4:10. DOI: 10.1038/s41523-018-0060-z.
308. Malik DE, David RM, Gooderham NJ. Ethanol potentiates the genotoxicity of the food-derived mammary carcinogen PhIP in human estrogen receptor-positive mammary cells: mechanistic support for lifestyle factors (cooked red meat and ethanol) associated with mammary cancer. Arch Toxicol 2018;92:1639-1655. DOI: 10.1007/s00204-018-2160-9.
309. ATCC. American Type Culture Collection, https://www.atcc.org/products/htb-26, accessed 10-14-2025.
310. Jungert A, Neuhäuser-Berthold M. The lower vitamin C plasma concentrations in elderly men compared with elderly women can partly be attributed to a volumetric dilution effect due to differences in fat-free mass. Br J Nutr 2015;113:859-864. DOI: 10.1017/s0007114515000240.
311. Greenlee H, White E, Patterson RE, et al. Supplement use among cancer survivors in the Vitamins and Lifestyle (VITAL) study cohort. J Altern Complement Med 2004;10:660-666. DOI: 10.1089/acm.2004.10.660.
312. Gebre-Medhin M, Adrian G, Engström P, et al. Chemoradiotherapy with cisplatin versus cetuximab in locoregionally advanced head and neck squamous cell cancer patients - mature results of the ARTSCAN III trial. Int J Radiat Oncol Biol Phys 2025. DOI: 10.1016/j.ijrobp.2025.05.086. *in press*
313. Sobin LH, Gospodarowic M.K, Wittekin C. . UICC TNM Classification of malignant tumors Seventh Edition. Chichester: Whiley-Blackwell, 2009.
314. Shen W, Punyanitya M, Wang Z, et al. Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. J Appl Physiol (1985) 2004;97:2333-2338. DOI: 10.1152/japplphysiol.00744.2004.
315. Mourtzakis M, Prado CM, Lieffers JR, et al. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab 2008;33:997-1006. DOI: 10.1139/h08-075.
316. Caan BJ, Cespedes Feliciano EM, Prado CM, et al. Association of Muscle and Adiposity Measured by Computed Tomography With Survival in Patients With Nonmetastatic Breast Cancer. JAMA Oncol 2018;4:798-804. DOI: 10.1001/jamaoncol.2018.0137.

317. Grossberg AJ, Chamchod S, Fuller CD, et al. Association of Body Composition With Survival and Locoregional Control of Radiotherapy-Treated Head and Neck Squamous Cell Carcinoma. JAMA Oncol 2016;2:782-789. DOI: 10.1001/jamaoncol.2015.6339.
318. Ufuk F, Herek D, Yüksel D. Diagnosis of Sarcopenia in Head and Neck Computed Tomography: Cervical Muscle Mass as a Strong Indicator of Sarcopenia. Clin Exp Otorhinolaryngol 2019;12:317-324. DOI: 10.21053/ceo.2018.01613.
319. Swartz JE, Pothen AJ, Wegner I, et al. Feasibility of using head and neck CT imaging to assess skeletal muscle mass in head and neck cancer patients. Oral Oncol 2016;62:28-33. DOI: 10.1016/j.oraloncology.2016.09.006.
320. Derstine BA, Holcombe SA, Ross BE, et al. Skeletal muscle cutoff values for sarcopenia diagnosis using T10 to L5 measurements in a healthy US population. Sci Rep 2018;8:11369. DOI: 10.1038/s41598-018-29825-5.
321. Olson B, Edwards J, Degnin C, et al. Establishment and Validation of Pre-Therapy Cervical Vertebrae Muscle Quantification as a Prognostic Marker of Sarcopenia in Patients With Head and Neck Cancer. Front Oncol 2022;12:812159. DOI: 10.3389/fonc.2022.812159.
322. Bril SI, Chargi N, Wendrich AW, et al. Validation of skeletal muscle mass assessment at the level of the third cervical vertebra in patients with head and neck cancer. Oral Oncol 2021;123:105617. DOI: 10.1016/j.oraloncology.2021.105617.
323. Vangelov B, Bauer J, Moses D, et al. The effectiveness of skeletal muscle evaluation at the third cervical vertebral level for computed tomography-defined sarcopenia assessment in patients with head and neck cancer. Head Neck 2022;44:1047-1056. DOI: 10.1002/hed.27000.
324. Derstine BA, Holcombe SA, Ross BE, et al. Optimal body size adjustment of L3 CT skeletal muscle area for sarcopenia assessment. Sci Rep 2021;11:279. DOI: 10.1038/s41598-020-79471-z.
325. Huiskamp LFJ, Chargi N, Devriese LA, et al. The predictive and prognostic value of low skeletal muscle mass for dose-limiting toxicity and survival in head and neck cancer patients receiving concomitant cetuximab and radiotherapy. Eur Arch Otorhinolaryngol 2020;277:2847-2858. DOI: 10.1007/s00405-020-05972-2.
326. Prado CM, Lieffers JR, McCargar LJ, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol 2008;9:629-635. DOI: 10.1016/s1470-2045(08)70153-0.
327. Fattouh M, Chang GY, Ow TJ, et al. Association between pretreatment obesity, sarcopenia, and survival in patients with head and neck cancer. Head Neck 2019;41:707-714. DOI: 10.1002/hed.25420.
328. Zwart AT, Pörtzgen W, van Rijn-Dekker I, et al. Sex-Specific Cut-Off Values for Low Skeletal Muscle Mass to Identify Patients at Risk for Treatment-Related Adverse Events in Head and Neck Cancer. J Clin Med 2022;11. DOI: 10.3390/jcm11164650.

329. Simonsson M, Markkula A, Bendahl PO, et al. Pre- and postoperative alcohol consumption in breast cancer patients: impact on early events. Springerplus 2014;3:261. DOI: 10.1186/2193-1801-3-261.
330. Bergman A. WP, Källmén H. AUDIT & DUDIT Identifiera problem med alkohol och droger, https://www.socialstyrelsen.se/globalassets/sharepoint-dokument/dokument-webb/ovrigt/audit-dudit-identifiera-problem-med-alkohol-och-droger.pdf 2014, accessed 10-10-2024.
331. Yang YC, Walsh CE, Johnson MP, et al. Life-course trajectories of body mass index from adolescence to old age: Racial and educational disparities. Proc Natl Acad Sci U S A 2021;118. DOI: 10.1073/pnas.2020167118.
332. Harborg S, Feldt M, Cronin-Fenton D, et al. Obesity and breast cancer prognosis: pre-diagnostic anthropometric measures in relation to patient, tumor, and treatment characteristics. Cancer Metab 2023;11:8. DOI: 10.1186/s40170-023-00308-0.
333. WHO Mean Body Mass Index Global Health Observator (GHO) data, https://www.who.int/gho/ncd/risk_factors/bmi_text/en/ 2019, accessed 10-10-2019.
334. WH0 Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. Geneva 2008, accessed 10-10-2019.
335. Rush EC, Goedecke JH, Jennings C, et al. BMI, fat and muscle differences in urban women of five ethnicities from two countries. Int J Obes (Lond) 2007;31:1232-1239. DOI: 10.1038/sj.ijo.0803576.
336. Lammers SWM, Geurts SME, van Hellemond IEG, et al. The prognostic and predictive effect of body mass index in hormone receptor-positive breast cancer. JNCI Cancer Spectr 2023;7. DOI: 10.1093/jncics/pkad092.
337. Kwan ML, Chen WY, Kroenke CH, et al. Pre-diagnosis body mass index and survival after breast cancer in the After Breast Cancer Pooling Project. Breast Cancer Res Treat 2012;132:729-739. DOI: 10.1007/s10549-011-1914-3.
338. Takenaka Y, Takemoto N, Nakahara S, et al. Prognostic significance of body mass index before treatment for head and neck cancer. Head Neck 2015;37:1518-1523. DOI: 10.1002/hed.23785.
339. Godina C, Ottander E, Tryggvadottir H, et al. Prognostic Impact of Menopausal Hormone Therapy in Breast Cancer Differs According to Tumor Characteristics and Treatment. Front Oncol 2020;10:80. DOI: 10.3389/fonc.2020.00080.
340. Persson M, Simonsson M, Markkula A, et al. Impacts of smoking on endocrine treatment response in a prospective breast cancer cohort. Br J Cancer 2016;115:382-390. DOI: 10.1038/bjc.2016.174.
341. Steffl M, Bohannon RW, Petr M, et al. Relation between cigarette smoking and sarcopenia: meta-analysis. Physiol Res 2015;64:419-426. DOI: 10.33549/physiolres.932802.
342. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415-421. DOI: 10.1038/nature12477.
343. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for

reporting observational studies. J Clin Epidemiol 2008;61:344-349. DOI: 10.1016/j.jclinepi.2007.11.008.
344. Altman DG, McShane LM, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies (REMARK): explanation and elaboration. BMC Med 2012;10:51. DOI: 10.1186/1741-7015-10-51.
345. Sørensen HT, Lash TL, Rothman KJ. Beyond randomized controlled trials: a critical comparison of trials with nonrandomized studies. Hepatology 2006;44:1075-1082. DOI: 10.1002/hep.21404.
346. Kennedy-Martin T, Curtis S, Faries D, et al. A literature review on the representativeness of randomized controlled trial samples and implications for the external validity of trial results. Trials 2015;16:495. DOI: 10.1186/s13063-015-1023-4.
347. Vandenbroucke JP, von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Ann Intern Med 2007;147:W163-194. DOI: 10.7326/0003-4819-147-8-200710160-00010-w1.
348. Altman N, Krzywinski M. Association, correlation and causation. Nat Methods 2015;12:899-900. DOI: 10.1038/nmeth.3587.
349. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. Int J Epidemiol 2012;41:514-520. DOI: 10.1093/ije/dyr218.
350. Shimonovich M, Pearce A, Thomson H, et al. Assessing causality in epidemiology: revisiting Bradford Hill to incorporate developments in causal thinking. Eur J Epidemiol 2021;36:873-887. DOI: 10.1007/s10654-020-00703-7.
351. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol 2008;8:70. DOI: 10.1186/1471-2288-8-70.
352. Gourgou-Bourgade S, Cameron D, Poortmans P, et al. Guidelines for time-to-event end point definitions in breast cancer trials: results of the DATECAN initiative (Definition for the Assessment of Time-to-event Endpoints in CANcer trials)†. Ann Oncol 2015;26:873-879. DOI: 10.1093/annonc/mdv106.
353. Lim AM, McDowell L, Hurt C, et al. Assessment of endpoint definitions in curative-intent trials for mucosal head and neck squamous cell carcinomas: Head and Neck Cancer International Group consensus recommendations. Lancet Oncol 2024;25:e318-e330. DOI: 10.1016/s1470-2045(24)00067-6.
354. Ording AG, Cronin-Fenton D, Ehrenstein V, et al. Challenges in translating endpoints from trials to observational cohort studies in oncology. Clin Epidemiol 2016;8:195-200. DOI: 10.2147/clep.S97874.
355. Gharzai LA, Morris E, Suresh K, et al. Surrogate endpoints in clinical trials of p16-positive squamous cell carcinoma of the oropharynx: an individual patient data meta-analysis. Lancet Oncol 2024;25:366-375. DOI: 10.1016/s1470-2045(24)00016-0.
356. Nout RA, Fiets WE, Struikmans H, et al. The in- or exclusion of non-breast cancer related death and contralateral breast cancer significantly affects estimated outcome probability in early breast cancer. Breast Cancer Res Treat 2008;109:567-572. DOI: 10.1007/s10549-007-9681-x.

357. Lacny S, Wilson T, Clement F, et al. Kaplan–Meier survival analysis overestimates cumulative incidence of health-related events in competing risk settings: a meta-analysis. Journal of Clinical Epidemiology 2018;93:25-35. DOI: 10.1016/j.jclinepi.2017.10.006.
358. Baer HJ, Glynn RJ, Hu FB, et al. Risk factors for mortality in the nurses' health study: a competing risks analysis. Am J Epidemiol 2011;173:319-329. DOI: 10.1093/aje/kwq368.
359. Krug D, Vladimirova V, Untch M, et al. Breast-conserving surgery is not associated with increased local recurrence in patients with early-stage node-negative triple-negative breast cancer treated with neoadjuvant chemotherapy. Breast 2024;74:103701. DOI: 10.1016/j.breast.2024.103701.
360. Stenckova M, Nenutil R, Vojtesek B, et al. Stat3 Tyrosine 705 and Serine 727 Phosphorylation Associate With Clinicopathological Characteristics and Distinct Tumor Cell Phenotypes in Triple-Negative Breast Cancer. Pathol Oncol Res 2022;28:1610592. DOI: 10.3389/pore.2022.1610592.
361. Ziadi W, Boussetta S, Elkamel S, et al. STAT3 polymorphisms in North Africa and its implication in breast cancer. Mol Genet Genomic Med 2021;9:e1744. DOI: 10.1002/mgg3.1744.
362. Vaclavicek A, Bermejo JL, Schmutzler RK, et al. Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer. Endocr Relat Cancer 2007;14:267-277. DOI: 10.1677/erc-06-0077.
363. Tell RW, Horvath CM. Bioinformatic analysis reveals a pattern of STAT3-associated gene expression specific to basal-like breast cancers in human tumors. Proc Natl Acad Sci U S A 2014;111:12787-12792. DOI: 10.1073/pnas.1404881111.
364. Liu Y, Huang J, Li W, et al. Meta-analysis of STAT3 and phospho-STAT3 expression and survival of patients with breast cancer. Oncotarget 2018;9:13060-13067. DOI: 10.18632/oncotarget.23962.
365. Yde CW, Emdal KB, Guerra B, et al. NFκB signaling is important for growth of antiestrogen resistant breast cancer cells. Breast Cancer Res Treat 2012;135:67-78. DOI: 10.1007/s10549-012-2053-1.
366. Moon SY, Lee H, Kim S, et al. Inhibition of STAT3 enhances sensitivity to tamoxifen in tamoxifen-resistant breast cancer cells. BMC Cancer 2021;21:931. DOI: 10.1186/s12885-021-08641-7.
367. Pedersen CG, Christensen S, Jensen AB, et al. Prevalence, socio-demographic and clinical predictors of post-diagnostic utilisation of different types of complementary and alternative medicine (CAM) in a nationwide cohort of Danish women treated for primary breast cancer. Eur J Cancer 2009;45:3172-3181. DOI: 10.1016/j.ejca.2009.09.005.
368. Roswall N, Olsen A, Christensen J, et al. Micronutrient intake and breast cancer characteristics among postmenopausal women. Eur J Cancer Prev 2010;19:360-365. DOI: 10.1097/cej.0b013e32833ade68.
369. Goode GD, Ballard BR, Manning HC, et al. Knockdown of aberrantly upregulated aryl hydrocarbon receptor reduces tumor growth and metastasis of

MDA-MB-231 human breast cancer cell line. Int J Cancer 2013;133:2769-2780. DOI: 10.1002/ijc.28297.
370. Li Y, Lin Q, Lu X, et al. Post-Diagnosis use of Antioxidant Vitamin Supplements and Breast Cancer Prognosis: A Systematic Review and Meta-Analysis. Clin Breast Cancer 2021;21:477-485. DOI: 10.1016/j.clbc.2021.09.001.
371. Harris HR, Orsini N, Wolk A. Vitamin C and survival among women with breast cancer: a meta-analysis. Eur J Cancer 2014;50:1223-1231. DOI: 10.1016/j.ejca.2014.02.013.
372. Chatterjee P, Banerjee S. Unveiling the mechanistic role of the Aryl hydrocarbon receptor in environmentally induced Breast cancer. Biochem Pharmacol 2023;218:115866. DOI: 10.1016/j.bcp.2023.115866.
373. Giordano D, Facchiano A, Moccia S, et al. Molecular Docking of Natural Compounds for Potential Inhibition of AhR. Foods 2023;12. DOI: 10.3390/foods12101953.
374. Paolini M, Abdel-Rahman SZ, Sapone A, et al. Beta-carotene: a cancer chemopreventive agent or a co-carcinogen? Mutat Res 2003;543:195-200. DOI: 10.1016/s1383-5742(03)00002-4.
375. Paolini M, Cantelli-Forti G, Perocco P, et al. Co-carcinogenic effect of beta-carotene. Nature 1999;398:760-761. DOI: 10.1038/19655.
376. Krüger K, Seimetz M, Ringseis R, et al. Exercise training reverses inflammation and muscle wasting after tobacco smoke exposure. Am J Physiol Regul Integr Comp Physiol 2018;314:R366-r376. DOI: 10.1152/ajpregu.00316.2017.
377. Gorphe P, Classe M, Ammari S, et al. Patterns of disease events and causes of death in patients with HPV-positive versus HPV-negative oropharyngeal carcinoma. Radiother Oncol 2022;168:40-45. DOI: 10.1016/j.radonc.2022.01.021.
378. Larsson L, Degens H, Li M, et al. Sarcopenia: Aging-Related Loss of Muscle Mass and Function. Physiol Rev 2019;99:427-511. DOI: 10.1152/physrev.00061.2017.
379. Marquardt JP, Roeland EJ, Van Seventer EE, et al. Percentile-based averaging and skeletal muscle gauge improve body composition analysis: validation at multiple vertebral levels. J Cachexia Sarcopenia Muscle 2022;13:190-202. DOI: 10.1002/jcsm.12848.
380. Roberti E, Merlano M, Ravanelli M, et al. Muscle quality and not quantity as a predictor of survival in head and neck squamous cell carcinoma. Oral Oncol 2023;145:106540. DOI: 10.1016/j.oraloncology.2023.106540.
381. Tralongo AC, Caspani F, Proserpio I, et al. Body mass index (BMI) influence on Cetuximab-induced antibody-dependent cellular cytotoxicity in advanced colon cancer. Intern Emerg Med 2023;18:297-303. DOI: 10.1007/s11739-022-03124-4.
382. Dubois SP, Miljkovic MD, Fleisher TA, et al. Short-course IL-15 given as a continuous infusion led to a massive expansion of effective NK cells: implications for combination therapy with antitumor antibodies. J Immunother Cancer 2021;9. DOI: 10.1136/jitc-2020-002193.

383. Yalcin A, Silay K, Balik AR, et al. The relationship between plasma interleukin-15 levels and sarcopenia in outpatient older people. Aging Clin Exp Res 2018;30:783-790. DOI: 10.1007/s40520-017-0848-y.
384. Farley MJ, Boytar AN, Adlard KN, et al. Interleukin-15 and high-intensity exercise: relationship with inflammation, body composition and fitness in cancer survivors. J Physiol 2024;602:5203-5215. DOI: 10.1113/jp286043.
385. Marin ND, Becker-Hapak M, Song WM, et al. Memory-like differentiation enhances NK cell responses against colorectal cancer. Oncoimmunology 2024;13:2348254. DOI: 10.1080/2162402x.2024.2348254.
386. Shrank WH, Patrick AR, Brookhart MA. Healthy user and related biases in observational studies of preventive interventions: a primer for physicians. J Gen Intern Med 2011;26:546-550. DOI: 10.1007/s11606-010-1609-1.
387. Heale R, Twycross A. Validity and reliability in quantitative studies. Evidence Based Nursing 2015;18:66-67. DOI: 10.1136/eb-2015-102129.
388. Pathak M, SNV SD, Dwivedi SN, et al. Comparison of hazard models with and without consideration of competing risks to assess the effect of neoadjuvant chemotherapy on locoregional recurrence among breast cancer patients. J Cancer Res Ther 2021;17:982-987. DOI: 10.4103/jcrt.JCRT_49_19.
389. Hill AB. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proc R Soc Med 1965;58:295-300.
390. Balshem H, Helfand M, Schünemann HJ, et al. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol 2011;64:401-406. DOI: 10.1016/j.jclinepi.2010.07.015.
391. Tano V, Jans DA, Bogoyevitch MA. Oligonucleotide-directed STAT3 alternative splicing switch drives anti-tumorigenic outcomes in MCF10 human breast cancer cells. Biochem Biophys Res Commun 2019;513:1076-1082. DOI: 10.1016/j.bbrc.2019.04.054.
392. Sparano JA, O'Neill A, Graham N, et al. Inflammatory cytokines and distant recurrence in HER2-negative early breast cancer. NPJ Breast Cancer 2022;8:16. DOI: 10.1038/s41523-021-00376-9.
393. Lindholm A, Abrahamsen ML, Buch-Larsen K, et al. Pro-inflammatory cytokines increase temporarily after adjuvant treatment for breast cancer in postmenopausal women: a longitudinal study. Breast Cancer Res 2024;26:142. DOI: 10.1186/s13058-024-01898-3.
394. Kim RK, Kaushik N, Suh Y, et al. Radiation driven epithelial-mesenchymal transition is mediated by Notch signaling in breast cancer. Oncotarget 2016;7:53430-53442. DOI: 10.18632/oncotarget.10802.
395. Esplugas R, Arenas M, Serra N, et al. Effect of radiotherapy on the expression of cardiovascular disease-related miRNA-146a, -155, -221 and -222 in blood of women with breast cancer. PLoS One 2019;14:e0217443. DOI: 10.1371/journal.pone.0217443.
396. Kober C, Roewe J, Schmees N, et al. Targeting the aryl hydrocarbon receptor (AhR) with BAY 2416964: a selective small molecule inhibitor for cancer immunotherapy. J Immunother Cancer 2023;11. DOI: 10.1136/jitc-2023-007495.

397. Dumbrava EE, Cecchini M, Zugazagoitia J, et al. Initial results from a first-in-human, phase I study of immunomodulatory aryl hydrocarbon receptor (AhR) inhibitor BAY2416964 in patients with advanced solid tumors. Journal of Clinical Oncology 2023;41:2502-2502. DOI: 10.1200/JCO.2023.41.16_suppl.2502.
398. Zwart AT, Cavalheiro VJ, Lamers MJ, et al. The validation of low-dose CT scans from the [(18)F]-FDG PET-CT scan to assess skeletal muscle mass in comparison with diagnostic neck CT scans. Eur J Nucl Med Mol Imaging 2023;50:1735-1742. DOI: 10.1007/s00259-023-06117-3.
399. Lortie J, Ufearo D, Hetzel S, et al. Validating a Practical Correction for Intravenous Contrast on Computed Tomography-Based Muscle Density. J Comput Assist Tomogr 2024. DOI: 10.1097/rct.0000000000001682.
400. Kim H, Baek S, Han S, et al. Low Skeletal Muscle Radiodensity Predicts Response to CDK4/6 Inhibitors Plus Aromatase Inhibitors in Advanced Breast Cancer. J Cachexia Sarcopenia Muscle 2025;16:e13666. DOI: 10.1002/jcsm.13666.
401. Lonkvist CK, Lønbro S, Vinther A, et al. Progressive resistance training in head and neck cancer patients during concomitant chemoradiotherapy -- design of the DAHANCA 31 randomized trial. BMC Cancer 2017;17:400. DOI: 10.1186/s12885-017-3388-0.

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