Elza Freiberga (Kuzņecova)

ORCID 0000-0002-6855-6414

Molecular Subtype-Specific Gene Expression Profiles in Breast Cancer

Doctoral Thesis – dissertation – for obtaining the scientific degree "Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences

Sector – Basic Medicine

Sub-Sector – Medicinal Genetics

Supervisors of the Doctoral Thesis:

Dr. med., Professor **Zanda Daneberga**,
Rīga Stradiņš University, Latvia

Dr. biol., Associate Professor **Miki Nakazawa-Miklaševiča**,
Rīga Stradiņš University, Latvia

Scientific advisor:

Dr. biol., Professor **Edvīns Miklaševičs**,
Rīga Stradiņš University, Latvia

Riga, 2026

Abstract

Breast cancer is the most diagnosed cancer among women worldwide, representing a significant and growing burden on global public health. Biologically and molecularly, breast cancer is a highly diverse disease. While its incidence continues to rise, mortality rates are gradually decreasing due to advancements in early detection and therapeutic interventions.

Breast cancer research field is increasingly focusing on transcriptomic research to uncover molecular pathways and prognostic biomarkers in order to improve disease management. This Thesis brings together findings of two linked studies, investigating triple negative breast cancer (TNBC) and transcriptomic signatures in monoallelic somatic *BRCA1* inactivation, to provide insights into potential therapeutic targets and prognostic markers.

The first part of the study focuses on TNBC, a highly aggressive subtype of breast cancer characterised by the absence of oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. The transcriptome of 19 breast cancers was sequenced and analysed to identify differentially expressed genes and enriched pathways specific to TNBC. A total of 229 differentially expressed genes were identified, with identified 4 hub genes – *FOXA1, ESR1, GATA3,* and *TFF1* as possible biomarkers. Functional enrichment and protein–protein interaction analyses indicated alterations in hormone signalling and extracellular matrix pathways, contributing to understanding of TNBC biology with potential to offer useful information for biomarker-driven therapies.

The second part of the study explores transcriptomic patterns linked with monoallelic somatic *BRCA1* inactivation and its correlation with event free survival in breast cancer patients. In this study, 36 breast cancer tissue samples were sequenced and categorised based on *BRCA1* inactivation status. Differential gene expression analysis revealed differential expression of genes, including *TPSD1, FABP4, CARTPT, GPX2* and *MMP9*, which are associated with homologous recombination deficient tumors. Kaplan-Meier analysis indicated a tendency towards improved event free survival for patients with monoallelic somatic *BRCA1* inactivation, suggesting this as a potentially favourable prognostic factor. Enrichment analysis showed significant involvement of extracellular proteins, providing additional insight into biology of the tumor microenvironment.

The acquired results highlight the importance of transcriptomics in breast cancer research, demonstrating the value of identifying cancer subtype-specific gene expression patterns. These findings contribute to better understanding of breast cancer biology and provide potential for the development of personalised treatment strategies, advancing care for breast cancer patients in the future.

Keywords: breast cancer, transcriptome, triple negative breast cancer, BRCA1 monoallelic inactivation.

Anotācija

Krūts vēža molekulārajiem apakštipiem raksturīga gēnu ekspresija

Krūts vēzis ir visbiežāk diagnosticētais vēža veids sievietēm visā pasaulē, un tas rada ievērojamu un pieaugošu slogu globālajai sabiedrības veselībai. Bioloģiski un molekulāri krūts vēzis ir ļoti daudzveidīga slimība. Lai gan tā izplatība turpina pieaugt, mirstības rādītāji pakāpeniski samazinās, pateicoties agrīnai diagnostikai un terapeitiskajām iespējām. Krūts vēža pētniecības joma arvien vairāk koncentrējas uz transkriptomikas pētījumiem, lai noteiktu molekulāros ceļus un prognostiskos biomarķierus, tādējādi uzlabojot pacientu aprūpi. Šajā disertācijā ir apkopoti secinājumi no divām savstarpēji saistītām pētījuma daļām, vienā pētot transkriptomu trīskārši negatīvam krūts vēzim (TNBC) un otrajā pētot transkriptomu monoalēliski somatiskas *BRCA1* inaktivācijas gadījumā, sniedzot ieskatu potenciālajos terapijas mērķos un prognostiskajos biomarķieros.

Pirmajā pētījuma daļā uzmanība vērsta uz TNBC, agresīvu krūts vēža apakštipu, ko raksturo estrogēna receptoru (ER), progesterona receptoru (PR) un cilvēka epidermālā augšanas faktora receptoru 2 (HER2) ekspresijas trūkums. Analizējot 19 krūts vēža transkriptomus, tika identificēti 229 atšķirīgi ekspresēti gēni, no kuriem *FOXA1, ESR1, GATA3* un *TFF1* iezīmējās kā potenciāli biomarķieri. Funkcionālās bagātināšanas un proteīnu mijiedarbības analīzes norādīja uz hormonu signālu un ekstracelulārās matricas signālceļu izmaiņām, sniedzot pienesumu TNBC bioloģijas izpratnē ar potenciāli vērtīgu informāciju biomarķieru vadītu terapiju attīstībai.

Otrajā pētījuma daļā analizēti transkriptomiskie modeļi, kas saistīti ar monogēno somatisko *BRCA1* inaktivāciju, un tās ietekme uz beznotikumu dzīvildzi krūts vēža pacientēm. Šajā pētījuma sadaļā sekvencēti 36 krūts vēža audu paraugi un kategorizēti pēc somatiskā *BRCA1* inaktivācijas statusa. Atšķirīgi ekspresēto gēnu analīze atklāja atšķirīgi ekspresētus gēnus, tostarp *TPSD1, FABP4, CARTPT, GPX2* un *MMP9*, kas saistīti ar homologās rekombinācijas trūkumu audzējos. Kaplana-Meiera analīze norādīja uz uzlabotu beznotikumu dzīvildzi pacientiem ar monogēno somatisko *BRCA1* inaktivāciju, kas liecina par šo kā potenciāli labvēlīgu prognostisko faktoru. Bagātināšanas analīze uzrādīja nozīmīgu ekstracelulārā matriksa proteīnu iesaisti, sniedzot papildu izpratni par audzēja mikrovides bioloģiju.

Šo pētījumu rezultāti uzsver transkriptomikas nozīmīgumu krūts vēža pētniecībā, parādot specifisku gēnu ekspresijas modeļu identificēšanas nozīmību. Šī darba rezultāti veicina dziļāku izpratni par krūts vēža bioloģiju potenciālu personalizētu ārstēšanas stratēģiju izstrādei, uzlabojot krūts vēža pacientu aprūpi nākotnē.

Atslēgvārdi: krūts vēzis, transkriptoms, trīskārši negatīvs krūts vēzis, *BRCA1* monoalēliska inaktivācija.

Table of Contents

Abbreviations used in the Thesis

AR	Androgen receptor
AMPK	AMP-activated protein kinase
BPA	Bisphenol A
BPM	Bisphenol M
BPP	Bisphenol P
BRCA1	BRCA1 DNA repair associated coding gene
BRCA2	BRCA2 DNA repair associated coding gene
BRK	Breast cancer kinase
CARTPT	CART Prepropeptide coding gene
CDKs	Cyclin dependent kinases
CGA	Glycoprotein Hormones, Alpha Polypeptide
CHGB	Chromogranin B
CI	Confidence interval
COL9A1	Collagen Type IX Alpha 1 Chain coding gene
CSC	Cancer stem cells
CSN3	Casein Kappa
DBD	DNA binding domain
DCIS	Ductal carcinoma in situ
DDR	DNA damage response
DEG	Differentially expressed genes
DNA	Deoxyribonucleic acid
DSB	Double-stranded break
ECM	Extracellular matrix
EMQN	European Molecular Genetics Quality Network
EMT	Dpithelial-to-mesenchymal transition
ER	Oestrogen receptor
ERBB2	Erb-b2 receptor tyrosine kinase 2
ERE	Oestrogen response elements
ERK	Oestrogen receptor kinase
ESR1	Oestrogen receptor 1
FABP4	Fatty Acid Binding Protein 4 coding gene
FDR	False discovery rate
FISH	Fluorescent in situ hybridisation
FOXA1	Forkhead Box A1 coding gene
GATA3	GATA binding protein 3 coding gene

GLI-2	Glioma-associated oncogene-2
GPX2	Glutathione Peroxidase 2 coding gene
HBOC	Hereditary breast and ovarian cancer
HER2	Human epidermal growth factor receptor 2
HR	Homologous recombination
HR	Hazard ratio
HRD	Homologous recombination deficiency
HRR	Homologous recombination DNA repair
HRT	Hormone replacement therapy
IDC	Invasive ductal carcinoma
IHC	Immunohistochemistry
ILC	Invasive lobular carcinoma
IRS4	Insulin receptor substrate 4 coding gene
LAR	Luminal androgen receptor
MAPK	Mitogen activated protein kinase
MC	Mast cells
MED1	Mediator Complex Subunit 1
MMP9	Matrix metalloproteinase 9
mRNA	Messenger RNA
mTOR	Mechanistic target of rapamycin signalling kinase pathway
NCCN	National Comprehensive Cancer Network
NICD	Notch intracellular domain
ORM1	Orosomucoid 1
PARP	poly (ADP-ribose) polymerase
PCA	Principal component analysis
PCR	Polymerase chain reaction
PI3K p	Phosphoinositide 3-kinase signalling pathway
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha coding gene
PPAR	Peroxisome proliferator-activated receptor signalling.
PPI	Protein–protein interaction
PR	Progesterone receptor
PRS	Polygenic risk score
PTCH-1	Patched homolog-1
PTEN	Phosphatase and tensin homolog coding gene
QC	Quality control
RNA	Ribonucleic acid

SHH	The Sonic Hedgehog pathway
SLC39A6	Solute Carrier Family 39 Member 6 coding gene
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins.
TFF1	Trefoil factor 1
TFF3	Trefoil factor 3
TKI	Tyrosine kinase inhibitor
TNBC	Triple negative breast cancer
TP53	Tumour protein p53 coding gene
TPSD1	Tryptase Delta 1
TRH	Thyroid releasing hormone
WHO	World Health Organisation

Introduction

Breast cancer is the most diagnosed malignancy among women globally, accounting for nearly 1 in 4 cancer cases in women, and representing significant public health challenges. In 2022 there were 2.3 million new cases of female breast cancer, making it the second leading cancer globally (11.6 % of all new cancer cases), and approximately 666 000 deaths, ranking it the fourth leading cause of cancer mortality (6.9 % of all cancer deaths) (Arnold et al., 2022; Bray et al., 2018). Although incidence rates are rising, mortality from breast cancer has gradually decreased due to improvement in early detection and therapeutic management (Kesson et al., 2012).

Triple negative breast cancer (TNBC) molecular subtype accounts for approximately 15–20 % of all breast cancer cases, characterised by absence of oestrogen (ER), progesterone (PR) and human epidermal growth factor receptor 2 (HER2) expression and is of particular concern due to its aggressive clinical behaviour and poor prognosis (Garrido-Castro et al., 2019; Shen et al., 2020). Predominantly TNBC affects younger women and is associated with higher relapse rates and higher likelihood of metastasis (Dent et al., 2007; Haffty et al., 2006). While most TNBC patients lack hormone receptor or HER2-targeted treatment options and therefore rely on chemotherapy, a subset of TNBC is associated with hereditary breast and ovarian cancer (HBOC) and may benefit from targeted therapies, highlighting the need for refined stratification and predictive biomarkers (Hwang et al., 2019).

Besides TNBC challenging management, breast cancer is heterogenous disease influenced by both genetic and environmental factors. Most common genetic factors include *BRCA1* and *BRCA2* genes, which play important role in genomic integrity through homologous recombination (HR) mediated DNA repair. Loss of function in these genes disrupts HR pathways which leads to genomic instability and tumour progression (Prakash et al., 2015). This can happen either through loss of function, or it can be inherited germline mutation or somatic alteration within tumour cells (Loboda et al., 2023). Somatic inactivation of *BRCA1* is known to be associated with distinctive molecular patterns, which includes genomic rearrangements and specific mutational signatures. These signatures are called “BRCAness” also known as predictive biomarkers for therapeutic response to platinum-based chemotherapies and PARP inhibitors, highlighting its clinical significance in treatment decision making (Bodily et al., 2020).

Development and advancement in high-throughput technologies such as RNA-sequencing has enabled scientists to look at transcriptomic profiles and molecular landscape of breast cancer (Chen et al., 2021; Hong et al., 2020; Rosati et al., 2024). This enables identification of differentially expressed genes and affected pathways within specific

cancer subtypes, including TNBC. Recent studies describe molecular heterogeneity of TNBC and highlights specific oncogenic drivers and evaluates tumour microenvironment (Kudelova et al., 2022; Shah et al., 2012). Transcriptomics also evaluates implications of somatic *BRCA1* inactivation therefore trying to clarify its role in tumour biology and therapy management (Arakelyan et al., 2021).

This Thesis integrates finding from two complementary studies. The first one explores transcriptome of TNBC, the hub genes and altered pathways associated with this particular breast cancer subtype, which can help in potential biomarkers and therapeutic targets identification. The second part of the study focuses on breast cancer with monoallelic somatic *BRCA1* inactivation, a condition of growing interest since even partial loss of *BRCA1* function may impair DNA repair and influence tumour behaviour. By examining its association with event-free survival, this study provides insights into the prognostic value of somatic *BRCA1* status. Together these studies help to enhance understanding of molecular mechanisms driving breast cancer progression and facilitate improvement in personalised treatment strategies which subsequently improve patient care outcome.

Aim of the Thesis

Characterise the gene expression profiles and altered pathways associated with distinct breast cancer molecular subtypes (triple-negative breast cancer and breast cancer with monoallelic somatic *BRCA1* inactivation) with the goal of identifying potential biomarkers and therapeutic targets, and to evaluate their potential clinical relevance.

Objectives of the Thesis

The following objectives are set to reach the aim of the Doctoral Thesis:

1. Perform RNA-seq, identify triple negative breast cancer (TNBC) subgroup and its specific transcriptome pattern.
2. Perform functional enrichment and protein–protein interaction analyses in TNBC.
3. Perform RNA-seq, identify transcriptomic pattern of tumours with monoallelic somatic *BRCA1* inactivation.
4. Perform functional enrichment and protein–protein interaction analyses in the *BRCA1* monoallelic inactivation group.
5. Analyse cancer free survival in the group with *BRCA1* monoallelic inactivation.

Hypothesis of the Thesis

Distinct transcriptomic signatures in breast cancer molecular subgroups may serve as predictive biomarkers, enabling personalised therapeutic strategies.

Novelty of the Thesis

Identification of gene expression alterations in TNBC and in breast cancers with BRCA1-associated homologous recombination impairment provides insight into tumour biology and may reveal biomarkers relevant for patient stratification and personalised therapeutic approaches.

Author's contribution

The author has performed and participated in all stages of the study, including the study design, RNA processing, molecular analysis, including sequencing, as well as the data analysis. The author also has prepared two scientific publications.

AI Disclosure/LLM statement

The author as a non-native English speaker, used LLM models and AI-assisted editing tools to refine the language in this PhD Thesis by correcting spelling and grammatical errors and improving the writing style. However, core authoring tasks, including the generation of scientific and medical insights and the drawing of conclusions, were carried out only by the author. Author takes full responsibility for the validity and integrity of this work.

1 Literature review

1.1 Breast cancer overview

1.1.1 Global breast cancer epidemiology

In Europe breast cancer is the most diagnosed cancer among women, with high incidence rates in Western, Northern, and Southern European countries. It is also the leading cause of cancer mortality in most European countries, accounting for 25 %, 19 %, and 15 % of deaths in women aged 0–44, 45–64, and 65 years or older, respectively. The risk of developing breast cancer before the age of 75 in Europe is estimated to be 8 %, while the risk of breast cancer-related death before the age of 75 is 1.6 %. Incidence rates of breast cancer in Europe range from 71 to 194 cases per 100 000 women, with the highest rates found in Belgium, the Netherlands, and Luxembourg, and the lowest rates in Albania, Bosnia and Herzegovina, and Montenegro (Dyba et al., 2021).

Globally, breast cancer is the most prevalent cancer and the leading cause of cancer-related deaths among women. In 2022, approximately 2.3 million new cases were diagnosed, accounting for 11.6 % of all cancer cases, with 666 000 deaths reported, or 6.9 % of all cancer deaths (Figures 1.1 and 1.2) (Sung et al., 2021, Arnold et al, 2022). According to recent statistics, the five-year survival rate for breast cancer in Europe is estimated to be around 85 %, with significant variations across different countries, however, survival rates for breast cancer are generally improving, due to advances in screening, diagnosis, and treatment (DeSantis et al., 2019).

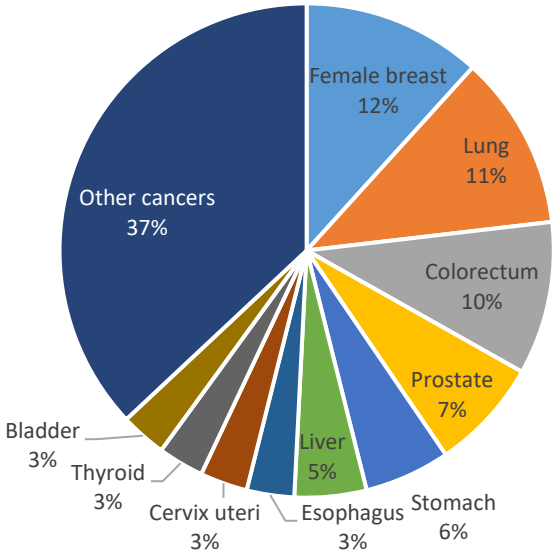

Figure 1.1 **Distribution of new cancer cases worldwide in 2020, both sexes, all ages according to GLOBOCAN** (Sung et al., 2021)

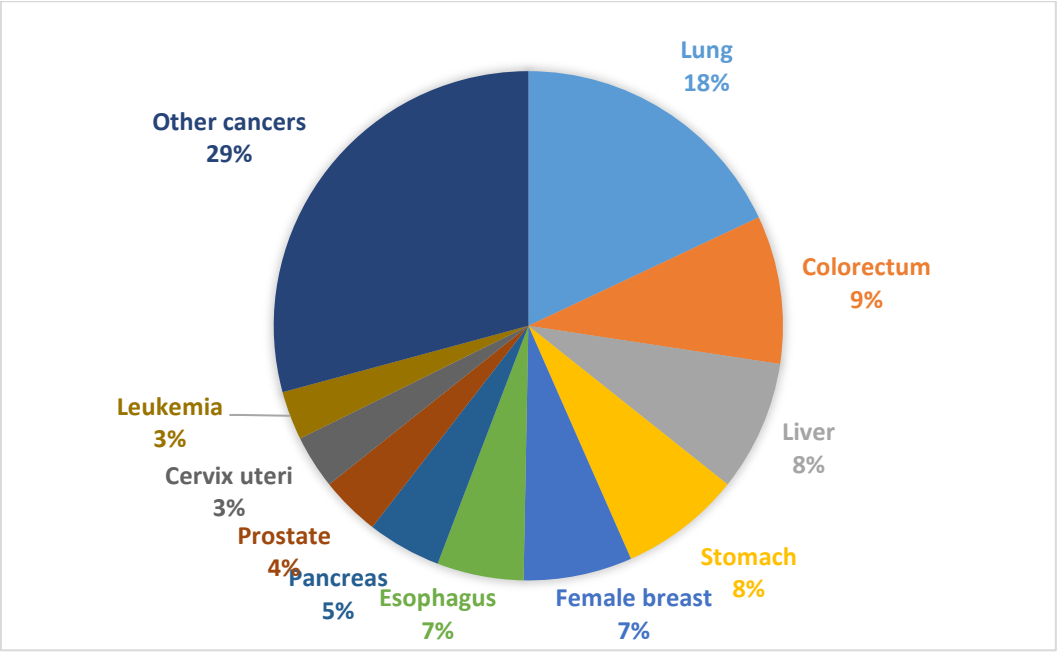

Figure 1.2 **Distribution of death cases worldwide in 2020, both sexes, all ages according to GLOBOCAN** (Sung et al., 2021)

Breast cancer occurs most often in women over the age of 50 (DeSantis et al., 2019). Risk factors include a family history of the disease, specific genetic variants, hormonal influences, obesity, and alcohol consumption (Bray et al., 2018). To facilitate breast cancer prevention and timely diagnosis, various guidelines and programs exist across different European countries. One of such programs is mammography screening, where regular check-up is recommended for women between the ages of 50 and 69, although some countries extend the age range for screening to 75 (Perry et al., 2008). In addition to age-based mammography screening, several countries are introducing risk-based screening approaches that incorporate individual risk factors, genetic testing, and polygenic risk scores (PRS) to better stratify women's risk of breast cancer (Mars et al., 2024).

Overall, breast cancer remains a significant public health challenge in Europe, but increased awareness and improved access to screening, diagnosis, and treatment can help to reduce the burden of this disease on individuals, their families, society and health care and social care system.

1.1.2 Epidemiology of breast cancer in Latvia

The incidence of newly diagnosed female breast cancer cases in Latvia has remained relatively stable between 2014. and 2023., fluctuating around 109–132 cases per 100 000 population (Figure 1.3). However, a marked increase is observed in 2024, reaching 132.6 cases per 100 000, the highest rate recorded during the examined period (Slimību Profilakses un Kontroles Centrs [SPKC], 2025).

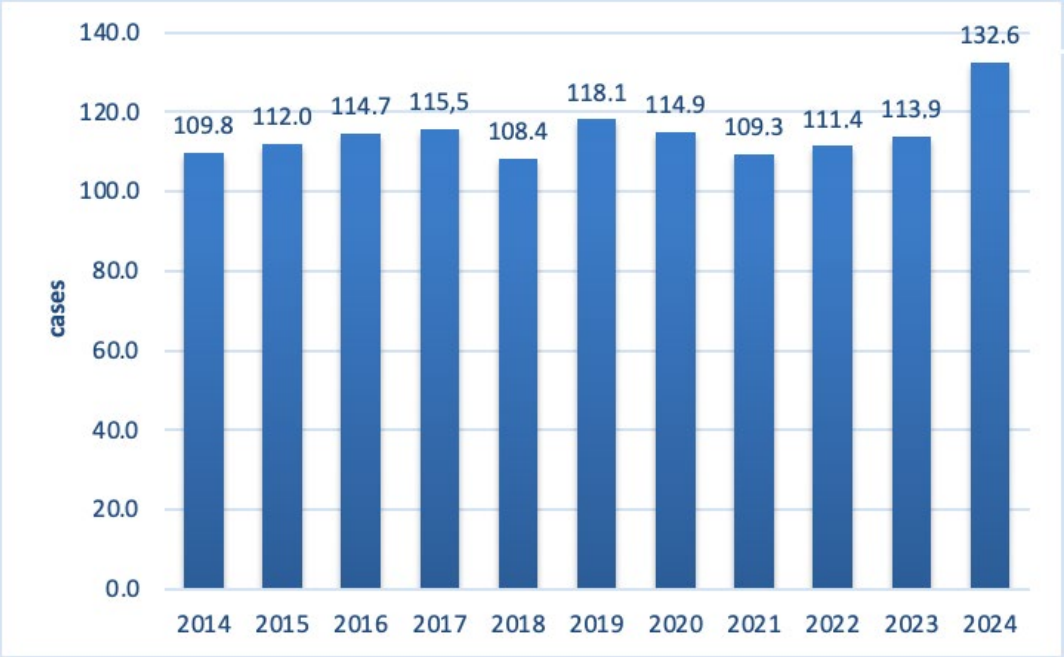

Figure 1.3 **Newly diagnosed (registered) female breast cancer cases in Latvia from 2013–2023 per 100 000 population** (SPKC, 2025)

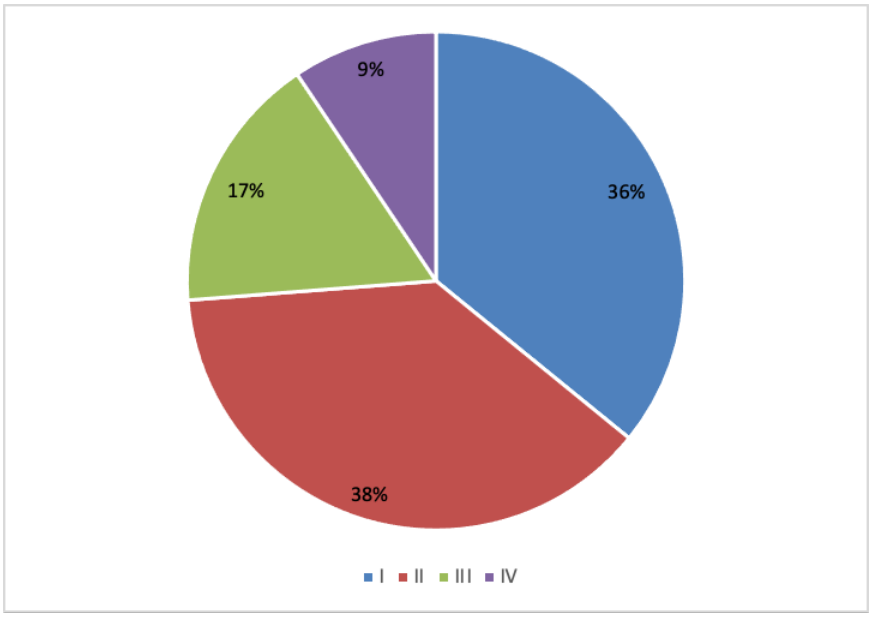

Figure 1.4 **Breast cancer (C50) diagnosis by stages in Latvia in 2023 in %** (SPKC, 2025)

The majority of breast cancer diagnoses in 2023 were at stage II, accounting for 38 % of cases. This suggests that a significant proportion of individuals were diagnosed with breast cancer at a relatively advanced stage followed by stage I diagnoses constituted the next largest proportion at 36 %, indicating a substantial number of early-stage diagnoses as well. Figure 1.4 also highlights a high proportion of diagnoses at more advanced stages. Stage III and IV diagnoses accounted for 17 % and 9 % of cases, respectively (Figure 1.4). These stages typically indicate more aggressive forms of breast cancer with a higher risk of metastasis and poorer prognosis.

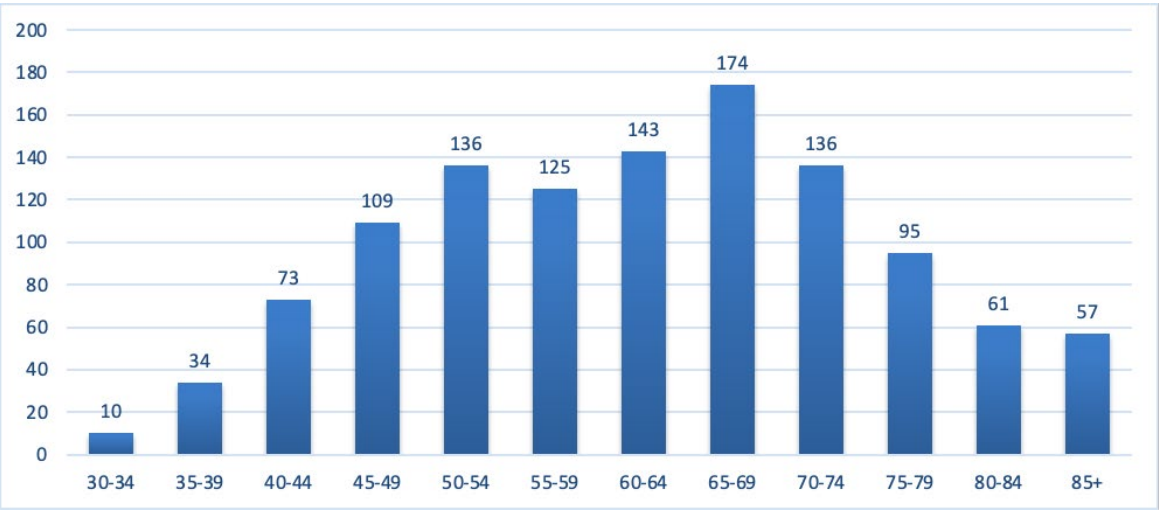

Figure 1.5 **Female breast cancer diagnosis by age groups in 2023**
(SPKC. Statistikas Dati. Slimību Profilakses Un Kontroles Centrs., 2025)

Increasing trend in the number of breast cancer cases is seen as age increases, with the highest number of cases being diagnosed in the 65–69 age group (Figure 1.5). This aligns with the common understanding that the risk of breast cancer generally increases with age, particularly after menopause. The data also suggests that while breast cancer can occur in younger age groups, it becomes more prevalent as women get older.

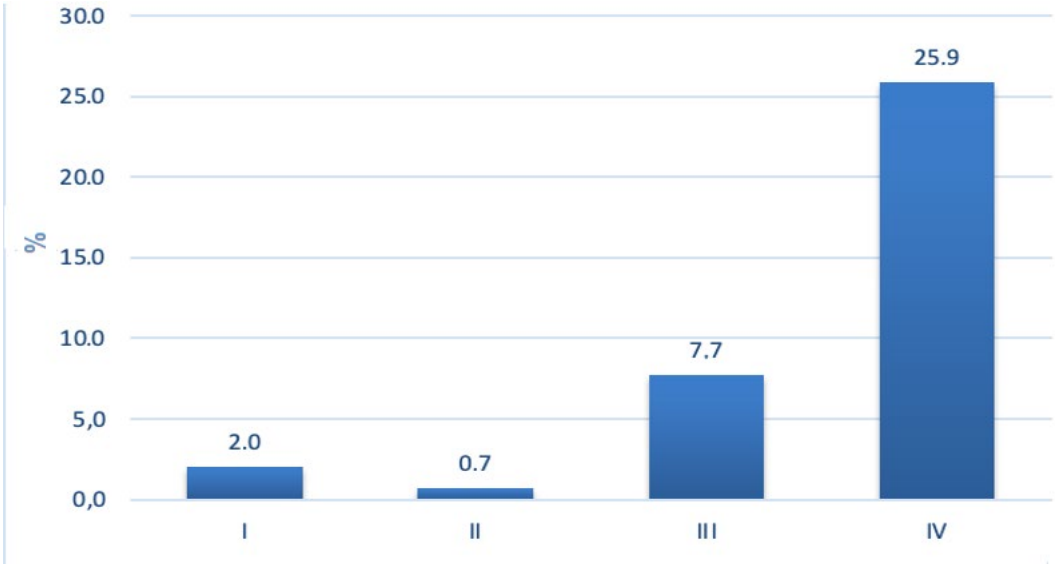

Figure 1.6 **Case-fatality rate of breast cancer patients depending on the stage of diagnosis in 2023 in %** (SPKC,2025)

The data indicates that the 1-year fatality rate (in 2023) increases as the cancer stage advances. Specifically, individuals diagnosed with Stage IV breast cancer have the highest fatality rate of 25.9 %, indicating a more advanced and often less treatable stage of the disease. Individuals diagnosed at earlier stages (stage I, II or III) have lower 1-year fatality rates, with stage I having the lowest fatality rate of 2 % (Figure 1.6). This Figure underscores the importance of early detection and intervention in breast cancer management, as early-stage

diagnoses are associated with significantly lower fatality rates compared to advanced-stage diagnoses.

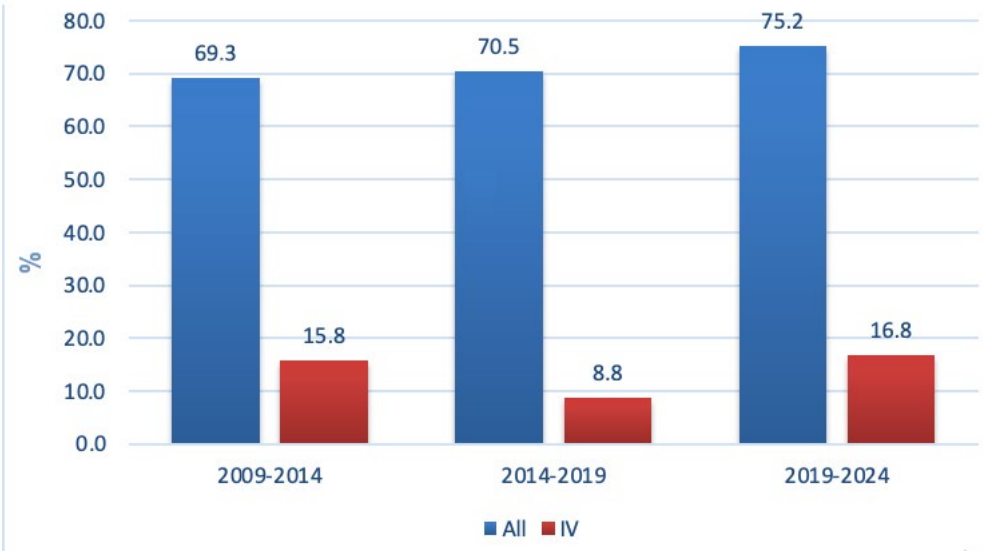

Figure 1.7 **5-year survival of breast cancer patients together and stage IV patients separately in different time periods in %** (SPKC, 2025)

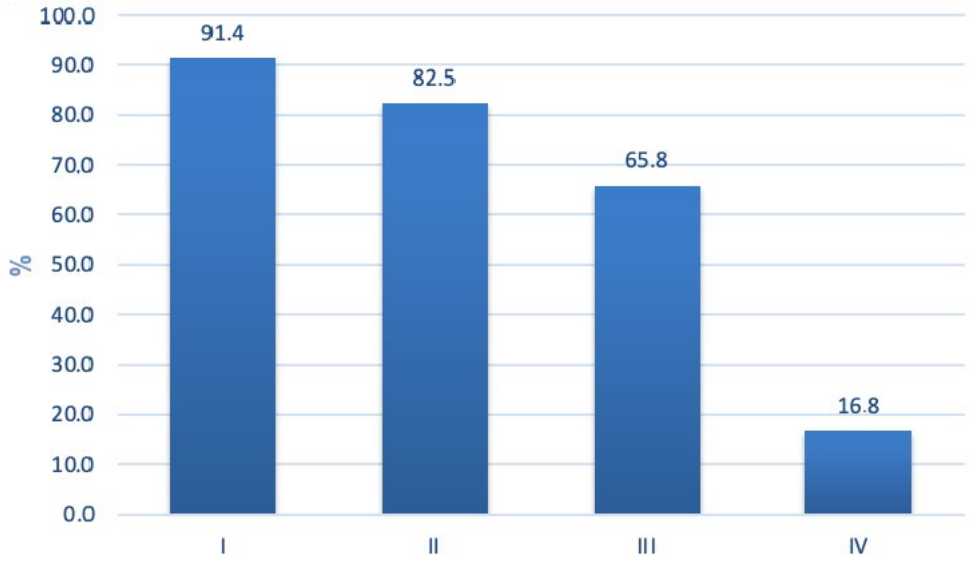

Figure 1.8 **Observed breast cancer patient's 5-year survival rate in %, diagnosed from 2019–2024 by stages** (SPKC, 2025)

Latvian registry data suggests that the 5-year survival rate remains relatively stable, at 69.3 % from 2009 to 2014, 70.5 % from 2014 to 2019, and 75.2 % from 2019–2024 for all breast cancer patients (SPKC, 2025). However, there is a notable change in the 5-year survival rate for patients diagnosed with stage IV breast cancer over time, increasing from 15.8 % from 2009 to 2014 to 16.8 % from 2019 to 2024 (Figure 1.7). Statistics from Latvia indicate a notable difference in the 5-year survival rate based on the stage of breast cancer at diagnosis, with a rate of 91.4 % for patients diagnosed in stage I and only 16.8 % for those diagnosed in stage IV (Figure 1.8), which is similar to European statistics, where 5-year survival. These findings

are comparable to European statistics, where the 5-year survival rate exceeds 90 % for early-stage breast cancers, while stage IV disease shows a survival rate of about 22 % (Tang et al., 2025).

1.1.3 Oncogenesis of breast cancer

Cancer oncogenesis is a complex phenomenon driven by genetic and epigenetic alterations, by which normal cells transform into cancer cells. At its core, oncogenesis involves the disruption of normal cellular regulatory mechanisms that govern processes such as cell proliferation, differentiation, and apoptosis. These disruptions can arise from various sources, including mutation in key regulatory genes, chromosomal rearrangements, and dysregulation of signalling pathways.

The hallmarks of cancer, proposed by Hanahan and Weinberg, include essential traits acquired by cancer cells to sustain their growth and survival. These hallmarks include self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, avoidance of apoptosis (also known as programmed cell death), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. The 2022 update further expands this framework by introducing additional hallmarks such as evasion of immune destruction and reprogramming of cellular metabolism, as well as emerging hallmarks includes phenotypic plasticity and dysregulated differentiation. (Hanahan, 2022; Hanahan & Weinberg, 2000; Peters & Gonzalez, 2018). By acquiring these capabilities, cancer cells can proliferate uncontrollably, evade the body's natural defences, and spread to distant sites, leading to the progression of the disease.

Cancer oncogenesis involves activation of proto-oncogenes, which play crucial role in maintaining normal cellular homeostasis by regulating essential processes like growth, proliferation, and survival. However, these gene can promote cell growth and proliferation, contributing to cancerogenesis when mutated or overexpressed (Hanahan & Weinberg, 2011). Conversely, the inactivation of tumour suppressor genes, which normally function to inhibit cell growth and promote apoptosis, is also a common feature of oncogenesis. These genetic alterations can occur spontaneously or may be induced by exposure to carcinogens such as chemicals, radiation, or infectious agents (Lee & Muller, 2010).

The activation of oncogenesis often begins with the accumulation of somatic mutations within cancer cells affecting critical genes involved in cell cycle regulation and DNA repair. These mutations provide a growth advantage to affected cells, allowing them to proliferate uncontrollably and evade normal regulatory mechanisms. As these abnormal cells continue to

divide and accumulate additional mutations, they undergo further genetic and phenotypic changes that drive tumour progression (Torgovnick & Schumacher, 2015).

In addition to genetic alterations, epigenetic modifications also play a crucial role in oncogenesis. Epigenetic changes, such as DNA methylation and histone modification, can alter the expression of genes involved in cell growth and differentiation without changing the underlying DNA sequence. These alterations can contribute to the dysregulation of signalling pathways and the development of cancerous phenotypes (Sadida et al., 2024; Sharma et al., 2010).

1.1.4 Sporadic breast cancer

Sporadic breast cancer is the most common form of breast cancer, representing approximately 85–90 % of all cases. Unlike hereditary breast cancer, sporadic breast cancer arises in individuals without inherited genetic allelic variants or a family history of the disease. Instead, it is triggered by somatic allelic variants, alterations occurring in the DNA of cells over person's lifetime, often due to environmental exposures like radiation or chemical agents (Fenga et al, 2016). Common somatic alterations in sporadic breast cancer include mutations in *TP53, PIK3CA,* and *PTEN*, along with recurrent alterations in *GATA3, CDH1, AKT1, RB1, ESR1,* and chromatin-regulating genes such as *KMT2C* and *KMT2D* (Angus et al., 2019; Kruk et al., 2019; Nik-Zainal et al., 2016).

Environmental and lifestyle factors play the main roles in the development of sporadic breast cancer. These encompass variables such as reproductive history, hormone exposure, obesity, physical inactivity, alcohol consumption and dietary patterns. Additionally, factors like smoking, exposure to environmental pollutants, and certain socioeconomic determinants also contribute to an individual's overall risk profile (Colditz & Bohlke, 2014; Kruk et al., 2019). While hereditary allelic variants in genes like *BRCA1* and *BRCA2* represent a minority of cases, sporadic breast cancer predominates and arises from a complex interplay of genetic, hormonal, and environmental influences (Michailidou et al., 2017). This complexity emphasises the heterogeneous nature of sporadic breast cancer, characterised by diverse molecular and histological subtypes that impact diagnosis, treatment strategies, and prognostic outcomes (Prat et al., 2015).

The diagnosis and treatment of sporadic breast cancer typically involve a combination of imaging tests, biopsies, surgery, radiation therapy, and chemotherapy, depending on the stage and subtype of the cancer. Treatment may also include hormone therapy, targeted therapy, and immunotherapy, depending on the specific characteristics of the tumour (Cardoso et al., 2020). Overall, while sporadic breast cancer is more common than hereditary forms of breast cancer,

it is still a complex and challenging disease that requires personalised treatment and management.

1.1.5 Hereditary breast and ovarian cancer (HBOC)

Hereditary breast cancer accounts for approximately 5–10 % of all breast cancer cases (Claus et al., 1996; Mahdavi et al., 2019). Breast cancer can be classified into two categories – familial and sporadic cases. In contrast to sporadic breast cancer the majority of hereditary breast cancer cases are caused by allelic variants in the *BRCA1* and *BRCA2* genes, which are tumour suppressor genes that play a key role in DNA repair (Ford et al., 1998a; Mahdavi et al., 2019). In addition to *BRCA1* and *BRCA2*, other genes such as *PALB2, TP53, PTEN, ATM, CHEK2,* and *BARD1* contribute to inherited breast cancer risk through their involvement in DNA repair and cell-cycle regulation (Mahdi et al., 2013). Breast cancer risk in general population is 12 %, whereas *BRCA1* and *BRCA2* variant carriers confer risk of developing breast cancer of 55–72 % by age 70 for *BRCA1* carriers, and 45–69 % for *BRCA2* carriers. Other high penetrance genes include *ATM, CDH1, PALB2, PTEN, STK11, TP53* which significantly increases lifetime risk of developing breast cancer. Moderate risk genes, including *ATM, BARD1* and *CHEK2* are associated with a 20–30 % lifetime risk depending on the variant (Adam et al., 1998). According to the most recent EMQN (2024) and NCCN v2.2025 guidelines, genetic testing panels should include the high-risk genes (*BRCA1, BRCA2, PALB2*) and, when clinically indicated, extend to moderate-risk genes such as *ATM, CHEK2, BARD1, RAD51C, RAD51D,* and *BRIP1* (Dwyer & Mary, 2024; McDevitt et al., 2024). Clinically, hereditary breast cancers with *BRCA1* pathogenic variants are diagnosed at a younger age and more frequently display the triple-negative (ER-/PR-/HER2-) phenotype, underscoring their distinct biological behaviour and clinical importance.

Genetic testing is available to identify individuals who may be at increased risk of developing inherited breast cancer. Guidelines for genetic testing have been established and are based on number of factors including personal and family history of cancer (Dubsky et al., 2024). Identifying individuals with inherited risk for breast cancer can enable early detection and better management of the disease, as well as inform decisions about prophylactic measures such as risk-reducing surgery and chemoprevention.

In recent years, there has been increasing interest in the use of targeted therapies for the treatment of inherited breast cancer, particularly in individuals with *BRCA1* and *BRCA2* allelic variants. PARP inhibitors and used for the treatment of germline BRCA-mutated breast cancer (Litton et al., 2020).

The differences between sporadic and hereditary breast cancer have important implications for treatment and prevention. Individuals with hereditary breast cancer may benefit from earlier and more intensive screening, prophylactic surgery, and targeted therapies such as PARP inhibitors. In contrast, individuals with sporadic breast cancer may benefit from chemotherapy, hormonal therapy, or targeted therapies such as HER2 inhibitors, depending on the molecular subtype of their tumour.

1.1.6 Classification of breast cancer

It is important to classify breast cancer in order to understand its complexity and choose treatment strategies. Historically, breast cancer classification was based on histopathological characteristics like tumour size, grade and lymph node involvement (Polyak, 2007).

Breast cancer can develop in different parts of the breast – the ducts, lobules, or the tissue in between. The type of breast cancer is determined by the cells that are affected therefore based on cell origin it is classified into two main types: carcinomas and sarcomas. Carcinomas arise from the cells that line the lobules and ducts, while sarcomas are rare (< 1 % of primary breast cancer) and develop from the stromal components of the breast. However, sometimes a single tumour can have a mix of cell types. Most breast cancers are carcinomas, which are further categorised based on their invasiveness. Understanding these subtypes is crucial for determining prognosis and treatment. They are broadly grouped into non-invasive (or in situ), invasive, and metastatic breast cancers (Figure 1.9) (Feng et al., 2018; Peters & Gonzalez, 2018; Polyak, 2007).

Breast cancer manifests in various forms, ranging from non-invasive to invasive and eventually to metastatic stages, each with distinct pathological characteristics and clinical implications. Non-invasive breast cancer, also known as carcinoma in situ, is confined to the ducts or lobules of the breast tissue without infiltrating the surrounding normal tissue. The most common type of non-invasive breast cancer is ductal carcinoma in situ (DCIS), where abnormal cells are found within the milk ducts but have not spread beyond the ductal walls. If left untreated, DCIS can progress to invasive breast cancer (Allred, 2010). Invasive breast cancer, on the other hand, occurs when cancerous cells break through the ductal or lobular walls and invade the surrounding breast tissue. This stage is marked by the potential for cancer cells to spread to nearby lymph nodes and other organs, posing a greater risk of metastasis. Metastatic breast cancer, also known as stage IV breast cancer, occurs when cancer cells spread from the breast to distant sites in the body, such as the bones, liver, lungs, or brain. Invasive Ductal Carcinoma (IDC) accounts for about 80 % of all breast cancers and involves cancer cells invading the surrounding breast tissue. It includes various subtypes like tubular, medullary,

mucinous, papillary, and cribriform carcinomas. Invasive Lobular Carcinoma (ILC) constitutes 10–15 % of breast cancers and typically affects older women. It presents distinct characteristics from IDC, often growing as single cells or in sheets, and has unique molecular features (Makki, 2015; Veronesi et al., 2005; Virnig et al., 2010)

Breast cancer classification into non-invasive, invasive, and metastatic forms enables the selection of appropriate treatment strategies according to tumour behaviour and disease progression. Early detection and timely intervention are crucial for preventing disease advancement and improving survival outcomes. Understanding the molecular and pathological characteristics of different subtypes, such as invasive ductal and lobular carcinomas, provides valuable insight for personalised therapeutic decisions and improved patient management (Redig & McAllister, 2013).

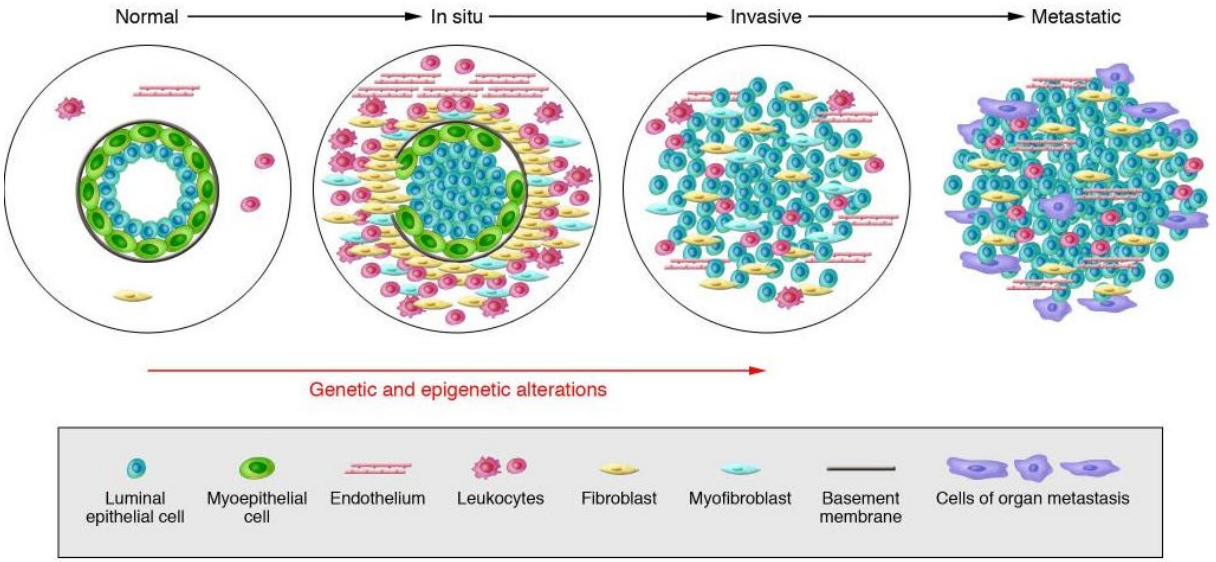

Figure 1.9 **Hypothetical model of breast tumour progression** (Polyak, 2007)

Breast cancer can be characterised by genetic and clinical diversity, encompassing multiple subtypes. Over time, the classification of these subtypes has undergone significant refinement. Immunohistochemistry is the most commonly used and widely recognised tool to classify breast cancers into biologically distinct subtypes and serve as prognostic and predictive tools (Zaha, 2014). It focuses on the expression of hormone receptors, including oestrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2). This method identifies four primary subtypes: luminal A, luminal B, HER2 and basal like (Zaha, 2014). Recent advancements in cancer research and a deeper molecular understanding of breast cancer suggest that the current clinical classification model could be further enhanced by incorporating additional molecular markers, such as specific miRNAs (e. g. let-7, miR-155, miR-150, miR-153) and pathogenic variants (e. g. p53, *BRCA1* and *BRCA2*) (Prat et al., 2015).

Luminal A breast cancer, representing approximately 40 % of cases, is oestrogen-receptor (ER) and/or progesterone-receptor (PR) positive and HER2 negative. It has low levels of Ki-67, grows slowly, and has a favourable prognosis. Treatment typically involves hormonal therapy (Cheang et al., 2009).

Luminal B breast cancer, accounting for less than 20 % of cases, shares similar hormone receptor status with luminal A but has higher levels of Ki-67 and may be HER2 positive. It grows slightly faster and has a slightly worse prognosis (Cheang et al., 2009).

HER2-enriched breast cancer, comprising 10–15 % of cases, lacks ER and PR expression but has high levels of HER2 and proliferation genes. These cancers grow rapidly and typically have a worse prognosis but can be targeted with HER2-directed therapies (Łukasiewicz et al., 2021).

Triple-negative/basal-like breast cancer (TNBC), making about 15–20 % of cases, lacks expression of ER, PR, and HER2. It tends to be more aggressive, higher grade, and more common in younger women and those with germline *BRCA1* pathogenic variants. Treatment options have traditionally been limited to chemotherapy, but recent advancements include the use of PARP inhibitors for *BRCA1* pathogenic variant carriers (Ensenyat-Mendez et al., 2021).

Normal-like breast cancer resembles luminal A disease but has a slightly worse prognosis. It is ER and/or PR positive, HER2 negative, and has low Ki-67 levels (Feng et al., 2018).

Understanding the molecular subtypes of breast cancer is crucial for tailoring treatment strategies and improving outcomes for patients. Advances in genomics and transcriptomics have enabled the identification of these subtypes and the development of diagnostic and prognostic tools to guide clinical decision-making. These tools, such as the Oncotype Dx, Breast Cancer Index, and PAM50 Risk of Recurrence score, provide valuable information for personalised treatment approaches and risk assessment (Yersal, 2014; Prat et al., 2015).

Both molecular and histological subtypes of breast cancer have important implications for diagnosis, treatment, and prognosis. Understanding the molecular and histological characteristics of a breast cancer tumour can help determine the appropriate course of treatment and provide valuable information about the likelihood of recurrence (Table 1.1).

Table 1.1

Molecular/intrinsic subtypes of breast cancers
adapted from Feng et al (Feng et al., 2018)

Subtypes	Molecular Signatures	Characteristics	Treatment options[a]
Luminal A	ER+, PR±, HER2-, Low Ki67	~70%, Most common	Hormonal Therapy
		Best prognosis	Targeted Therapy
Luminal B	ER+, PR±, HER2±,	10%–20%	Hormonal Therapy
	High Ki67	Lower survival than Luminal A	Targeted Therapy
HER2	ER-, PR-, HER2+	5%–15%	Targeted Therapy
Triple Negative	ER-, PR-, HER2-	15%–20%	Limited Targeted Therapy
		More common in black women	
		Diagnosed at younger age	
		Worst prognosis	
Normal-like	ER+, PR±, HER2-, Low Ki67	Rare	Hormonal Therapy
		Low proliferation gene cluster expression	Targeted Therapy

[a] Besides conventional surgical and non-surgical treatment.

1.2 Molecular pathways in breast cancer

Normal human development relies on intricate signalling pathways that facilitate communication between cells and their environment. These pathways, essential for regulating cell proliferation, survival, and migration, are often targeted by cancer cells and cancer stem cells (CSCs) (Sever & Brugge, 2015). Cancer arises from genetic and epigenetic changes that disrupt the normal controls on cell behaviour, allowing uncontrolled growth and spread. Many of these changes affect signalling pathways governing cell division, death, differentiation, and motility. For instance, activating mutations in proto-oncogenes can lead to overactivation of these pathways, while inactivation of tumour suppressors removes crucial inhibitors of signalling. Key signalling pathways involved in normal mammary gland development and breast cancer stem cell activities includes oestrogen receptor (ER) signalling, HER2 signalling, and canonical Wnt signalling.

1.2.1 Oestrogen receptor signalling and breast cancer

Oestrogen receptors (ERs) play a crucial role in breast cancer biology, representing a key target for therapeutic intervention. These receptors exist in two main forms: membrane oestrogen receptors, primarily G protein-coupled receptors, and nuclear oestrogen receptors, including ERα and ERβ. ERα and ERβ function as transcription factors, modulating the expression of target genes upon ligand binding. Encoded by the *ESR1* gene, ERα is widely expressed in breast tissue and is implicated in the development and progression of breast cancer. Similarly, ERβ, encoded by the *ESR2* gene, exhibits distinct but overlapping functions with

ERα, contributing to the regulation of breast cancer growth and survival (Cheskis et al., 2007; V. Kumar et al., 1987; Renoir et al., 2013)

Structurally, ERα and ERβ share common features, including six functional domains with varying degrees of similarity. Notably, the DNA-binding domain (DBD) facilitates the interaction between ER dimers and oestrogen response elements (EREs) within the promoter regions of target genes. This interaction initiates a cascade of events leading to the transcriptional activation or repression of genes involved in cell proliferation, differentiation, and survival (Figure 1.10) (Cheskis et al., 2007; Renoir et al., 2013).

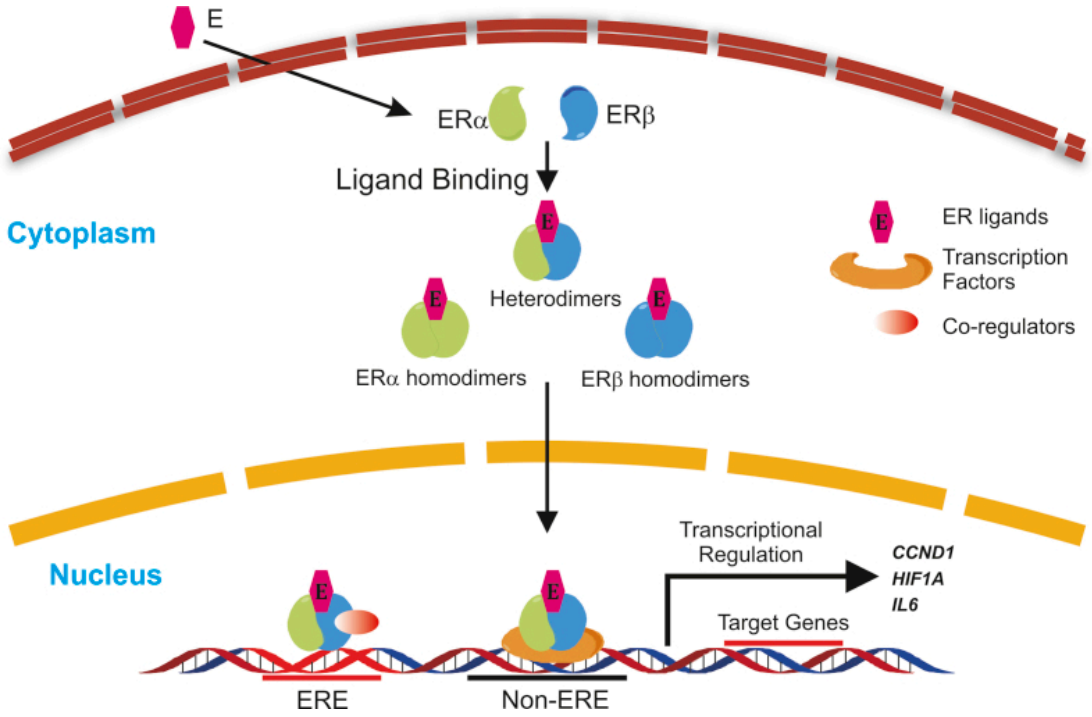

Figure 1.10 **Oestrogen receptor signalling** (Feng et al., 2018)

In addition to the classical ERE-dependent pathway, ERs can regulate gene expression through ERE-independent mechanisms, involving a complex interplay of co-activators and co-repressors. These molecular interactions modulate ER-mediated transcriptional activity and contribute to the diverse biological functions of ERs in breast cancer. Notably, *BRCA1*, a tumour suppressor gene frequently mutated in hereditary breast ovarian cancer, plays a critical role in regulating ERα signalling. Loss of *BRCA1* function can disrupt the balance between ERα and its co-regulators, leading to aberrant oestrogen signalling and promoting breast cancer development (Fan et al., 1999, 2001; Fuentes & Silveyra, 2019).

ERα-driven signalling pathways are implicated in various aspects of breast cancer progression, including tumour growth, metastasis, and therapy resistance. One well-characterised mechanism by which ERα promotes tumour growth is through its interaction with cyclin D1, a key regulator of cell cycle progression. Cyclin D1 activates cyclin-dependent

kinases (CDKs) 4 and 6, facilitating cell cycle entry and promoting cell proliferation (Cicatiello et al., 2004; Said et al., 1997). The cross talk between ERα and cyclin D1 pathways underscores the complexity of oestrogen signalling in breast cancer and highlights potential therapeutic targets for intervention.

Moreover, the role of ER isoforms, particularly ERα36, in breast cancer pathogenesis is gaining attention. ERα36, a truncated isoform of ERα, has been associated with aggressive tumour phenotypes and poor prognosis in breast cancer patients. Its aberrant expression can modulate the response to endocrine therapy, contributing to treatment resistance and disease progression. Understanding the distinct functions of ER isoforms and their implications for breast cancer biology may provide insights into novel therapeutic strategies and personalised treatment approaches for ER-positive breast cancer patients (Thiebaut et al., 2020).

1.2.2 HER2 signalling and breast cancer

Human epidermal growth factor receptors (EGFRs), including HER1, HER2, HER3 and HER4, constitute a family of tyrosine kinase receptors present in both normal tissues and various cancer types. Among them, human epidermal growth factor receptor-2 (HER2) is a key member, functioning as a receptor tyrosine kinase with distinct structural domains: an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain. The constitutive activity of HER2 allows it to form dimers with other molecules, influencing multiple cellular functions through various pathways (Roskoski, 2014; Sergina & Moasser, 2007).

Upon ligand binding, HER2 undergoes dimerisation, leading to phosphorylation of tyrosine residues in its intracellular domain and subsequent activation of downstream signalling pathways like the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) pathways (Figure 1.11), heavily implicated in breast tumorigenesis (Burgess, 2008; Mayer & Arteaga, 2016) .

Amplification of HER2 is observed in various human breast cancer cell lines, resulting in HER2 protein overexpression, which drives tumour cell proliferation and cancer progression (Cheng, 2024; Iqbal & Iqbal, 2014; Slamon et al., 1987). Targeted therapies have been developed to bind specific molecules in these signalling pathways, offering effective treatment for appropriately selected patients. Recent studies have unveiled novel mechanisms underlying HER2-driven tumorigenesis, including the influence of intermediary factors like *MED1* and its association with inflammation and cancer stem-like cell expansion. Additionally, epigenetic mechanisms such as DNA methylation and histone modifications play a role in regulating HER2 expression (Singla et al., 2017).

HER2 status, determined through immunohistochemistry (IHC) or fluorescent *in situ* hybridisation (FISH), identifies patients who may benefit from HER2-targeted therapies, such as monoclonal antibodies or tyrosine kinase inhibitors (TKIs). These treatments have significantly improved the prognosis of patients with HER2-positive neoplasms (Romond et al., 2005; Untch et al., 2018). However, breast cancer cells expressing HER2 are more prone to metastasis, highlighting the importance of HER2 testing in selecting appropriate patients for targeted therapy (Schwartz & Erban, 2017). Progesterone-induced paracrine signals may induce migration in early primary tumour cells, activating mammary stem cells, consistent with the stem-cell-like qualities stimulated by HER2.

HER2 molecular analysis has become integral in the diagnostic work-up of breast cancer patients, aiding in the selection of targeted therapies. As research continues to elucidate the complex interplay between HER2 signalling and breast cancer progression, further advancements in targeted therapies and diagnostic strategies are anticipated to improve patient outcomes and treatment specificity (Hosseini et al., 2016; Nwabo et al., 2014; Schwartz & Erban, 2017).

Human epidermal growth factor receptors (EGFRs), including HER1 to HER4, constitute a family of tyrosine kinase receptors present in both normal tissues and various cancer types. Among them, human epidermal growth factor receptor-2 (HER2/NEU, c-ERBB2) is a key member, functioning as a receptor tyrosine kinase with distinct structural domains: an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain. The constitutive activity of HER2 allows it to form dimers with other molecules, influencing multiple cellular functions through various pathways. Upon ligand binding, HER2 undergoes dimerisation, leading to phosphorylation of tyrosine residues in its intracellular domain and subsequent activation of downstream signalling pathways like the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) pathways, heavily implicated in breast tumorigenesis (Gutierrez & Schiff, 2011).

Amplification of HER2 is observed in various human breast cancer cell lines, resulting in HER2 protein overexpression, which drives tumour cell proliferation and cancer progression. Targeted therapies have been developed to bind specific molecules in these signalling pathways, offering effective treatment for appropriately selected patients. Recent studies have unveiled novel mechanisms underlying HER2-driven tumorigenesis, including the influence of intermediary factors like MED1 and its association with inflammation and cancer stem-like cell expansion. Additionally, epigenetic mechanisms such as DNA methylation and histone modifications play a role in regulating HER2 expression (Feng et al., 2018).

HER2 status, determined through immunohistochemistry (IHC) or fluorescent in situ hybridisation (FISH), identifies patients who may benefit from HER2-targeted therapies, such as monoclonal antibodies or tyrosine kinase inhibitors (TKIs). These treatments have significantly improved the prognosis of patients with HER2-positive neoplasms. However, breast cancer cells expressing HER2 are more prone to metastasis, highlighting the importance of HER2 testing in selecting appropriate patients for targeted therapy. Progesterone-induced paracrine signals may induce migration in early primary tumour cells, activating mammary stem cells, consistent with the stem-cell-like qualities stimulated by HER2 (Feng et al., 2018).

HER2 molecular analysis has become integral in the diagnostic work-up of breast cancer patients, aiding in the selection of targeted therapies. As research continues to elucidate the complex interplay between HER2 signalling and breast cancer progression, further advancements in targeted therapies and diagnostic strategies are anticipated to improve patient outcomes and treatment specificity (Cheng, 2024).

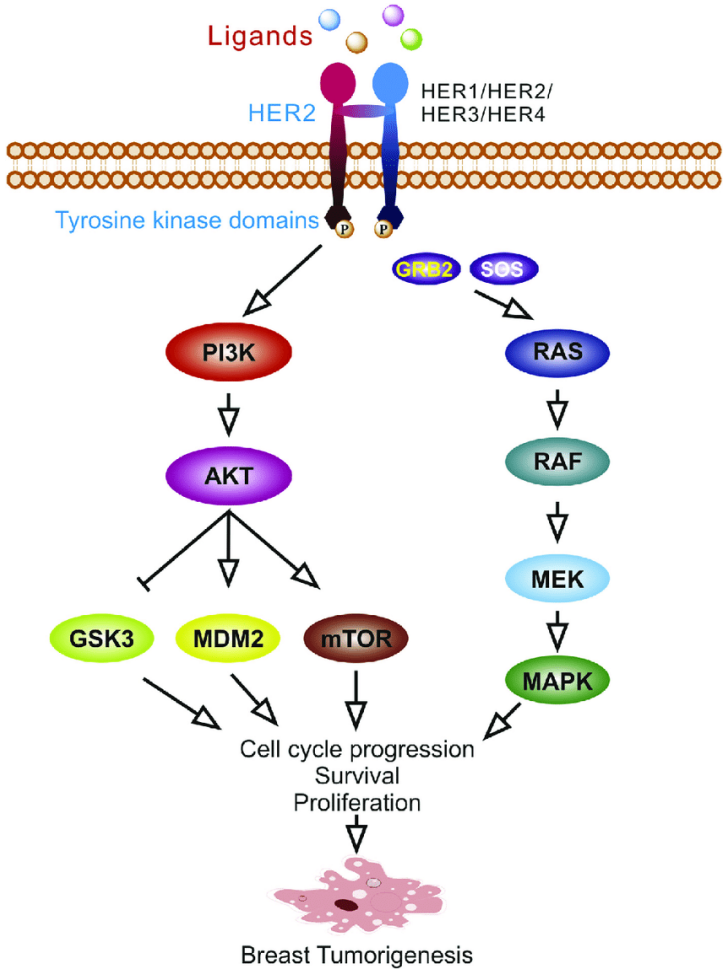

Figure 1.11 **HER2 signalling** (Feng et al., 2018)

1.2.3 Other signalling pathways affected in breast cancer

Besides dysregulated pathways discussed previously, there are many other pathways which if dysregulated, can significantly impact normal mammary development and contribute to breast cancer progression. Among these pathways are Cyclin dependent kinases (CDKs), Notch signalling, Sonic Hedgehog (SHH) signalling, Breast tumour kinase (BRK), PI3K/AKT/mTOR pathway and many others (Kamdje, 2014)

CDKs, which regulate cell cycle progression, are overexpressed in breast cancer, with cyclin D1 amplification observed in more than 50 % of cases (Casimiro et al., 2012). Oestrogen utilises cyclin D1 to exert its mitogenic effects, and high tumour expression of cyclin D1, along with overexpression of HER2, is associated with reduced recurrence-free survival and tamoxifen responsiveness (Ahlin et al., 2017). Oral CDK4/6 inhibitors have shown promise in inhibiting cell cycle progression, particularly in ER-positive cell lines (Murphy & Dickler, 2015; Wang et al., 2024)

Notch signalling, involving Notch receptors and ligands, plays a role in breast cancer pathogenesis. Aberrant activation of Notch signalling is a common feature in breast cancer, evidenced by increased NICD (Notch intracellular domain) accumulation and target gene expression observed in various breast cancer cell lines and primary samples (Mittal et al., 2009; Stylianou et al., 2006; Weijzen et al., 2002). Notably, overexpression of Notch receptors and ligands is prevalent in breast tumours and correlates with poorer patient prognosis (Reedijk et al., 2005). Dysregulation of Notch signalling is particularly seen in the triple-negative breast cancer (TNBC) subtype, where Notch receptor overexpression is associated with its aggressive, metastatic, and therapy-resistant phenotype (Zhong et al., 2016; Giuli et al., 2019). Specifically, Notch4 expression is notably elevated in TNBC, with one study reporting its presence in 55.6 % of TNBC samples compared to only 25.5 % of ER+ samples (Wang et al., 2018).

The Sonic Hedgehog (SHH) signalling pathway plays a critical role in embryonic tissue patterning and organ development. In breast cancer, dysregulation of the SHH pathway has been implicated in tumorigenesis and disease progression (Jiang & Hui, 2008). SHH signalling is involved in organizing cell growth and differentiation during mammary gland development. Disruption of downstream targets of the SHH pathway, such as Patched homolog-1 (PTCH-1) or glioma-associated oncogene-2 (GLI-2), can lead to defects in ductal morphogenesis (Jiang & Hui, 2008). Moreover, aberrant activation of SHH signalling has been associated with invasive breast cancer phenotypes and poor prognosis (Jeng et al., 2013). Higher expression levels of SHH and its downstream targets have been correlated with increased breast cancer invasiveness (Doheny et al., 2020).

Breast tumour kinase (BRK) is a non-receptor tyrosine kinase that is commonly overexpressed in about 60–85 % of human breast carcinomas. This overexpression is not observed in normal mammary glands or benign lesions, underscoring its significance in breast cancer pathology. Studies have demonstrated that BRK plays a crucial role in promoting cell proliferation and migration in breast cancer cells (Barker et al., 1997; Miah et al., 2012). Depletion of BRK has been shown to impair epidermal growth factor receptor (EGFR)-regulated signalling pathways, suggesting that BRK is intricately involved in mediating EGFR signalling cascades that contribute to breast cancer progression (Li et al., 2012). The inhibition or downregulation of BRK activity may therefore represent a promising therapeutic strategy for attenuating breast cancer cell growth and metastasis.

The PI3K/AKT/mTOR pathway is a crucial signalling cascade involved in regulating various cellular processes, including cell growth, proliferation, survival, and metabolism. Dysregulation of this pathway has been implicated in the development and progression of breast cancer (Hoxhaj & Manning, 2020). Oncogenic pathogenic variants in phosphatidylinositol 3-kinase (*PI3K*), particularly *PIK3CA* mutations are frequently observed in breast cancer and can lead to the dedifferentiation of mammary progenitor cells, facilitating tumorigenesis (deGraffenried et al., 2004; Isakoff et al., 2005) . Hyperactivation of AKT and subsequent mTOR activation downstream of PI3K signalling contribute to resistance to endocrine therapies and poor clinical outcomes in breast cancer patients (Pérez-Tenorio & Stål, 2002). Activation of AKT is associated with reduced response rates to endocrine therapy and worse overall survival. Therefore, targeting the PI3K/AKT/mTOR pathway represents a promising therapeutic strategy for breast cancer treatment, with ongoing research focused on developing inhibitors and combination therapies to effectively disrupt aberrant signalling in breast cancer cells and improve patient outcomes.

Understanding these signalling pathways and their interactions provides valuable insights into breast cancer biology and potential therapeutic targets for improving patient outcomes.

1.2.4 DNA repair mechanisms and double strand breaks

Double-strand breaks (DSBs) are a type of DNA damage that can occur spontaneously or be caused by exposure to environmental factors such as ionizing radiation or certain types of chemotherapy drugs. DSBs can lead to genetic mutations, chromosomal rearrangements, and ultimately, cancer. In order to repair DSBs, cells have evolved several mechanisms, one of which is homologous recombination (HR) (Jackson & Bartek, 2009).

HR is a high-fidelity DNA repair pathway that uses a homologous DNA molecule as a template to repair DSBs. The process of HR is initiated when a DSB is recognised by the MRN complex, which recruits ATM and ATR kinases to the site of damage. The ATM and ATR kinases phosphorylate downstream targets, leading to the activation of several HR factors, including BRCA1 and BRCA2. These proteins facilitate the resection of the broken DNA ends, allowing for the invasion of a homologous DNA molecule and the formation of a joint molecule. The joint molecule is then processed and resolved, resulting in the repair of the DSB (Symington & Gautier, 2011).

1.2.5 *BRCA1* and *BRCA2* genes in breast cancer development

BRCA1 and *BRCA2* genes are important in the pathogenesis of breast cancer. Allelic variants in these genes are associated with a high risk of developing breast and ovarian cancer. *BRCA1* and *BRCA2* are tumour suppressor genes that encode proteins involved in DNA repair through the homologous recombination pathway (Kuchenbaecker et al., 2017; Lord & Ashworth, 2012).

BRCA1 allelic variants are linked to an increased risk of developing triple-negative breast cancer, which is a more aggressive subtype of breast cancer that lacks oestrogen, progesterone, and HER2 receptors. Studies have shown that *BRCA1*-mutated breast tumours have distinct molecular characteristics, including high-grade histology, high proliferation rate, and frequent *TP53* allelic variant (Arimura et al., 2024; Colomer et al., 2024; Manié et al., 2009). Transcriptomic profiling of TNBCs has identified the upregulation of genes involved in cell proliferation, metastasis, and epithelial-to-mesenchymal transition (EMT), such as *MMP9* and *ZEB1* (Jang et al., 2015; Kalavska et al., 2020; Maturi et al., 2018). These molecular characteristics contribute to the high metastatic potential and aggressive behaviour of TNBC. *BRCA1* mutated tumours also might display a high tumour mutational burden (TMB) and is associated with increased expression of immune checkpoints like PD-1 and PD-L1 (Vugt & Parkes, 2022), which suggests that these patients might benefit from checkpoint blockade therapies in combination with chemotherapy.

In addition, *BRCA1*-mutated tumours have been found to be more sensitive to chemotherapy, particularly platinum-based agents (Tutt et al., 2010).

BRCA2 allelic variant are also associated with an increased risk of developing breast cancer, although the risk is not as high as that associated with *BRCA1* allelic variant. *BRCA2* allelic variant are more commonly associated with the development of oestrogen receptor-positive breast cancer (Ford et al., 1998; Vidarsdottir et al., 2023). Similar to *BRCA1*-mutated tumours, *BRCA2*-mutated tumours also have distinct molecular

characteristics, including a high frequency of *TP53* allelic variant and genomic instability (Nik-Zainal et al., 2016). Unlike *BRCA1*-mutated tumours, however, *BRCA2*-mutated tumours have been found to be less sensitive to platinum-based chemotherapy (Chen et al., 2023).

BRCA1 and *BRCA2* pathogenic variant have important implications for the treatment and management of breast cancer. Women with a known *BRCA1* or *BRCA2* pathogenic variants may be offered risk-reducing mastectomy or oophorectomy to reduce their risk of developing breast and ovarian cancer (Bertozzi et al., 2023; Rebbeck et al., 2009). In addition, *BRCA1* and *BRCA2* allelic variant may also impact treatment decisions for breast cancer. For example, women with *BRCA1*-mutated tumours may be more likely to benefit from platinum-based chemotherapy, while women with *BRCA2*-mutated tumours may not (Byrski et al., 2009; Chen et al., 2023; Tutt et al., 2010).

Testing for *BRCA1* and *BRCA2* allelic variant is now widely available and recommended for women with a family history of breast and ovarian cancer or those diagnosed with breast cancer at a young age. In addition, genetic testing may also be recommended for men with a family history of breast cancer, as *BRCA1* and *BRCA2* allelic variant can also increase the risk of developing male breast cancer (Dubsky et al., 2024) .

1.2.6 *BRCA1* and *BRCA2* gene pathogenic variants in Latvian population

Maksimenko et al. examined a small, founder-negative, high-risk Latvian cohort with a 26-gene panel and found pathogenic and likely pathogenic variants in 44 % of probands (6 in *BRCA1* and 1 in *BRCA2*), several of which were novel for the Latvian population, in that way demonstrating the added yield of multigene testing beyond the three common *BRCA1* founder variants (Maksimenko et al., 2018). Complementing this, Loza et al. screened 9,543 individuals for *BRCA1* founder variants and then sequenced 164 founder-negative, high-risk cases by NGS, identifying pathogenic/likely pathogenic *BRCA1/2* variants in 26.8 % and describing a recurrent *BRCA1* c.5117G>A (p.Gly1706Glu) variant. The authors suggest expanding local testing strategies in appropriate patients (Loza et al., 2021). Together, these Latvian data support international practice that early identification of germline *BRCA1/2* variants informs surveillance and risk-reduction and, in eligible HER2-negative breast cancer patients, can guide targeted therapy with PARP inhibitors.

1.3 Breast cancer treatment options

Breast cancer is treated in different ways and treatment is tailored for each patient individually based on tumour subtype, stage and molecular characteristics. Surgery still remains the main strategy for local disease management, from lumpectomy to mastectomy, which is

often combined with lymph node biopsy or axillary dissection in order to access regional spread of diseases (Moo et al., 2018).

Breast cancer molecular subtypes play essential role in treatment decisions because different subtypes respond differently to various therapeutic approaches. Luminal A and B breast cancers, which express oestrogen and/or progesterone receptors, are generally treated with endocrine therapy, such as tamoxifen or aromatase inhibitors. These agents work by blocking oestrogen's effect cancer cells, reducing tumour growth and recurrence. Additionally, chemotherapy may also be recommended for some patients with luminal breast cancer, depending on factors such as tumour size, lymph node involvement, and patient age (Loibl et al., 2024; Mohammed, 2021)

HER2-enriched breast cancer, which overexpresses the HER2 protein, may be treated with various HER2-targeted therapies that have been developed over time. These include monoclonal antibodies like pertuzumab and margetuximab, trastuzumab-based antibodies-drug conjugates such as T-DM1 and T-DXd, as well as tyrosine kinase inhibitors including lapatinib and tucatinib among other options. These drugs specifically target HER2-positive cancer cells and can improve survival rates in patients with HER2-positive breast cancer. These targeted therapies have revolutionised treatment landscape for HER2-positive breast cancer by improving outcomes and reduced recurrence when used in combination with standard chemotherapeutic regimens (Mercogliano et al., 2023).

Triple-negative breast cancer (TNBC) poses significant treatment challenges due to molecular heterogeneity and aggressive behaviour. This tumour type lacks expression of oestrogen, progesterone and HER2 receptors, and it is generally treated with systemic chemotherapy as primary treatment option with agents such as anthracyclines and taxanes administered in both – neoadjuvant and adjuvant settings (Obidiro et al., 2023). Chemotherapy remains the first-line approach due to the unavailability of endocrine or HER2-targeted therapies, however, other options exist such as immunotherapy, radiotherapy and surgery. Recent advances indicate that PARP inhibitors show promise by exploiting deficiencies in DNA repair mechanisms, particularly in tumours with *BRCA* allelic variants (Dibitetto et al., 2024; Lord & Ashworth, 2017). Furthermore, immunotherapy agents are increasingly being studies and integrates into treatments regiments by offering potential benefits for patients with high tumour mutation burden (Kwa & Adams, 2018).

Treatment decisions are complex and highly individualised. Apart from molecular subtypes there are other factors that play significant role in determining the most appropriate treatment plan, such as tumour size, location, extent of lymph node involvement. Some patients may benefit from combination of surgery, radiotherapy and systemic agents in order to treat

both – local and systemic diseases. Other patients may benefit from more conservative approach based on their risk profile and treatment tolerance.

Moreover, ongoing advances in molecular diagnostics and transcriptomic profiling are helping clinicians to refine and tailor treatment strategies. Healthcare teams can identify biomarkers that predict treatment responses and tailor therapies accordingly by integrating and analysing detailed gene expression data with clinical parameters. This personalised approach not only maximises therapeutic efficacy but also minimises unnecessary toxicity in that way improving patient outcomes.

1.4 Triple negative breast cancer

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterised by the absence of oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression (Aysola et al., 2012; Pal et al., 2011). TNBC accounts for approximately 15–20 % of all breast cancer cases and occurs more frequently in younger women, individuals of African ancestry, and carriers of *BRCA1* pathogenic (Boyle, 2012; Sporikova et al., 2018). Although the precise causes of TNBC are not fully understood, both genetic and environmental factors are known to contribute to its development (Fabbri et al., 2020; Suba, 2014). Several genetic alterations have been identified that increase the risk of TNBC, including variants in *BRCA1*, *BRCA2*, and *TP53*. More than 75 % of women with *BRCA1* related breast cancer develop the TNBC subtype (Bayraktar et al., 2011; Choi et al., 2023). Pathogenic variants in *TP53* are also associated with a higher risk of TNBC, reflecting the gene's crucial role in regulating cell growth and maintaining genomic stability (Arimura et al., 2024).

In addition to genetic predisposition, environmental exposures may influence TNBC risk. Studies have shown that contact with endocrine-disrupting compounds such as bisphenol A (BPA)**,** bisphenol P (BPP)**,** and bisphenol M (BPM)can increase TNBC risk (Liu et al., 2023; Zhang et al., 2016). Similarly, exposure to ionizing radiation, including chest radiotherapy for Hodgkin lymphoma, has been linked to an elevated likelihood of developing TNBC (Horst et al., 2014).

Clinically, TNBC presents major therapeutic challenges due to its aggressive behaviour and lack of responsiveness to conventional targeted treatments. Unlike other breast cancer subtypes, TNBC does not respond to endocrine or HER2-targeted therapies, making chemotherapy the primary systemic treatment option. Standard regimens typically include anthracycline and taxane based agents, which are administered in both adjuvant and neoadjuvant settings. In recent years, platinum-based compounds such as *carboplatin* and

cisplatin have seen increasing use, as growing evidence shows that they can enhance pathological complete response (pCR) rates**,** particularly in tumours with *BRCA1/2* alterations or homologous recombination repair deficiency (Poggio et al., 2018).

Beyond conventional chemotherapy, treatment strategies for TNBC are being reshaped by molecularly targeted and immune-based approaches. PARP inhibitors have emerged as an important therapeutic class, especially for patients carrying germline or somatic *BRCA1/2* pathogenic variants. By exploiting the defective DNA repair pathways characteristic of these tumours, PARP inhibition induces synthetic lethality, leading to selective tumour cell death while sparing normal cells (Dibitetto et al., 2024; Lord & Ashworth, 2017).

At the same time, immunotherapy has revolutionised TNBC treatment. The PD-1 inhibitor pembrolizumab, when administered with chemotherapy, has demonstrated significant clinical benefit in high-risk, early-stage TNBC. Findings from the KEYNOTE-522 trial revealed substantial improvements in both pathological complete response and event-free survival**,** resulting in its incorporation into current clinical guidelines for use in neoadjuvant and adjuvant treatment regimens (Schmid et al., 2022). These advances illustrate an ongoing shift from uniform chemotherapy-based management towards biomarker-based precision therapy aimed at improving long-term outcomes in TNBC.

In conclusion, TNBC remains an aggressive breast cancer subtype with complex genetic and environmental determinants. Pathogenic variants in *BRCA1*, *BRCA2*, and *TP53* increase susceptibility, while environmental exposures may further contribute to disease onset. Chemotherapy remains basis of treatment. However, emerging targeted and immune based therapies such as PARP inhibitors and immune checkpoint inhibitors, are showing considerable promise. Ongoing research continues to emphasise the need for robust molecular biomarkers capable to predict treatment response and identifying therapeutic vulnerabilities. By characterizing the unique gene expression and mutational landscapes of TNBC, researchers are uncovering the mechanisms underlying its aggressiveness and therapy resistance. Such insights pave the way for more refined personalised treatment strategies and improved patient outcomes (Kudelova et al., 2022).

1.5 Molecular breast cancer biomarkers

1.5.1 Mutational signature

Mutational signatures are distinctive patterns of somatic mutations that reflect the underlying DNA damage and repair processes active during the development and progression of breast cancer (BC). These signatures are identified through comprehensive genomic sequencing and bioinformatics analyses, this enables researchers to uncover molecular

mechanisms driving different BC subtypes. Each mutational signature is characterised by the type, context, and distribution of pathogenic variants across the genome, providing valuable insights into the etiological factors and biological pathways implicated in tumorigenesis (Alexandrov et al., 2013).

Different BC subtypes exhibit unique mutational signatures that correlate with their distinct biological behaviours and clinical outcomes. For instance, TNBC often display signatures associated with homologous recombination deficiency (HRD), frequently linked to allelic variants in *BRCA1* and *BRCA2* genes. These HRD-related signatures indicate affected ability to repair double-strand DNA breaks, leading to genomic instability and increased mutation rates (Nik-Zainal et al., 2016). In contrast, luminal subtypes, such as luminal A and luminal B, typically exhibit mutational signatures related to hormone-driven proliferation and specific DNA repair deficiencies, reflecting their reliance on oestrogen receptor signalling and associated regulatory mechanisms (Puppe et al., 2020).

The identification of mutational signatures has significant clinical implications, particularly in the area of precision oncology. Understanding the mutational landscape of BC subtypes facilitates the development of targeted therapies tailored to the specific genetic alterations present in a tumour. For example, TNBCs with HRD signatures are more likely to respond to PARP inhibitors, which uses the defective DNA repair pathways to induce synthetic lethality in cancer cells (Helleday, 2011). Additionally, mutational signatures can serve as prognostic biomarkers, correlating with patient survival rates and the likelihood of disease recurrence, thereby informing treatment decisions and therapeutic strategies (Alexandrov et al., 2013).

Furthermore, mutational signatures provide valuable insights into historical exposures to mutagens, such as ionizing radiation or tobacco carcinogens, contributing to the etiological understanding of BC. This information is crucial for identifying risk factors and developing preventive measures which can reduce incidence of specific BC subtypes. As research advances, the integration of mutational signature analysis with other omics data, including transcriptomics and proteomics, promises to enhance the comprehensive molecular characterisation of breast cancer, ultimately leading to improved diagnostic accuracy, personalised treatment plans, and better patient outcomes (Bailey et al., 2018).

1.5.2 Transcriptome in breast cancer research

Transcriptomics, the comprehensive study of RNA transcripts produced by the genome, has become an important area of breast cancer (BC) research, providing deep insights into gene expression patterns and how these patterns drive tumour development, progression, and

heterogeneity. While genomics focuses on static DNA alterations, transcriptomics analyses the real-time functional state of cells by examining RNA expression profiles, thereby clarifying how genetic information is translated into cellular behaviour. This approach is crucial for breast cancer, a highly heterogeneous disease with distinct molecular subtypes that require precise molecular classification for effective patient management.

Gene expression refers to the process by which genetic information is used to synthesise proteins, the building blocks of cells. RNA expression patterns are pivotal in the development and progression of breast cancer. Altered RNA expression can lead to changes in cellular behaviours such as proliferation, differentiation, and survival, driving processes like tumour growth, metastasis, and therapy resistance (Ciriello et al., 2013; Shi et al., 2024). Studies have identified dysregulated pathways in breast cancer, including those involved in cell cycle regulation, DNA repair, and cell signalling (Ortega et al., 2020). For instance, overexpression of HER2 is associated with aggressive BC subtypes, while reduced expression of the tumour suppressor gene *BRCA1* is linked to both sporadic and inherited forms of the disease (Mavaddat et al., 2012).

Transcriptomics provides a comprehensive view of the molecular pathways that characterise different BC subtypes by examining RNA profiles from tumour tissues. These include luminal A, luminal B, HER2-positive, and triple-negative breast cancer (TNBC). Each subtype displays unique clinical features and therapeutic responses, making precise molecular classification essential for effective patient management This approach, which is based on data analysis, enhances our understanding of tumour biology and plays a critical role in the discovery of biomarkers – specific genes or gene signatures that can predict patient outcomes, assess therapy responses, and guide targeted treatment strategies (Nolan et al., 2023).

Advances in transcriptomic technologies, such as RNA sequencing, have facilitated the identification of differentially expressed genes (DEGs) with exceptional accuracy, providing insights into the distinct molecular features of breast cancer. Aberrant gene expression during BC progression underscores the need for actionable molecular markers. Recent studies have highlighted several genes as potential diagnostic, prognostic, and therapeutic targets, emphasizing the importance of identifying effective gene signatures for tailoring therapies, particularly for high-risk patients. These findings contribute to the development of innovative diagnostic tools and personalised treatments, advancing precision oncology and improving patient outcomes (Rosati et al., 2024).

1.5.3 Differential gene expression

A key application of transcriptomics in cancer research, including breast cancer, is the identification of differentially expressed genes (DEGs). These DEGs exhibit significant changes in expression levels between cancerous and normal tissues or among different breast cancer subtypes. Differential gene expression analysis provides insights into the molecular mechanisms driving tumorigenesis, progression and heterogeneity, which is helpful in diagnostics, prognostics and therapeutic strategy (Tuly et al., 2023).

Differential gene expression reveals specific expression patterns associated with molecular subtypes of breast cancer and is helping to define molecular characteristics of specific breast cancer types, such as triple-negative breast cancer (TNBC) or hormone receptor-positive subtypes. Transcriptomic data also enable the classification of breast cancer into subtypes like luminal A, luminal B, HER2-positive, and TNBC, each distinguished by specific clinical features and therapeutic outcomes (Perou et al., 2000; Shan et al., 2024). For examples, luminal subtypes typically exhibit high expression of hormone receptor genes like *ESR1* (oestrogen receptor) and *PGR* (progesterone receptor), which are linked to hormone driven proliferation and better prognosis. Whereas TNBC is characterised by the absence of oestrogen, progesterone, and HER2 receptors, and interestingly they often show upregulation of genes involved in cell proliferation, metastasis and DNA repair deficiencies (Hu et al., 2024).

Altered RNA expression patterns are associated with changes in cellular behaviour, including proliferation, survival, and metastasis. For example, overexpression of the oncogene HER2 is linked to aggressive breast cancer phenotypes, while reduced expression of *BRCA1/2* has been tied to both inherited and sporadic forms of the disease. Advances in RNA sequencing technologies now allow researchers to analyse these patterns with high precision, facilitating the discovery of novel diagnostic, prognostic, and predictive biomarkers. These findings are driving the development of innovative diagnostic tools and personalised therapeutic strategies, improving patient outcomes and advancing the field of precision oncology.

1.5.4 Pathway enrichment analysis

Once the differentially expressed genes (DEGs) have been identified between two groups of samples, pathway enrichment analysis can be used to explore the biological pathways significantly associated with these genes. This computational method highlights pathways or processes that are enriched in DEGs or are closely linked to specific samples or diseases.

Pathway enrichment analysis offers multiple benefits in biomarker identification and validation. It aids in in potential biomarkers identification by pinpointing genes that are overrepresented in particular pathways. Furthermore, it helps validating the biological importance of these genes by examining their roles within the enriched pathways, followed by

analysing their interactions at the protein level. This analysis also connects gene expression changes to specific diseases, therefore providing insights into potential disease markers and uncovering new therapeutic targets (Rosati et al., 2024).

For examples, in a study by Chen et al. (Chen et al., 2021) pathway enrichment analysis was employed to explore the biological functions of differentially expressed genes (DEGs) in breast cancer. The results showed that upregulated DEGs were notably associated with pathways such as p53 signalling, progesterone-mediated oocyte maturation, protein degradation, and cellular uptake processes. In contrast, downregulated DEGs were primarily linked to pathways like AMP-activated protein kinase (AMPK), adipocytokine, and peroxisome proliferator-activated receptor (PPAR) signalling. Through protein–protein interaction (PPI) network analysis, researchers identified 12 genes associated with breast cancer prognosis. Their findings highlighted the critical roles these pathways and genes play in the progression and outcomes of the disease. The study offered valuable insights into the molecular mechanisms driving breast cancer and underscored the potential of these genes and pathways as prognostic biomarkers and therapeutic targets, leading way for future research and clinical applications.

1.5.5 Transcriptomics in Biomarker discovery

Transcriptomic studies have significantly deepened understanding of the molecular landscape of breast cancer, revealing novel biomarkers and therapeutic targets. In particular, transcriptomic profiling in TNBC has uncovered the upregulation of genes involved in cell proliferation, metastasis, and epithelial-to-mesenchymal transition (EMT) (Grasset et al., 2022). For example, expression of matrix metalloproteinase 9 (*MMP9*) is associated with absence neoadjuvant chemotherapy response in this aggressive breast cancer subtype (Błaszczak et al., 2025; Kalavska et al., 2020; Lejeune et al., 2023).

TNBC is also frequently associated with homologous recombination deficiencies (HRD), often linked to *BRCA1/2* allelic variants. This deficiency not only results in the upregulation of DNA repair genes such as *RAD51* but also renders tumours particularly vulnerable to DNA-damaging agents, including platinum-based chemotherapies and poly (ADP-ribose) polymerase (PARP) inhibitors (Llop-Guevara et al., 2021). In *BRCA1/2*-deficient tumours, the heightened activity of DNA damage response pathways creates a context for synthetic lethality, which PARP inhibitors effectively exploit (Helleday, 2011). Moreover, breast cancers harbouring *BRCA1* allelic variants often exhibit increased *PD-L1* and *PD-1* expression along with enhanced immune cell infiltration. This immunogenic profile not only supports the potential efficacy of immune checkpoint inhibitors but also provides a rationale

for integrating these agents with conventional treatments to achieve synergistic therapeutic effects (Han et al., 2023).

Apart from protein-coding genes, transcriptomics has highlighted the critical roles of non-coding RNAs as biomarkers. MicroRNAs (miRNAs) such as miR-21 are consistently upregulated in breast cancer and have been linked to poor prognosis and chemotherapy resistance (Dan et al., 2021). Similarly, long non-coding RNAs (lncRNAs) like HOTAIR contribute to metastasis and tumour progression by altering chromatin structure and gene expression (Sideris et al., 2022).

In clinical practice, comprehensive gene expression panels such as Oncotype DX and PAM50 have leveraged transcriptomic data to classify breast cancer into intrinsic subtypes and predict treatment responses. Oncotype DX, which assesses the expression of 21 genes, estimates the risk of recurrence and potential benefit from chemotherapy in early-stage, hormone receptor-positive breast cancer (Davey et al., 2022). PAM50 evaluates 50 genes to classify tumours into subtypes luminal A, luminal B, HER2-enriched, and basal-like – providing crucial prognostic information that guides personalised therapy (Kensler et al., 2019).

Furthermore, integrating transcriptomic data with other omics layers, such as genomics and proteomics, enhances the identification and validation of robust biomarkers. For instance, combining mutational signatures with gene expression profiles can elucidate how specific genetic alterations influence regulatory networks and therapeutic responses, thereby uncovering novel targets for precision oncology (Rosati et al., 2024).

These advances in biomarker discovery not only underscore the power of transcriptomic approaches in breast cancer biology, but also lay the groundwork for their application in routine clinical practice. By translating detailed molecular insights into actionable diagnostic and prognostic tools, transcriptomics would be able to transform patient management and therapy selection.

1.5.6 Transcriptomics potential in clinical practice

Comprehensive study of RNA transcripts in a cell has improved out understanding of disease biology, including breast cancer. By uncovering gene expression patterns and molecular pathways driving tumour progression, transcriptomics is a powerful tool in biomarker identification, predicting treatment responses and developing targeted therapies. Integrating transcriptomics in clinical practice has huge potential for improving precision medicine and patient outcome.

In breast cancer transcriptome analysis have established identification of intrinsic subtypes, such as luminal A, luminal B, HER2-enriched, and triple negative breast cancer. Each has distinct biological characteristics and treatment responses. Clinical practices also use

gene expression assays such as Oncotype DC and MammaPrint in order to assess recurrence risk and guide decisions on adjuvant chemotherapy in early-stage breast cancer(Andre et al., 2022).

Transcriptomics also plays role in therapeutic response prediction. Tumours with homologous recombination deficiency (HRD) are often linked with *BRCA1/2* pathogenic variants, and therefore exhibit specific transcriptomic signatures associated with sensitivity to DNA damaging agents such as platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors. Studies have shown that transcriptomics patterns reflecting BRCAness or HDR-related features can guide selection of PARP inhibitors for patients regardless of *BRCA1/2* allelic variants at the same time showing similar molecular vulnerabilities (Oshi et al., 2022).

Transcriptomics has also uncovered novel therapy targets. As an example, in TNBC via transcriptomic profiling was identified pathways like PI3K/AKT/mTOR and JAK/STAT as drivers of tumour growth and evasion of immune system, which allows to develop targeted inhibitors. Also, discovery of immune-related gene expression signatures has facilitated the use on immune checkpoint inhibitors in tumours with high immune activity, such as PD-L1 expression (Serrano García et al., 2024).

Despite the promising role of transcriptomics, its integration into routine clinical practice has many challenges, including high costs, complexity of data, the need for robust validation in diverse patient populations (Tsimberidou et al., 2022). However, technologies like next generation sequencing become more accessible and computational tools for data analysis improves over time. It is expected that transcriptomics in clinical workflow will expand offering hope for more precise and effective cancer care through personalised treatment strategies, disease progression monitoring, understanding mechanisms of resistance (Cuppen et al., 2022).

In summary, transcriptomics has rapidly evolved into a powerful tool that has deepened out understanding of the molecular mechanisms driving breast cancer and it also holds promise for transforming clinical practice. Its capacity to identify intrinsic subtypes, predict therapeutic responses, and uncover novel targets underlines its potential to guide personalised treatment strategies. Despite current challenges like high costs, data complexity and the need to future validations, the accessibility of next generation sequencing and advances in bioinformatics are steadily bridging the gap between research and routine clinical application.

Based on these insights our study aims to leverage transcriptomic profiling to elucidate the molecular landscape associated with TNBC in one part and monoallelic somatic *BRCA1* inactivation in breast cancer in second part of the study. By exploring the differential gene expression patterns and associated pathways in this specific context, we hope to uncover potential biomarkers that could improve prognosis and inform therapeutic decision-making.

2 Materials and methods

2.1 Study group

A total of 65 fresh-frozen breast cancer samples, obtained from patients diagnosed and surgically treated between 2012 and 2017, were collected from the tissue repository of the Rīga Stradiņš University Institute of Oncology (RSU IO) and the study design and workflow are shown in Figure 2.1. Ten patients were excluded from the analysis due to predefined exclusion criteria, including the presence of germline *BRCA1* mutations, unconfirmed histopathological diagnosis, and insufficient sequencing quality. Remaining 55 patients had a histologically confirmed breast cancer diagnosis and had not received neoadjuvant chemotherapy, hormone therapy, or radiotherapy prior to surgery. Clinical data including age at diagnosis, therapeutic interventions, and survival outcomes, were available through medical reports.

Following surgery, most patients received adjuvant chemotherapy, with or without hormone therapy (including trastuzumab/Herceptin) and/or radiotherapy. In some cases, patients received hormone therapy or radiotherapy alone or in combination.

The obtained cancer samples were used for two main research components of this Thesis:

1. Transcriptome profiling of TNBC – aiming to identify differentially expressed genes and alterations in signalling pathways associated with triple negative breast cancer vs non-triple-negative breast cancer (n = 19).

2. Transcriptome profiling of tumours with monoallelic somatic *BRCA1* inactivation vs tumours with both active *BRCA1* alleles, including Kaplan-Meier survival analysis to evaluate the impact of *BRCA1* status on event-free survival (n = 36).

2.2 Ethics

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Central Medical Ethics Committee (No 1/18-09-19 (19 September 2018)). Informed consent was obtained from all subjects involved in the study (Annex 1; Annex 2; Annex 3).

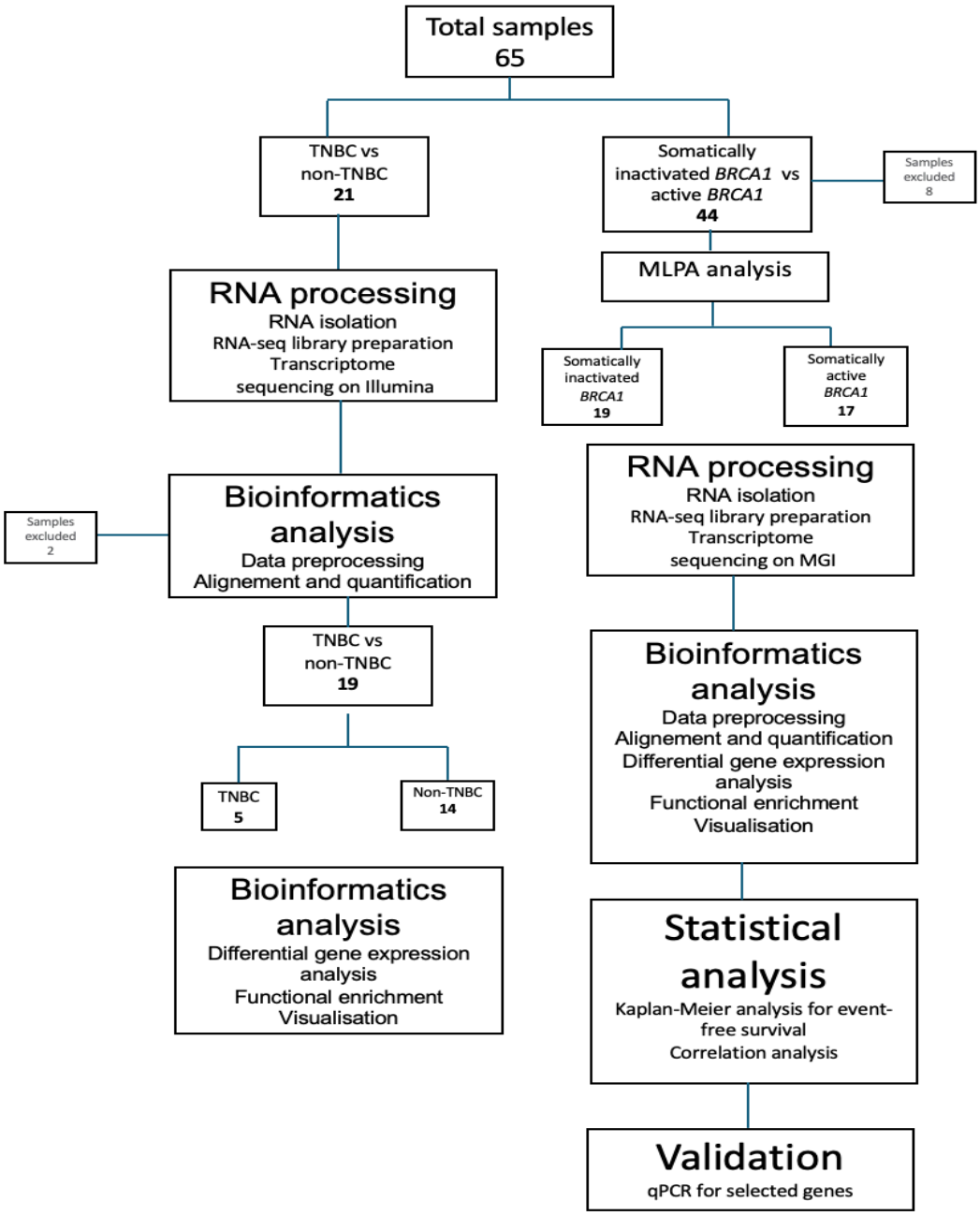

Figure 2.1 **Study design and analytical workflow**

2.3 DNA extraction and MLPA analysis

MLPA analysis was performed on 44 breast cancer samples to detect copy number variations (CNVs) and promotor region methylation in the *BRCA1* genes. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, cat no 56304), and MLPA was conducted with the ME001 Tumor Suppressor Probemix 1 (MRC Holland, cat no ME001-025R; supplementary Table 2) following the manufacturer's protocol. Amplified products were separated by capillary electrophoresis using Sanger sequencing platform ABI 3500 (Thermo Fisher Scientific, Waltham, MA, USA) and analysed with Coffalyser software (Version: v.250317.1029). Samples with *BRCA1* promoter deletion and/or methylation were considered to have monoallelic somatic *BRCA1* inactivation.

2.4 RNA sequencing

2.4.1 Isolation of RNA

The RNA isolation was performed using TRIzol reagent, cat.no 15596026 (Life Technologies, Carlsbad, CA, USA) followed by RNA purification using Direct-zol™ RNA MiniPrep kit, cat.no R2050 (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. The isolated RNA was stored at −80 °C until further analysis. The concentration of RNA was determined using Qubit 4.0 Fluorometer and Qubit RNA Assay kit, cat no Q32852 (Thermo Fisher Scientific/Life Technologies) and NanoDrop 2000. RNA quality and degradation was evaluated with Qubit RNA IQ assay kit (Thermo Fisher Scientific/Life Technologies) and Qubit 4.0 Fluorometer.

2.4.2 Library preparation and transcriptome sequencing

A total of 57 tumour samples were subjected to RNA sequencing across two experimental groups. 21 samples for subsequent triple-negative breast cancer (TNBC) and non-TNBC group analysis were sequenced on the Illumina HiScanSQ platform (later during analysis 2 samples were excluded due to low sequencing quality), while 36 samples corresponding to the somatically inactivated *BRCA1* and *BRCA1* active groups were sequenced on MGI DNBSEQ-G50 and G200 platforms.

For the Illumina workflow, cDNA libraries were prepared using the TruSeq Stranded mRNA Library Preparation Kit (Illumina, San Diego, USA). Library quality and fragment size distribution were assessed using the QIAxcel Advanced System (QIAGEN GmbH, Hilden, Germany) prior to sequencing. Libraries that met quality control criteria were hybridised on a flow cell using the TruSeq PE Cluster Kit v3-cBot-HS (Illumina, San Diego, USA), and sequencing was performed with the TruSeq SBS Kit v3-HS according to the manufacturer's instructions.

For the MGI workflow, all 36 samples were processed using MGI reagent kits. cDNA libraries were constructed using the MGIEasy RNA Directional Library Prep Kit V2.1, and all libraries were circularised using the MGIEasy Circularisation Module. Library quality and fragment size distribution were assessed using the QIAxcel Advanced System (QIAGEN GmbH, Hilden, Germany) prior to sequencing. Sequencing was performed with DNBSEQ-G50RS and DNBSEQ-G200RS platforms using the DNBSEQ-G50RS or SNBSEQ-G200RS High-Throughput Sequencing Kits (MGI, Wuhan, China), following the manufacturer's recommended protocols.

2.4.3 Bioinformatics analysis

Upon obtaining the raw reads, CLC Genomics Workbench (version 23.0.5, Qiagen) was used to assess sequencing quality (Q20 and Q30), evaluate GC content, and perform downstream transcriptomic analyses. High-quality reads were aligned to the reference genome GRCh37.p13 (hg19), gene expression levels were quantified as RPKM, and TNBC versus non-TNBC groups were defined based on transcriptome profiles. Differential expression analysis was conducted using a negative binomial generalised linear model within CLC for 19 Illumina HiScanSQ sequenced samples and 36 MGI G50/G200 sequenced samples comparing monoallelically somatically inactivated BRCA1 to BRCA1-active tumours. Genes with a Bonferroni-adjusted p-value < 0.05 were considered significantly differentially expressed and subjected to further analysis.

2.4.4 Functional and pathway enrichment analysis

GO enrichment analysis of three categories, such as biological process (BP), cellular component (CC) and molecular function (MF) was performed to understand biological functions of identified DEGs. Analysis was performed with "ToppFun" from the "ToppGene Suite" Gene Ontology tool, (Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, USA, https://toppgene.cchmc.org) $p<0.05$ was set as the threshold value.

2.4.5 Construction of PPI network and the identification of hub genes

The online tool STRING (Search Tool for the Retrieval of Interacting Genes, https://string-db.org) (Szklarczyk et al., 2019) was utilised to develop a protein–protein interaction (PPI) network of selected differentially expressed genes (DEGs) with the aim of analysing their possible role in triple-negative breast cancer (TNBC) pathogenesis and in cancer with *BRCA1* inactivation. Interactions were filtered using a minimum required confidence score of 0.4 (medium confidence), and edges were restricted to evidence derived from experimental data, curated databases, and co-expression. The STRING database provides information about predicted and experimental interactions of proteins, and only interactions with a combined score greater than 0.04 were considered significant and used to construct a network model, which was visualised using Cytoscape (version 3.8.2). Subsequently, significant functional modules were screened using the MCODE plug-in (Gary et al 2003) for Cytoscape, and hub genes were identified using Cytohubba (Chin et al 2014). The MCC and DMNC ranking methods were applied and their outputs compared.

2.4.6 Target transcript validation by qPCR

For the technical validation of target transcripts from the same cancer samples, complementary DNA (cDNA) was synthesised using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™, Cat. No 4368814, Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. Quantitative real-time PCR (qPCR) was subsequently performed with TaqMan™ Gene Expression Assays (FAM-labeled, Cat. No 4331182, Thermo Fisher Scientific) together with the TaqMan™ Fast Advanced Master Mix (Cat. No 4444557, Thermo Fisher Scientific). Amplification and detection were carried out on the Applied Biosystems™ ViiA™ 7 Real-Time PCR System. Expression levels were normalised to the geometric mean of two reference genes, RPLP1 and RPL13A, and further analysed with Python (version 3.10.7) using scipy.stats, NumPy and Pandas libraries.

2.4.7 Survival analysis

Survival analysis was performed using the Kaplan-Meier method to evaluate differences between study groups. Event-free survival was defined as the time from the date of diagnosis to the date of documented disease recurrence. Patients who had not experienced an event at the time of the last follow-up were censored at that point. Differences between groups were assessed using the log-rank test. Statistical analyses were conducted in R (v4.4.2) using the 'survival' package for Kaplan-Meier analysis, with log-rank tests evaluated by the Peto and Peto method.

2.4.8 Statistical analysis of clinical variables

P-values were calculated using two-sided statistical tests selected according to variable type and data distribution. Continuous variables were compared between groups using the Mann–Whitney U test due to small sample sizes and non-normal distributions, while categorical variables were analysed using Fisher's exact test because of small expected counts.

3 Results

The study of this Thesis consists of two parts. The first part is focused on transcriptome analysis with the aim to reveal differentially expressed genes (DEGs) between triple negative breast cancer (TNBC) group and the non-TNBC group. The second part examined DEGs in patient samples with somatic monoallelic *BRCA1* inactivation compared to those with both active *BRCA1* alleles.

3.1 TNBC vs non-TNBC

3.1.1 Clinical and pathological characteristics of the study groups

Table 3.1 summarises the clinical and pathological characteristics of the TNBC study cohort (n = 19), stratified into TNBC (n = 5) and non-TNBC (n = 14) groups based on acquired RNA-seq data. The median age was lower in the TNBC group (52 years) compared with the non-TNBC group (65.5 years), although this difference did not reach statistical significance. No significant differences were detected between the groups with respect to tumour (T) stage, nodal (N) status, or overall clinical stage. Most tumours in both groups were classified as T2 and clinically staged as I or II. Molecular subtype distribution reflected the expected biology: luminal and HER2-positive tumours were observed exclusively in the non-TNBC group, whereas the TNBC group consisted solely of triple-negative cases. Adjuvant treatment patterns, including chemotherapy, endocrine therapy, and radiotherapy, were comparable between groups. Trastuzumab therapy was administered only in HER2-positive non-TNBC cases, as expected. Although not statistically significant, overall survival tended to be lower in the TNBC subgroup at the last follow-up in 2025.

Table 3.1

Clinical characteristics of TNBC and non-TNBC groups

Characteristics	**Total (n = 19)**	**TNBC (n = 5)**	**Non-TNBC (n = 14)**	***p*-value**
Age, median (range)	63.0 (34–85)	52.0 (39–71)	65.5 (34–85)	0.19
T stage	n (%)	n (%)	n (%)	1.00
T1	7 (36.8)	2 (40.0)	5 (35.7)	
T2	10 (52.6)	3 (60.0)	7 (50.0)	
T3	2 (10.5)	0 (0.0)	2 (14.3)	
N stage	n (%)	n (%)	n (%)	0.84
N0	10 (52.6)	2 (40.0)	8 (57.1)	
N1	4 (21.1)	2 (40.0)	2 (14.3)	
N2	1 (5.3)	0 (0.0)	1 (7.1)	
N3	4 (21.1)	1 (20.0)	3 (21.4)	

Table 3.1 continued

Characteristics	Total (n = 19)	TNBC (n = 5)	Non-TNBC (n = 14)	*p*-value
Clinical stage	n (%)	n (%)	n (%)	0.92
IA	6 (31.6)	1 (20.0)	5 (35.7)	
IIA	4 (21.1)	2 (40.0)	2 (14.3)	
IIB	4 (21.1)	1 (20.0)	3 (21.4)	
IIIA	1 (5.3)	0 (0.0)	1 (7.1)	
IIIC	4 (21.1)	1 (20.0)	3 (21.4)	
Molecular subtype*	n (%)	n (%)	n (%)	0.07
Luminal	10 (52.6)	2 (40.0)	8 (57.1)	
HER2-positive	7 (36.8)	1 (20.0)	6 (42.9)	
TNBC	2 (10.5)	2 (40.0)	0 (0.0)	
Adjuvant treatment	n (%)	n (%)	n (%)	
Chemotherapy	10 (52.6)	2 (40.0)	8 (57.1)	0.63
Endocrine therapy	7 (36.8)	2 (40.0)	5 (35.7)	1.00
Radiotherapy	18 (94.7)	4 (80.0)	14 (100.0)	0.26
Trastuzumab	4 (21.1)	1 (20.0)	3 (21.4)	1.00
Overall survival (status at last follow-up 2025)	n (%)	n (%)	n (%)	0.06
Alive	10 (52.6)	1 (20.0)	9 (64.3)	
Decease	9 (47.4)	4 (80.0)	5 (35.7)	

* Based on pathology reports (from patient medical history data).

3.1.2 Transcriptome analysis

The study included sequencing of 19 samples, yielding a median Q30 quality score of 85.4 % and an average read count of 40 million per sample. The TNBC samples were sequenced at approximately 300× depth. Based on global transcriptome analysis, samples were classified into two distinct groups: the triple-negative breast cancer (TNBC) group, which consisted of 5 samples with low or not identified transcripts of ER, PR, and HER2 genes, and the non-TNBC group, comprising the remaining 14 samples. The sequencing results identified a total of 53,854 (DEGs). After applying Bonferroni correction ($p < 0.05$), 229 DEGs were statistically significant, including 124 genes that were downregulated and 105 genes that were upregulated in the TNBC group.

3.1.3 Gene ontology (GO) enrichment analysis

Gene Ontology enrichment analysis of the 42 upregulated genes revealed significant associations across molecular function, biological process, and cellular component categories (Table 3.2). In the biological process group, 13 genes including *GDF*, *MYF5*, *COL9A1*, *COL11A2*, *COL19A1*, *DLK1*, *EXTL1*, *ZIC1*, *ALX1*, *NCAN*, *DLX6*, *MMP9* and *EN1* were

enriched in skeletal system development ($p = 2.06 \times 10^{-7}$). A set of 10 genes (*COL9A1, MYF5, COL11A2, COL19A1, COL5A3, NCAN, LOXL4, LOXL3, MMP9, MIA*) showed strong enrichment for extracellular matrix and collagen organisation ($p = 4 \times 10^{-6}$), while *CD163, LOXL4, LOXL3,* and *MARCO* were implicated in scavenger receptor and cargo receptor activity ($p < 1 \times 10^{-4}$). At the molecular function level, enrichment was observed for extracellular matrix structural constituents conferring tensile strength and protein-lysine oxidase activity, driven largely by collagen and *LOXL* family members. Within the cellular component category, the most significant terms included collagen trimer (*COL11A2, COL19A1, COL5A3, C1QL2, C1QL4, FCN1, MARCO*; $p = 1.23 \times 10^{-9}$) and collagen-containing extracellular matrix ($p = 1.64 \times 10^{-5}$).

Table 3.2

Biological annotation of upregulated genes in TNBC versus non-TNBC groups

Genes from input		**GO: Molecular function**		***p*-value**
4/42	*COL9A1, COL11A2, COL19A1, COL5A3*	GO:0030020	extracellular matrix structural constituent conferring tensile strength	2.66×10^{-5}
4/52	*CD163, LOXL4, LOXL3, MARCO*	GO:0005044	scavenger receptor activity	6.23×10^{-5}
2/5	*LOXL4, LOXL3*	GO:0004720	protein-lysine 6-oxidase activity	1.66×10^{-5}
4/82	*CD163, LOXL4, LOXL3, MARCO*	GO:0038024	cargo receptor activity	3.66×10^{-5}
2/11	*L1CAM, GFRA3*	GO:0008046	axon guidance receptor activity	9.00×10^{-5}
Genes from input		**GO: Biological process**		***p*-value**
13/583	*GDF , MYF5, COL9A1, COL11A2, COL19A1, DLK1, EXTL1, ZIC1, ALX1, NCAN, DLX6, MMP9, EN1*	GO:0001501	skeletal system development	2.06×10^{-7}
10/431	*COL9A1, MYF5, COL11A2, COL19A1, COL5A3, NCAN, LOXL4, LOXL3, MMP9, MIA*	GO:0030198	extracellular matrix organisation	4.21×10^{-6}
10/432	*COL9A1, MYF5, COL11A2, COL19A1, COL5A3, NCAN, LOXL4, LOXL3, MMP9, MIA*	GO:0043062	extracellular structure organisation	4.30×10^{-6}
10/434	*COL9A1, MYF5, COL11A2, COL19A1, COL5A3, NCAN, LOXL4, LOXL3, MMP9, MIA*	GO:0045229	external encapsulating structure organisation	4.48×10^{-6}
4/63	*COL11A2, COL5A3, LOXL4, LOXL3*	GO:0030199	collagen fibril organisation	8.82×10^{-6}
6/198	*MYF5, MYF6, COL19A1, POPDC2, SOX8, CHRNA1*	GO:0007519	skeletal muscle tissue development	9.41×10^{-5}
6/211	*MYF5, MYF6, COL19A1, POPDC2, SOX8, CHRNA1*	GO:0060538	skeletal muscle organ development	1.33×10^{-4}
2/5	*LOXL4, LOXL3*	GO:0018057	peptidyl-lysine oxidation	1.35×10^{-4}

Table 3.2 continued

Genes from input		GO: Cellular components		*p*-value
8/87	*COL11A2, COL19A1, C1QL4, COL5A3, C1QL2, FCN1, MARCO*	GO:0005581	collagen trimer	1.23×10^{-9}
10/498	*COL9A1, COL11A2, COL19A1, L1CAM, COL5A3, TMEFF2, FCN1, NCAN, CSPG4, MMP9*	GO:0062023	collagen-containing extracellular matrix	1.64×10^{-5}
Genes from input		**GO: Cellular components**		***p*-value**
10/633	*COL9A1, COL11A2, COL19A1, L1CAM, COL5A3, TMEFF2, FCN1, NCAN, CSPG4, MMP9*	GO:0031012	extracellular matrix	1.23×10^{-4}
10/635	*COL9A1, COL11A2, COL19A1, L1CAM, COL5A3, TMEFF2, FCN1, NCAN, CSPG4, MMP9*	GO:0030312	external encapsulating structure	1.26×10^{-4}
12/1053	*CRLF1, L1CAM, MUC16, DLK1, CHRNA1, CD163, GFRA3, FCN1, CSPG4, LY6K, DCBLD2, MSLN*	GO:0009986	cell surface	5.19×10^{-4}
4/106	*MUC16, DEFA3, NCAN, CSPG4*	GO:0005796	Golgi lumen	6.79×10^{-4}
2/12	*COL11A2, COL5A3*	GO:0005583	fibrillar collagen trimer	8.94×10^{-4}
2/12	*COL11A2, COL5A3*	GO:0098643	banded collagen fibril	8.94×10^{-4}
6/323	*PCSK2, COL9A1, COL11A2, COL19A1, COL5A3, MSLN*	GO:0005788	endoplasmic reticulum lumen	1.34×10^{-4}
6/345	*SOX10, NPTX2, MUC16, CDH19, GFRA3, FCN1*	GO:0019898	extrinsic component of membrane	1.87×10^{-3}
7/480	*CRLF1, L1CAM, MUC16, DLK1, CD163, GFRA3, FCN1*	GO:0009897	external side of plasma membrane	2.14×10^{-3}
2/21	*COL11A2, COL5A3*	GO:0098644	complex of collagen trimers	2.78×10^{-3}
2/22	*MPO, SCGB3A2*	GO:0071682	endocytic vesicle lumen	3.05×10^{-3}

In contrast, the 11 downregulated genes (*ABCC8, KCNJ11, SHROOM3, RAB17, PDZK1, ABCC11, SHROOM1, P2RY2, RAB27B, GP2, TJP3*) showed enrichment only within cellular component categories, including inward-rectifying potassium channels and the apical plasma membrane (Table 3.3)

Table 3.3

Biological annotation of down-regulated genes in TNBC versus non-TNBC groups

Genes from input		GO: cellular components		*p*-value
2/4	*ABCC8, KCNJ11*	GO:0008282	inward rectifying potassium channel	1.41×10^{-4}
9/414	*SHROOM3, RAB17, PDZK1, ABCC11, SHROOM1, P2RY2, RAB27B, GP2, TJP3*	GO:0016324	apical plasma membrane	1.94×10^{-4}

3.1.4 PPI network analysis and hub gene detection

To examine the interaction among proteins encoded by the differentially expressed genes (DEGs), a protein–protein interaction (PPI) network was created using data from the STRING database. This network consisted of 188 nodes and 182 edges (Figure 3.1). Following this, clustering analysis identified three distinct functional modules within the network. Figure 3.1 marks the positions of these modules within the full network, while the extracted module subnetworks are shown in Figures 3.2–3.4. Module 1 (Figure 3.2) is enriched in collagen and basement-membrane components, forming a tight extracellular matrix interaction cluster consistent with structural remodelling. Its connectivity suggests coordinated regulation of matrix organisation that can facilitate changes in tissue architecture linked to invasion. Module 2 (Figure 3.3) represents a canonical luminal/oestrogen receptor regulatory circuit, with *ESR1* linked to key cooperating transcription factors *GATA3* and *FOXA1* and downstream luminal markers (*TFF1*/*TFF3*, *SLC39A6*). Its presence within the network points to an ER/luminal-like program captured in the data, suggesting biological heterogeneity within the analysed samples. Module 3 (Figure 3.4) contains two connected subgroups: a cluster of tumour-associated surface proteins (*MSLN*, *LY6K*, *GP2*, *LYPD6B*) and a cluster cantered on *MMP9* that includes *MPO*, *HMOX1*, and *CD163*. The two subgroups are linked through *MSLN*, forming a single module with a denser interaction core around *MMP9* and *MSLN*.

Hub genes were then selected leading to the identification of eight key hub genes: *FOXA1, ESR1, TFF1, GATA3, TFF3, AR, SLC39A6,* and *COL9A1* (Figure 3.5). Notably, seven of these hub genes (*FOXA1, ESR1, TFF1, GATA3, TFF3, AR, SLC39A6*) showed reduced expression in the TNBC group when compared to the non-TNBC group, while *COL9A1* was the only gene with increased expression in the TNBC group. The genes were ranked according to their importance within the network: *ESR1* was the highest ranked, followed by *FOXA1* and *GATA3* in shared second position, *TFF1* and *TFF3* in third, *SLC39A6* in fourth, and both *COL9A1* and *AR* in fifth place.

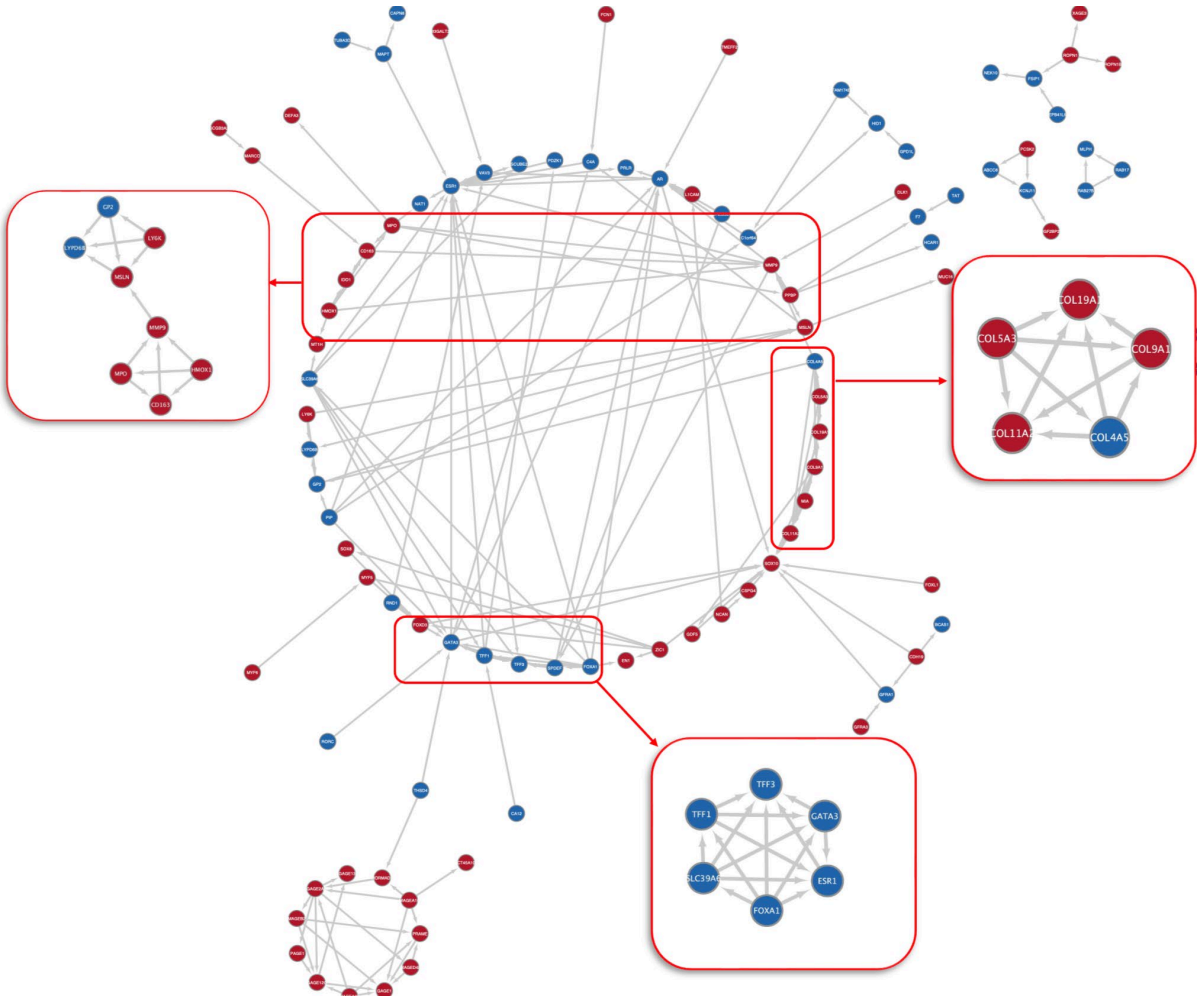

Figure 3.1 **Protein–protein interaction (PPI) network constructed from differentially expressed genes (DEGs). Nodes represent proteins encoded by DEGs. Red nodes indicate upregulated and blue nodes downregulated expression in TNBC group**

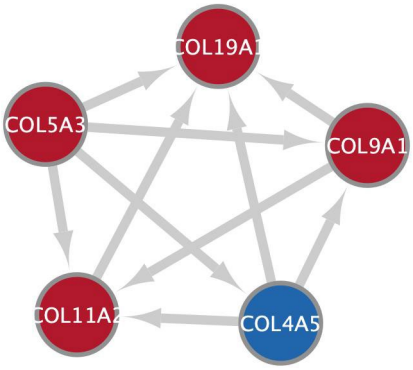

Figure 3.2 **Significant module-1 selected from PPI network including genes *COL5A3, COL19A1, COL9A1, COL5A5, COL11A2***

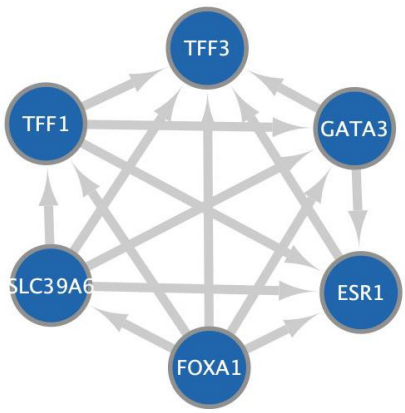

Figure 3.3 **Significant module-2 selected from PPI network including genes *TFF1, TFF3, GATA3, FOXA3, ESR1, SLC39A6***

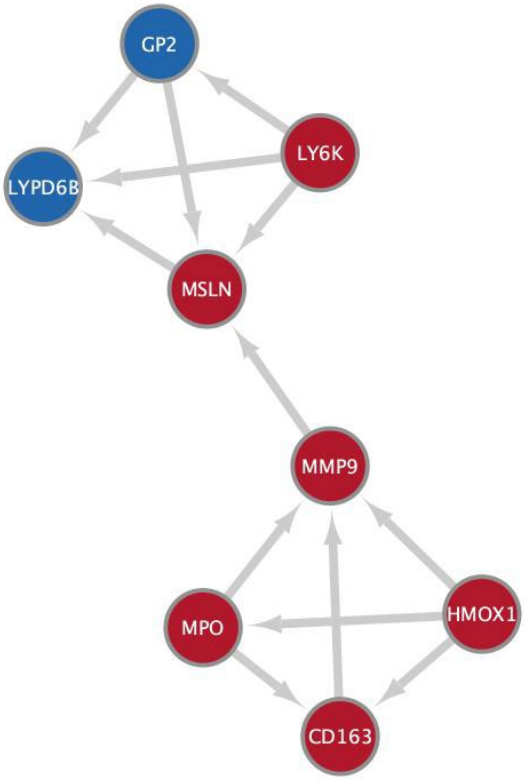

Figure 3.4 **Significant module-3 selected from PPI network including genes GP*2, LY6K, MSLN, LYPD6B, MMP9, HMOX1, CD163, MPO***

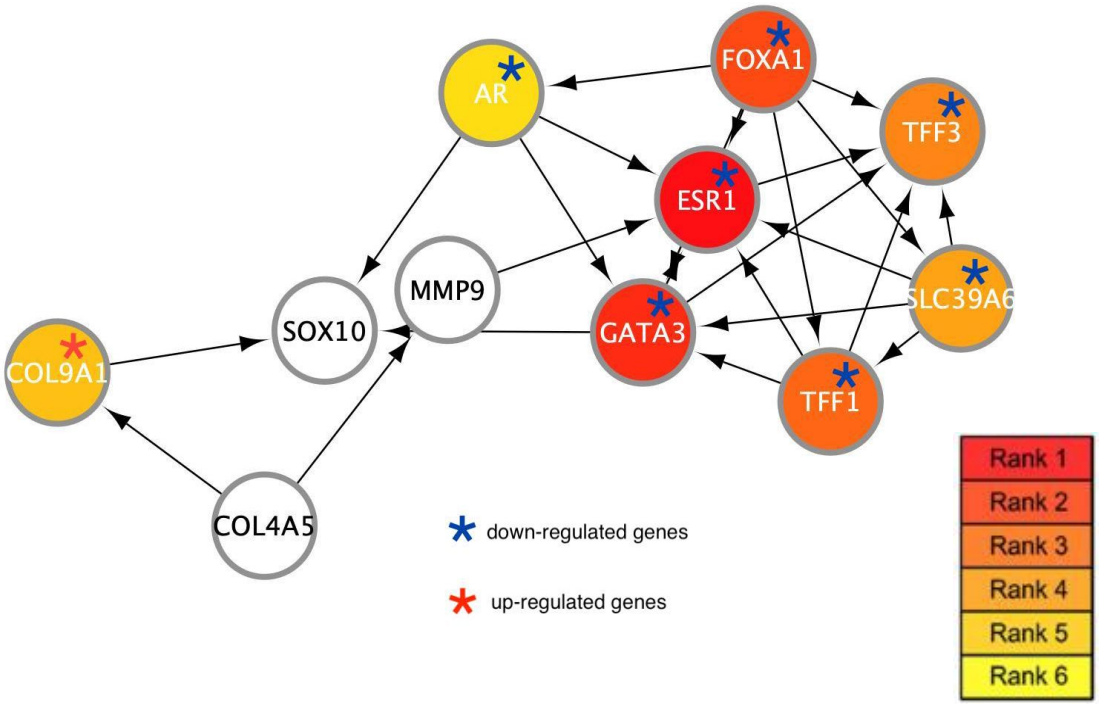

Figure 3.5 **Subnetwork of 8 hub genes (*ESR1*, *FOXA1*, *GATA3*, *TFF1*, *TFF3*, *SLC39A6*, *AR*, *COL9A1*)**

3.2 *BRCA1* monoallelic somatic inactivation molecular profile

3.2.1 Clinical and pathological characteristics of the study groups

A cohort of 36 tumour samples was classified into two study groups based on *BRCA1* status, which was determined using MLPA analysis: those with monoallelic somatic inactivation and those without. Among these, 16 samples exhibited a monoallelic deletion within the *BRCA1* promoter region, while one sample demonstrated promoter hypermethylation. The remaining 19 samples showed no evidence of deletion or methylation in the *BRCA1* promoter region (Supplementary Table 1). Accordingly, the cohort was divided into two groups: *BRCA1*−, representing cases with monoallelic somatic inactivation, and *BRCA1*+, representing tumours with two active *BRCA1* alleles.

Table 3.4 summarises the clinical and pathological characteristics of the study cohort (n = 36), stratified into *BRCA1*− (n = 17) and *BRCA1*+ (n = 19) groups. The median age was slightly higher in the *BRCA1*+ group (68 years) compared with the *BRCA1*− group (58 years). No statistically significant differences were observed between groups regarding tumour (T) or nodal (N) stage, clinical stage, histological grade, Ki-67 proliferation index, or molecular subtype. Most tumours were classified as T2 stage, grade 2, and luminal A or luminal B molecular type. With respect to treatment, both groups received chemotherapy, endocrine therapy, and radiotherapy at comparable frequencies, while trastuzumab was administered only in the *BRCA1*− subgroup.

Table 3.4

Clinical characteristics of *BRCA1*− and *BRCA1*+ groups

Characteristics	Study population, n = 36	*BRCA1*− group (n = 17)	*BRCA1*+ group (n = 19)	*p*-value
Age, median (range)	59.5 (37–81)	58 (38–81)	68 (37–76)	
BMI, kg/m^2, median (range)	28.9 (20.5–44.6)	27.1 (21.9–43.0)	29.0 (20.5–44.6)	0.78
T stage, n(%)	**n (%)**	**n (%)**	**n (%)**	0.16
T_{is}	1 (2.78)	0 (0.00)	1 (5.26)	
T1	10 (27.78)	4 (23.53)	6 (31.58)	
T2	21 (58.33)	13 (76.47)	8 (42.11)	
T3	3 (8.33)	0 (0.00)	3 (15.79)	
T4	1 (2.78)	0 (0.00)	1 (5.26)	
N stage, n(%)	**n (%)**	**n (%)**	**n (%)**	0.24
N0	26 (72.22)	13 (76.47)	13 (68.42)	
Nmic	2 (5.56)	2 (11.76)	0 (0.00)	
N1	7 (19.44)	2 (11.76)	5 (26.32)	
N3	1 (2.78)	0 (0.00)	1 (5.26)	

Table 3.4 continued

Characteristics	Study population, n = 36	*BRCA1*− group (n = 17)	*BRCA1*+ group (n = 19)	*p*-value
Clinical stage, n(%)	**n (%)**	**n (%)**	**n (%)**	
0	1 (2.78)	0 (0.00)	1 (5.26)	
IA	9 (25.00)	3 (17.65)	6 (31.58)	
IB	1 (2.78)	1 (5.88)	0 (0.00)	
IIA	14 (38.99)	10 (58.82)	4 (21.05)	0.25
IIB	8 (22.22)	3 (17.65)	5 (26.32)	
IIIA	1 (2.78)	0 (0.00)	1 (5.26)	
IIIB	1 (2.78)	0 (0.00)	1 (5.26)	
IIIC	1 (2.78)	0 (0.00)	1 (5.26)	
Grade	**n (%)**	**n (%)**	**n (%)**	
G1	5 (13.89)	3 (17.65)	2 (10.53)	
G2	22 (61.11)	10 (58.82)	12 (63.16)	0.74
G3	6 (16.67)	2 (11.76)	4 (21.05)	
not known	3 (8.33)	2 (11.763)	1 (5.26)	
Ki67 index:	*n* (%)	*n* (%)	*n* (%)	0.34
< 20%	15 (41.67)	5 (29.41)	10 (52.63)	
> 20%	15 (41.67)	9 (52.94)	6 (31.58)	
Unknown	6 (16.67)	3 (17.65)	3 (15.79)	
Molecular subtype:	**n (%)**	**n (%)**	**n (%)**	0.28
Luminal A	10 (27.78)	3 (17.65)	7 (36.84)	
Luminal B	13 (36.11)	6 (35.29)	7 (36.84)	
TNBC	7 (19.44)	4 (23.53)	3 (15.79)	
HER2 positive	3 (8.33)	3 (17.65)	0 (0.00)	
Unknown	3 (8.33)	1 (5.88)	2 (10.53)	
Adjuvant treatment:	**n (0%)**	**n (0%)**	**n (0%)**	
Chemotherapy	14 (38.89)	7 (41.18)	7 (36.84)	1
Endocrine therapy (Tamoxifen or AI)	13 (36.11)	6 (35.29)	7 (36.84)	1
Radiotherapy	26 (72.22)	11 (64.71)	15 78.95)	0.69
Trastuzumab	2 (5.56)	2 (11.76)	0 (0.00)	0.76

3.2.2 Differentially expressed genes

Analysis of RNA sequencing (RNA-seq) data identified 39 differentially expressed genes (DEGs) between the study groups, using Bonferroni-adjusted $p < 0.05$ and a maximum group mean > 10 as selection criteria. Of these, 23 genes were upregulated (Table 3.5) and 16 were downregulated (Table 3.6) in the *BRCA1*− group. It's noteworthy that, except for four transcripts, all identified DEGs were protein-coding genes. The remaining four corresponded to lncRNAs or rRNAs (not included in the Tables).

Table 3.5

The list of upregulated DEGs in *BRCA1−* group

Gene	Log_2 fold change	Fold change	*p*-value	HGNC
NRIP3	2.37	5.17	1.81×10^{-08}	HGNC:1167
TUBGCP3	2.42	5.37	7.66×10^{-08}	HGNC:18598
GPX2	2.72	6.60	6.25×10^{-08}	HGNC:4554
PXDNL	2.84	7.15	3.21×10^{-07}	HGNC:26359
FSIP1	3.06	8.32	5.60×10^{-09}	HGNC:21674
IL20	3.35	10.22	1.31×10^{-07}	HGNC:6002
MMP9	3.75	13.46	6.45×10^{-08}	HGNC:7176
TPSD1	3.81	14.06	3.600×10^{-07}	HGNC:14118
TPSAB1	3.85	14.43	1.45×10^{-08}	HGNC:12019
TRH	4.36	20.56	2.56×10^{-10}	HGNC:12298
AKR1B10	4.37	20.75	5.45×10^{-07}	HGNC:382
ORM1	4.52	22.86	4.80×10^{-07}	HGNC:8498
CGA	4.75	26.90	1.18×10^{-06}	HGNC:1885
BEX1	4.87	29.27	5.66×10^{-10}	HGNC:1036
TBX10	5.72	52.82	9.21×10^{-10}	HGNC:11593
FGG	5.81	56.16	5.42×10^{-08}	HGNC:3694
CASP14	6.27	77.34	4.16×10^{-10}	HGNC:1502
CRISP3	7.06	133.61	1.41×10^{-16}	HGNC:16904
CSN3	7.10	137.58	1.92×10^{-10}	HGNC:2446
HTN1	9.80	891.01	1.74×10^{-12}	HGNC:5283
ALPI	10.61	1566.05	4.67×10^{-16}	HGNC:437

Table 3.6

List of downregulated DEGs in *BRCA1−* group

Gene	Log_2 fold change	Fold change	*p*-value	HGNC
CARTPT	−9.88	−944.90	4.29×10^{-20}	HGNC:24323
SBSN	−8.98	−504.29	2.71×10^{-19}	HGNC:24950
IRS4	−8.00	−256.83	1.06×10^{-16}	HGNC:6128
CHGB	−7.93	−243.21	2.68×10^{-17}	HGNC:1930
CYP2A7	−7.44	−173.43	4.19×10^{-22}	HGNC:2611
KRT6A	−5.37	−41.46	4.43×10^{-10}	HGNC:6443
DCD	−5.27	−38.53	4.59×10^{-09}	HGNC:14669
OBP2B	−4.04	−16.48	1.14×10^{-10}	HGNC:23381
KRT86	−3.62	−12.30	7.03×10^{-09}	HGNC:6463
DMKN	−3.30	−9.83	1.16×10^{-08}	HGNC:25063
FABP4	−3.09	−8.49	2.50×10^{-07}	HGNC:3559
IFITM10	−2.23	−4.68	3.22×10^{-08}	HGNC:40022
AOC3	−2.03	−4.08	3.70×10^{-07}	HGNC:550
ITGA7	−1.85	−3.61	1.25×10^{-06}	HGNC:6143

The volcano plot (Figure 3.6) displays the overall distribution of gene expression alterations, represented by log2 fold change. Among the most strongly upregulated transcripts were *TRH*, *MMP9*, *TPSD1*, and *CGA*, while *CARTPT*, *CHGB*, and *IRS4* showed notable downregulation.

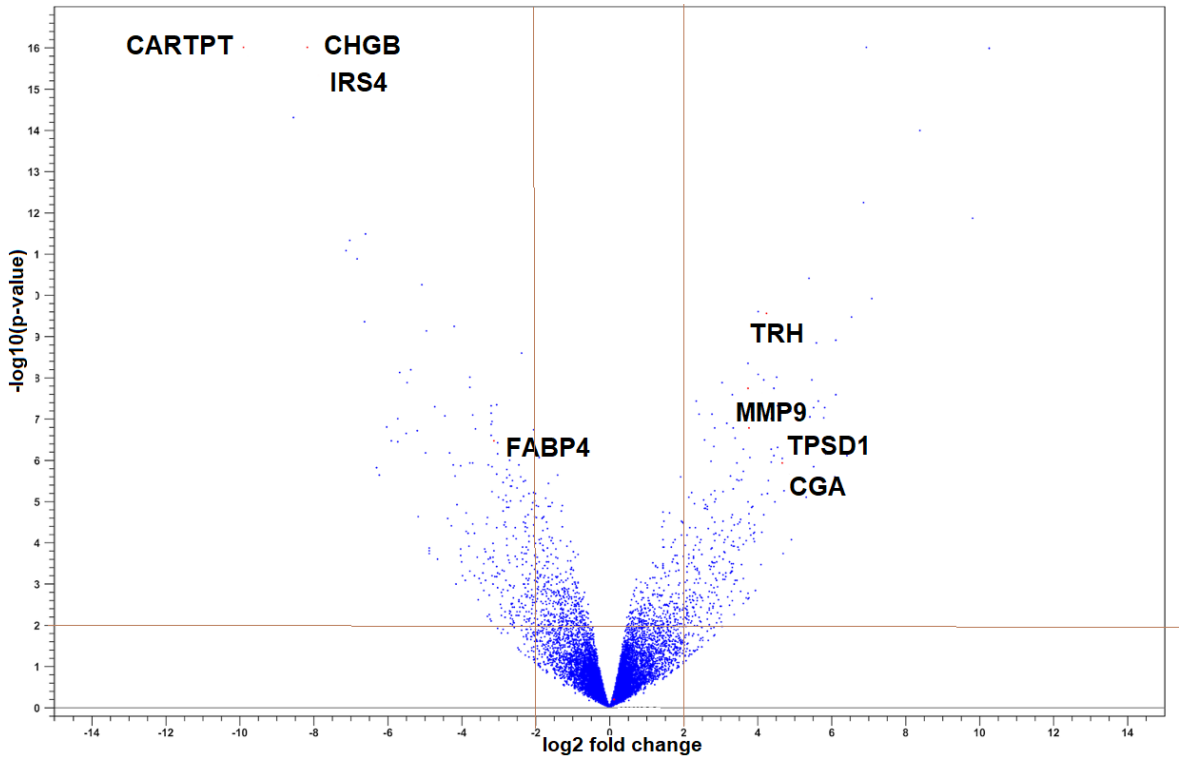

Figure 3.6 **Volcano plot of differentially expressed genes between *BRCA1*− and *BRCA*+ groups**

To investigate the functional connections among proteins encoded by the identified DEGs, we employed the STRING tool (Szklarczyk et al., 2019). Through this analysis, we gained valuable insights into the molecular pathways and processes possibly linked to monoallelic somatic inactivation of the *BRCA1* gene in breast cancer, as shown in Figure 3.7. Following hub gene analysis identified *MMP9* and *GPX2* as central nodes with the network.

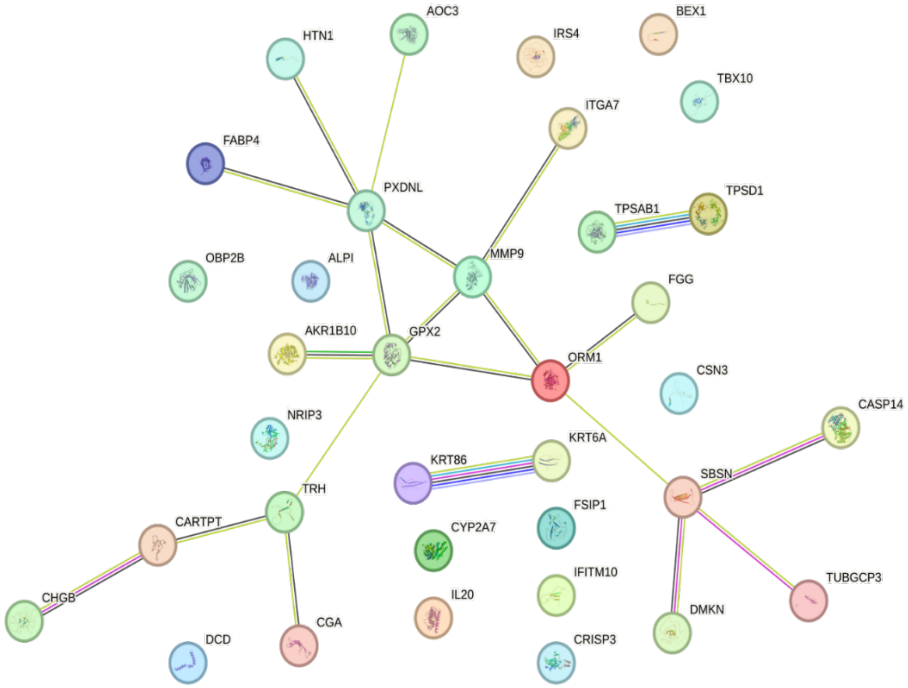

Figure 3.7 **Protein-protein interaction (PPI) network of DEGs in the *BRCA1* monoallelic inactivation group**

Gene Ontology (GO) cellular component analysis of the differentially expressed genes (DEGs) showed enrichment in the extracellular region and extracellular space (Figure 3.8). These terms exhibited the largest number of associated genes and the lowest false discovery rates (FDR), suggesting a strong overrepresentation of extracellular protein-encoding genes.

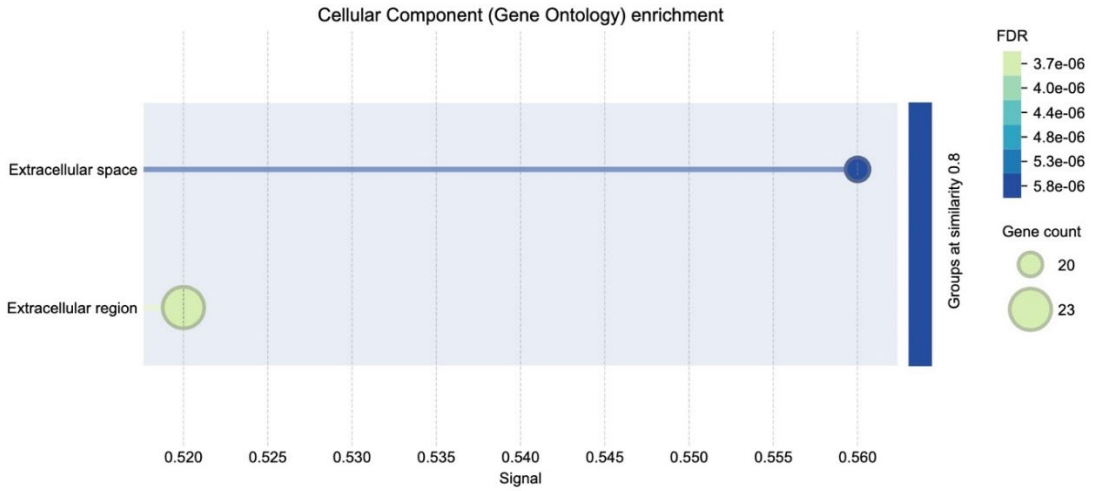

Figure 3.8 **Functional Enrichment of Genes in Extracellular Space and Extracellular Region (GO Analysis)**

Analysis of subcellular localisation with the COMPARTMENTS database revealed that most DEGs were associated with the extracellular space (Figure 3.9). This alignment with the GO results highlights the significance of extracellular functions in the group with inactivated *BRCA1* gene.

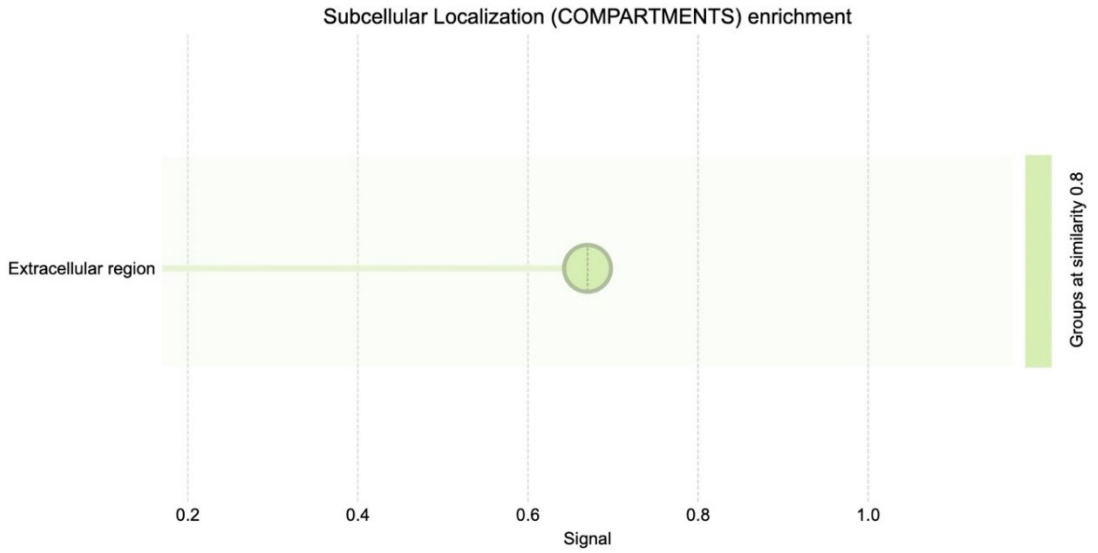

Figure 3.9 **Subcellular localisation enrichment of differentially expressed genes (DEGs) in *BRCA1* monoalelically inactive group using COMPARTMENTS database**

Principal component analysis (PCA) was carried out to evaluate overall gene expression profiles in the two groups. The results did not reveal a distinct separation, indicating that transcriptomic differences are likely modest and not well represented by the main principal components (Figure 3.10).

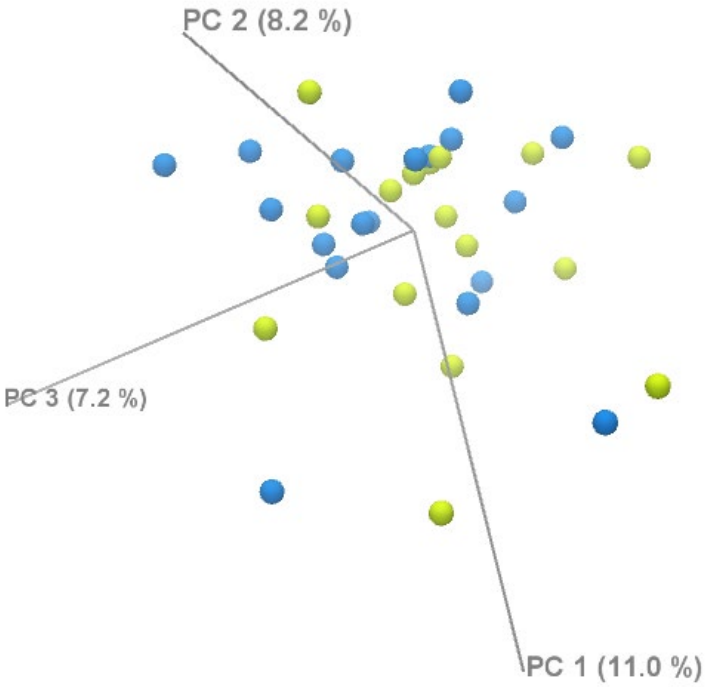

Figure 3.10 **PCA analysis of DEGs**

Blue dots represent samples without *BRCA1* inactivation;
green dots represent samples with *BRCA1* inactivation.

The enrichment analysis conducted using the STRING tool revealed significant enrichment of proteins within the extracellular region (GO:0005576) and extracellular space (GO:0005615)

(Table 3.7). Notable genes associated with these categories include *TPSD1, FABP4, ORM1, ALPI, CARTPT, TRH, CSN3,* and *MMP9,* among others. Additionally, the identification of thyroid gland disease as an enriched category suggests potential connections between breast cancer and thyroid dysfunction, with enriched genes such as *TRH, IRS4, CHGB,* and *CGA* possibly contributing to the interaction between breast cancer and thyroid-related processes.

Table 3.7

Biological annotation of upregulated genes in *BRCA1-* group

#Category	Term ID	Term description	Genes from input	Strength	False discovery rate	Matching proteins in network (labels)
GO Component	GO:0005576	Extracellular region	23/4175	0.49	3.74×10^{-05}	TPSD1, FABP4, ORM1, ALPI, CARTPT, TRH, CSN3, FGG, DMKN, TPSAB1, PXDNL, AKR1B10, IL20, MMP9, CHGB, KRT6A, CRISP3, DCD, HTN1, SBSN, KRT86, CGA, OBP2B
GO Component	GO:0005615	Extracellular space	20/3247	0.54	5.90×10^{-05}	TPSD1, FABP4, ORM1, CARTPT, CSN3, FGG, DMKN, TPSAB1, PXDNL, IL20, MMP9, CHGB, KRT6A, CRISP3, DCD, HTN1, SBSN, KRT86, CGA, OBP2B

Table 3.7 continued

#Category	Term ID	Term description	Genes from input	Strength	False discovery rate	Matching proteins in network (labels)
DISEASES	DOID:50	Thyroid gland disease	4/50	1.65	0.0113	TRH, IRS4, CHGB, CGA
COMPARTMENTS	GOCC:0005576	Extracellular region	17/2079	0.66	4.18×10^{-05}	FABP4, ORM1, ALPI, CARTPT, TRH, CSN3, FGG, TPSAB1, PXDNL, AKR1B10, IL20, MMP9, CHGB, CRISP3, DCD, HTN1, CGA

Hub gene analysis performed with the MCC and DMNC algorithms (Chin et al., 2014) identified *MMP9* and *GPX2* as central nodes in the interaction network. Notably, *GPX2* ranked highest in both approaches, while *MMP9* shared the top position with *GPX2* in the DMNC method and was placed second by MCC.

To confirm the RNA-seq results, quantitative PCR (qPCR) was conducted on a selected panel of genes (*FABP4*, *CARTPT*, *MMP9*, *TPSD1).* Although the qPCR analysis demonstrated expression trends for *FABP4*, *MMP9*, and *GPX2* that were directionally consistent with the RNA-seq data, none of the assessed genes showed statistically significant differences between the BRCA-active and BRCA-inactive groups (Table 3.8; supplementary Table 3). This discrepancy may be attributed to probe-specific detection bias in quantifying transcript abundance using the TaqMan qPCR system.

Table 3.8

Selected transcripts technical validation by qPCR

Gene	Log_2FC	Fold Change	*p*-Value
FABP4	−1.80	0.29	0.07
CARTPT	0.39	1.31	0.61
MMP9	0.36	1.29	0.66
GPX2	1.92	3.77	0.28
TPSD1	−0.28	0.82	0.55

The transcriptomic profiles of breast cancer cases with *BRCA1* promoter hypermethylation (n = 1) and those with promoter deletions (n = 16) were compared to assess whether the mechanism of *BRCA1* loss influences downstream gene expression. Analysis of key homologous recombination related genes (*RAD51, BRCA2, PALB2, CHEK1, CDKN1A, ATM*) revealed no statistically significant differences between the two groups (Table 3.9). While modest fold changes were detected (e. g. *BRCA2*, FC = 1.28; *RAD51*, FC = 1.15), none reached significance ($p < 0.05$). These findings indicate that the specific mode of *BRCA1* inactivation has minimal impact on the expression of downstream DNA repair genes.

Table 3.9

Expression of genes involved in HR pathway downstream from *BRCA1* gene

Gene	Log_2FC	Fold Change	*p*-Value
RAD51	0.20	1.15	0.85
BRCA2	0.36	1.28	0.65
CHEK1	−0.41	−1.33	0.63
CDKN1A	0.16	1.12	0.85
ATM	−0.43	−1.35	0.55

3.2.3 Survival analysis

Kaplan-Meier analysis revealed a trend towards prolonged event-free survival in the BRCA1 inactivation group ($p < 0.09$), as illustrated in Figure 3.11. The hazard ratio (HR = 5.17; 95 % CI: 0.60–44.3) indicates a potential survival difference between groups, though it did not reach statistical significance. Overall, patients with monoallelic *BRCA1* inactivation (BRCA1−) tended to have longer event-free survival compared with those with two active *BRCA1* alleles (BRCA1+).

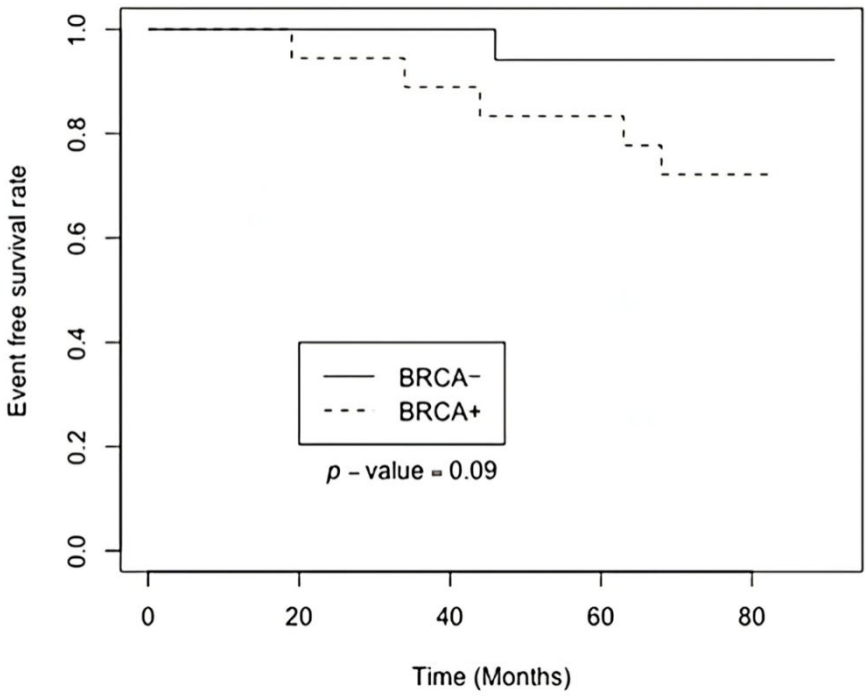

Figure 3.11 **Kaplan-Meier plot of event free survival based on *BRCA1* monoallelic inactivation status** HR 5.17, 95% CI 0.60 and 44.3.

Patients were stratified into MMP9-high and MMP9-low groups based on *MMP9* expression. Kaplan-Meier curves (Figure 3.12) showed no significant difference in event-free survival (log-rank $p = 0.4$). Patients with high *MMP9* expression show a trend toward longer event-free survival, though the difference is not statistically significant.

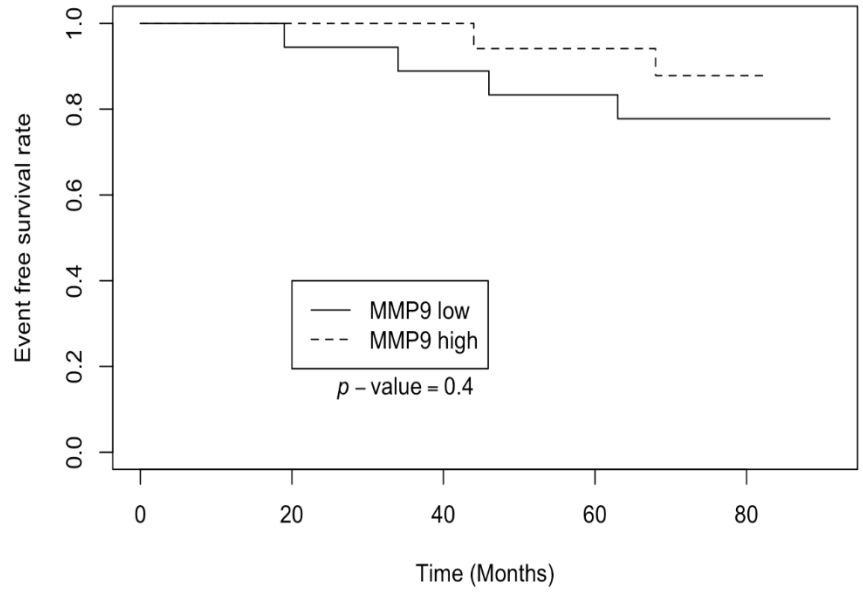

Figure 3.12 **Kaplan-Meier plot of event-free survival based on *MMP9* expression status (high vs low)** HR 0.48, 95% CI 0.09–2.64 ($p = 0.4$).

Patients were divided into FABP4-high and FABP4-low groups based on *FABP4* expression. Kaplan-Meier analysis (Figure 3.13) showed no statistically significant difference

(log-rank $p = 0.13$), but there was a visible trend toward better event-free survival in the FABP4-low group. The HR estimate supports this direction but is not significant.

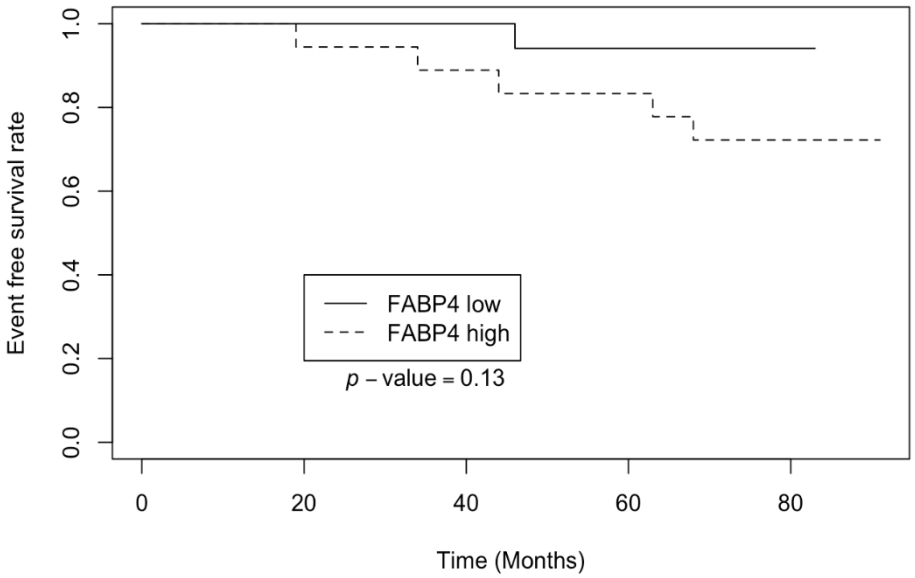

Figure 3.13 **Kaplan-Meier plot of event-free survival based on *FABP4* expression status (high vs low)** HR 5.17, 95% CI 0.60–44.3 ($p = 0.13$)

Patients were divided into TPSD1-high and TPSD1-low groups based on *TPSD1* expression. Kaplan-Meier analysis (Figure 3.14) showed no statistically significant difference in event-free survival between groups (log rank $p = 0.30$), although the curve for the TPSD1-high group remained higher over follow-up. The hazard ratio was 0.41 (95 % CI 0.07–2.23; p = 0.30), but the estimate was not statistically significant.

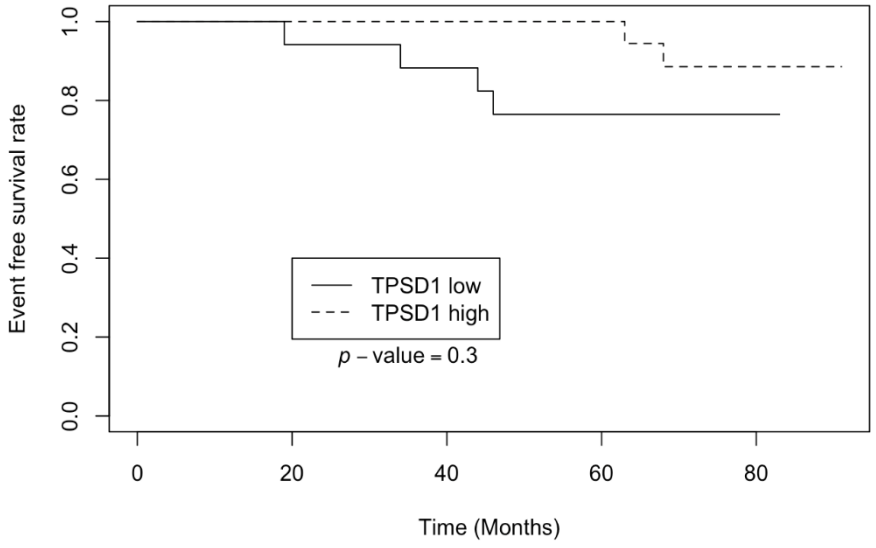

Figure 3.14 **Kaplan-Meier plot of event-free survival based on *TPSD1* expression status (high vs low)** HR 0.41, 95% CI 0.07–2.23 ($p = 0.30$).

Patients were divided into GPX2-high and GPX2-low groups based on *GPX2* expression. Kaplan-Meier analysis (Figure 3.15) showed no difference in event-free survival between groups (log-rank $p = 0.90$), with largely overlapping survival curves throughout follow-up. The hazard ratio was 0.91 (95 % CI 0.18–4.53; $p > 0.90$), indicating no statistically significant association.

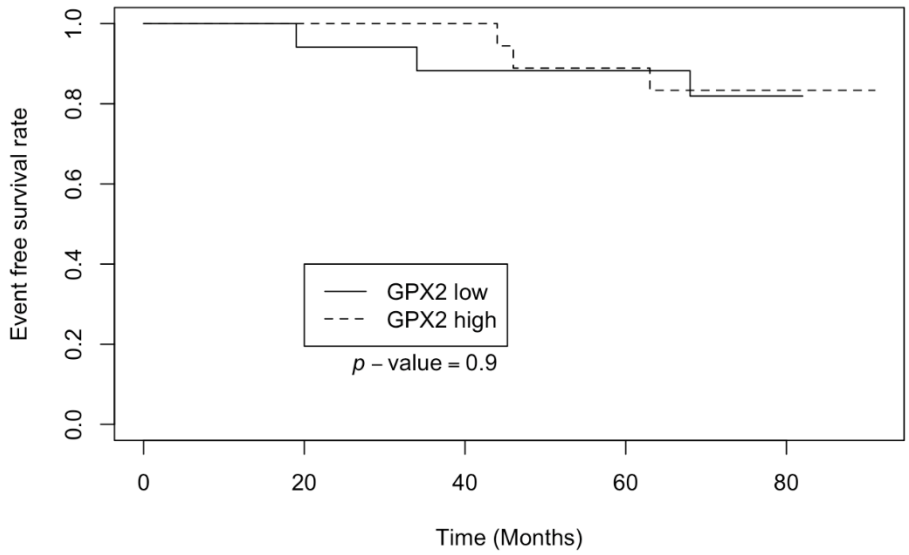

Figure 3.15 **Kaplan-Meier plot of event-free survival based on *GPX2* expression status (high vs low)**. HR 0.91, 95% CI 0.18–4.53 (p > 0.90).

4 Discussion

The first section is dedicated to analysing the transcriptome of triple-negative breast cancer (TNBC), while the second examines the transcriptome of breast cancers with somatic inactivation of *BRCA1* gene, with a particular focus on evaluating the transcriptome's potential as a prognostic and predictive tool. Despite significant advancements, breast cancer remains a life-threatening disease, underscoring the need for continued research to identify reliable biomarkers for improved patient outcomes.

4.1 Transcriptome analysis of triple negative breast cancer (TNBC) samples compared to non-TNBC

Transcriptome analysis of triple negative breast cancer (TNBC) samples compared to non-TNBC samples has revealed differentially expressed genes (DEGs), with many of them being involved in extracellular matrix (ECM) organisation and structure. GO analysis of the DEGs have confirmed that pathways related to collagen trimer formation, extracellular matrix structural constituents that confer tensile strength, and scavenger receptor activity are significantly enriched in TNBC.

Additionally, the protein–protein interaction (PPI) network analysis has identified several hub genes critical for ECM integrity. For instances, *COL9A1* has emerged as key structural gene, while transcription factors and secreted proteins like, *ESR1*, *GATA3*, *TFF1*, and *TFF3* were found to be downregulated in TNBC samples. This pattern of dysregulation points to an altered tumour microenvironment in TNBC, which is associated with more aggressive behaviour and poorer clinical outcomes (Yu & Di, 2017). Targeting ECM related pathways is now being explored as promising therapeutic strategy to reduce tumour invasion and metastasis (Babar et al., 2023).

Differentially expressed gene (DEG) association with TNBC

ESR1

The *ESR1* gene encodes the oestrogen receptor alpha (ERα), a transcription factor that plays a crucial role in the development and progression of breast cancer (Miziak et al., 2023) In TNBC, *ESR1* expression is typically low or absent, which is the opposite for receptor–positive tumours where high *ESR1* levels correlate with responsiveness to endocrine therapy. The absence of *ESR1* in TNBC is linked to an aggressive tumor phenotype, increased metastatic potential, and limited options for targeted endocrine therapies (Dustin et al., 2019; Li et al., 2021).

Recent studies have also suggested that, in a subset of TNBC cases, *ESR1* expression can be reactivated through epigenetic modifications, raising the possibility that epigenetic

therapies might restore hormone sensitivity and open new treatment avenues (Al-Kabariti & Abbas, 2023). This reactivation could potentially convert a portion of TNBC into a more treatable, hormone-responsive state, highlighting the pivotal role of epigenetic regulation in maintaining the balance of key signalling pathways involved in tumor suppression. Furthermore, the loss of *ESR1* not only deprives tumors of the benefits associated with oestrogen receptor signalling but may also contribute to an altered tumor microenvironment by disrupting normal cell–cell communication and immune surveillance. Such disruptions can create conditions that favour tumor growth, invasion, and metastasis, ultimately exacerbating disease progression (Lopez et al., 2025). As research continues to understand these molecular intricacies, integrating epigenetic therapies could emerge as a new strategy in managing TNBC by offering new hope for improved clinical outcomes in patients with limited treatment options.

GATA3

Similarly, transcription factor GATA3 plays a critical role in the development and differentiation of luminal epithelial cells in the breast and is frequently lost in TNBC. In breast cancer, *GATA3* expression is typically associated with hormone receptor-positive and HER2-positive subtypes and is considered a marker of luminal differentiation. The loss of *GATA3* not only disrupts normal differentiation process but also is associated with markedly reduced overall survival and an increased risk of metastasis (Querzoli et al., 2021a). Apart from maintaining luminal identity, *GATA3* regulates downstream gene involved in cell adhesion, immune response and migration, therefore loss of GATA3 factor can lead to disorganised cell architecture and impaired cell-cell communication, which in turn promote tumour invasiveness and immune evasion (Bacha et al., 2024; Bai et al., 2022).

However, in triple-negative breast cancer (TNBC), *GATA3* expression is frequently lost, which is thought to contribute to the more aggressive phenotype of this subtype. Studies have shown that loss of *GATA3* expression in TNBC is associated with decreased overall survival and increased risk of metastasis. Therefore, *GATA3* is considered a potential prognostic marker and therapeutic target for TNBC (Querzoli et al., 2021b) In our study, *GATA3* appeared to be downregulated in the DEG set, which is in-line with other published data, suggesting that it could be a potential marker for differentiation of TNBC from non-TNBC subtypes. Notably, in the TNBC absence of *GATA3* may reflect a loss of specific progenitor markers, therefor increasing tumour aggressiveness. While *GATA3* expression is generally associated with more favourable prognosis in hormone receptor positive breast cancers, its role in TNBC is more complex. For example, some studies show association between low expression of *GATA3* and poor prognosis in TNBC (Yang et al., 2017), while other studies suggests that some rare TNBC

subtypes can still express *GATA3* (Yoon et al., 2022). Our data by showing significant downregulation of *GATA3* in TNBC samples align with these observations that TNBC show decreased *GATA3* expression and underscore its potential as biomarker for distinguishing TNBC from other breast cancer subtypes. Some research is exploring strategies to restore *GATA3* activity through gene therapy and epigenetic modulations, which may help re-induce differentiation and reduce tumour aggressiveness (Garcia-Martinez et al., 2021).

FOXA1

Out study have demonstrated that *FOXA1* is significantly downregulated in TNBC samples when compared to non-TNBC samples, therefore underscoring its potential role in driving tumour development. As a member of forkhead box family, FOXA1 functions as a pioneer factor and binds to chromatin and allows recruitment of other transcription factors to regulatory regions. FOXA1 interacts with oestrogen and androgen receptors, which is essential for the proper transcription activation of luminal genes, therefore involved in breast cancer development and progression. *FOXA1* expression has been linked to TNBC characteristics such as tumour size, lymphovascular invasion, histological grade, and HER2 status, suggesting a stratification value for TNBC patients without ER expression (De Lara et al., 2019)

FOXA1 expression is typically linked with more differentiated phenotype and improved clinical outcomes in ER-positive breast cancer (Metovic et al., 2022). In contrast our finding along with recent literature indicate that low *FOXA1* expression in TNBC correlates with enhanced cancer malignancy traits such as cell proliferation, invasion and the acquisition of stem-like properties (Dai et al., 2019; U. Kumar et al., 2021).

Furthermore, low levels *FOXA1* may disrupt critical signalling pathways that governs cell cycle progression and apoptosis, therefore contributing to chemoresistance (Metovic et al., 2022). The combined assessment of *FOXA1* and *GATA3* expression has shown promise in refining molecular classification of TNBC (Albergaria et al., 2009; Govindan et al., 2020). Based on these observations, the combined expression of *FOXA1* and *GATA3* may enhance TNBC molecular characterisation and provide prognostic insights (Albergaria et al., 2009).

AR

In recent years, due to advances in molecular profiling have refined the subclassification of TNBC into distinct subtypes – basal like 1, basal like 2, mesenchymal and luminal androgen receptor (LAR) variants (Lehmann et al., 2011, 2016). The LAR subtype is characterised by the expression of the androgen receptor (AR), this subtype has unique biological features like lower proliferation rate and more differentiated phenotype (Vtorushin et al., 2022). AR is expressed in majority of primary breast cancer cases (Fan et al., 2023) and is more frequently

expressed in hormone receptor positive breast cancer compared to TNBC cases (Vera-Badillo et al., 2014). Some studies report that AR-positive TNBC is associated with improved outcomes (Asano et al., 2017), while others have found little or even no prognostic benefit (Collins et al., 2011). In our TNBC cohort, *AR* expression was significantly reduced with *AR* emerging as a hub gene, which indicates a link between low AR levels and more aggressive tumour behaviour. What is interesting that more studies show that co-expression of *AR* and *GATA3* can influence chemotherapy response, with some data indicating that this co-expression may influence chemotherapeutic effectiveness (Kim et al., 2016). However, TNBC with increased expression of *AR* is correlated with better clinical outcomes, but with reduced sensitivity to chemotherapy and lower rates of pathological complete response following neoadjuvant chemotherapy (Gerratana et al., 2018; Mohammed et al., 2020). Given our cohort's low *AR* and *GATA3* expression, a poorer prognosis may be inferred.

These findings have increased interested in AR-targeted therapies which are currently being evaluated in clinical trials (Sridhar et al., 2025), that way offering potential therapies for patients with AR-positive TNBC. Overall, while the prognostic significance of AR in TNBC is still under debate, its role as a biomarker and therapeutic target continues to be a focus of active investigation, underscoring the need for personalised treatment strategies in this heterogeneous disease.

Despite controversies around AR's role, certain findings align the AR subtype with improved outcomes, reduced chemotherapy sensitivity, and less pathologic complete response after neoadjuvant therapy. This subtype may benefit from AR-targeted treatments (Gerratana et al., 2018).

Combined with findings from existing literature, key genes such as *GATA3*, *FOXA1*, *AR*, and *SOX10* emerge as relevant molecular markers in TNBC (Hu et al., 2024). Notably, the combination of low *GATA3* expression with high *SOX10* levels appears to define a distinct TNBC subgroup with a particularly aggressive phenotype. Similarly, reduced *FOXA1* expression, has been linked to features of malignancy and stemness in TNBC, suggesting that the joint evaluation of *GATA3* and *FOXA1* could refine TNBC classification and provide valuable prognostic information (Albergaria et al., 2009).

TFF1 and *TFF3*

Additional hub genes also offer promising insights. *TFF1* and *TFF3* both encodes proteins associated with mucin production and gastrointestinal tissues, these genes show decreased expression in TNBC tumours in our data set. While elevated serum levels of these trefoil factors have been observed in breast cancer patients (Elnagdy et al., 2018), lower tissue

expression of TFF1 in particular has been correlated with TNBC and improved prognosis (Yi et al., 2020). This difference between serum and tissue levels highlights the need for further research, but our data suggests the potential of *TFF1* and *TFF3* as biomarker for TNBC.

Furthermore, gene *SLC39A6*, which is commonly overexpressed in ER-positive breast cancers, is downregulated in TNBC group in our study, underscoring its specificity for hormone receptor positive subtypes. In contrast, the upregulation of *COL9A1*, which encodes the collagen type IX alpha chain, points to a possible role for altered collagen dynamics in TNBC biology, although the impact of collagen remodelling on tumour behaviour remains an area of active investigation.

Altogether these finding emphasise the molecular heterogeneity of TNBC and emphasise the potential of integrating multiple biomarkers, including *GATA3, FOXA1, AR, SOX10, TFF1, TFF3, SLC39A6* and *COL9A1*, into a comprehensive diagnostic and prognostic framework that may ultimately guide personalised therapeutic strategies.

4.2 Transcriptomics alterations associated with monoallelic somatic *BRCA1* inactivation

Investigation in this study further explored distinct transcriptomics alterations associated with monoallelic somatic *BRCA1* inactivation. The study was based on global transcriptome sequencing of breast cancer tissue samples to identify differentially expressed genes and signalling pathways associated with monoallelic somatic *BRCA1* inactivation. The Kaplan-Meier analysis assessed the differences in event free survival between two distinct subgroups, and it was observed that patients with *BRCA1* promotor inactivation tended to have longer event free survival ($p < 0.09$). This finding implies that *BRCA1* inactivation may be linked to better clinical outcomes. Therefore, future investigation into these finding could offer valuable insights into the underlying mechanisms driving the disease and would help to develop more targeted and effective therapeutic strategies for breast cancer patients.

Additionally, the STRING database analysis revealed functional interaction among differentially expressed genes in the context of *BRCA1* inactivation. By focusing on proteins localised in the extracellular region and space, several key genes like *TPSD1, FABP4, CARTPT, TRH, CSN3, MMP9, GPX, ORM1* were identifies that have been linked to cancer progression in previous published data (An et al., 2024; Gonzalez-Avila et al., 2019; Hao et al., 2018; Sobiepanek et al., 2022). These proteins appear to play pivotal roles in shaping the tumour microenvironment by influencing extracellular matrix composition and mediating cell-to-cell communication. Such interactions may affect processes like cell adhesion, migration, and invasion, which are critical processes for tumour development metastasis (Popova & Jücker,

2022). Understanding roles of these molecules could provide new insight into molecular mechanisms driving breast cancer progression.

The extracellular region and extracellular space are one of main components in the tumour microenvironment, contributing significantly to cancer progression and metastasis (Winkler et al., 2020). Proteins identified in these categories often participate in intricate signalling networks, modulating cell behaviour, angiogenesis, and immune responses within the tumour microenvironment. Furthermore, the study on the transcriptome of TNBC tumours revealed that differentially expressed genes (DEGs) were associated with processes such as extracellular matrix organisation, collagen fibril organisation, and the composition of collagen-c matrix (Popova & Jücker, 2022).

Differentially expressed gene (DEG) association with monoallelic somatic *BRCA1* inactivation in breast cancer

TPSD1

Our results indicate *TPSD1* gene to be upregulated in tumours with monoallelic somatic *BRCA1* inactivation. The *TPSD1* gene encodes protease tryptase delta, which is predominantly secreted by mast cells, which play different roles in tumour biology. Mast cells assist to the remodelling of the extracellular matrix (ECM) through release of tryptases, which are bioactive substances and are able to degrade ECM components and facilitate angiogenesis – a crucial process for tumour growth and metastasis (Sobiepanek et al., 2022). Previous study by Kankkunen et al demonstrated a significant increase in tryptase containing mast cells in malignant breast carcinomas when compared to benign lesions (Kankkunen et al., 1997). Other research has shown that mast cells often accumulate in tumours prior to neovascularisation and are frequently located near the newly formed blood vessels. Higher mast cell density have been correlated with increased microvessel density and poorer patient prognosis, which was supported by the study where experimental models in pancreatic cancer revealed that genetic inactivation of mast cells led to reduced intratumoral microvessel density and slower tumour growth. Moreover, higher mast cell activity together with elevated tryptase release, has been correlated with cancer growth, particularly enhanced angiogenesis (Iamaroon et al., 2003; Wroblewski et al., 2017). Studies also suggests that mast cells can directly and indirectly decrease tumour sensitivity to anti-angiogenic therapies, suggesting that mast cell activity could increase the efficacy of these therapies (Wroblewski et al., 2017).

In the studied cohort, elevated *TPSD1* expression was associated with improved event-free survival, a finding that differs from the conventional view that mast cell tryptases function as pro-tumorigenic molecules. This observation might indicate that in the context of BRCA1-deficient tumours tryptase delta could be involved in alternative pathways that

modulate immune responses or promote tissue repair, thereby contributing to a less aggressive tumour phenotype. However, the precise mechanism remains unknown by which *TPSD1* influences the tumour microenvironment and clinical outcomes in BRCA1-inactivated breast cancer.

FABP4

Fatty Acid Binding Protein 4 (FABP4), also known as adipocyte protein 2 (aP2), is a member of the FABP family, playing a crucial role in lipid metabolism and cellular signalling. *FABP4* is primarily expressed in adipocytes and macrophages, where it facilitates the transportation of fatty acids and other lipophilic molecules within cells (Furuhashi et al., 2014).

Typically, high levels of FABP4 are observed in breast cancer tissues, where they are associated with aggressive tumour behaviour (Hao et al., 2018). Elevated *FABP4* expression has been linked with increased cell proliferation, migration, invasion, which subsequently promotes tumour growth and metastasis. *FABP4* also contributes to angiogenesis by modulating lipid signalling pathways, which are essential for the formation of new blood vessels and maintenance of solid tumours. Studies have demonstrated that highly expressed *FABP4* in adipocytes and macrophages can enhance the transfer of fatty acids to tumour cells, therefore fuelling metabolic pathways that support rapid tumour growth and resistance to apoptosis (Liu et al., 2022; Sun & Zhao, 2022a).

The transcriptomic analysis in this study revealed a noticeable reduction in *FABP4* expression in breast cancer patients with monoallelic somatic *BRCA1* inactivation. This observation suggests potential regulatory interplay between *BRCA1* and *FABP4*, where the loss of *BRCA1* function may lead to alterations in lipid metabolism and associated with signalling cascades. Also, when comparing all patient event free survival based on *FABP4* expression, patients with low expression showed tendency towards better event free survival. The *FABP4* downregulation in this context could contribute to a less aggressive tumour phenotype, potentially explaining the improved event free survival observed in BRCA- group. It is conceivable that reduced *FABP4* levels diminish supply of lipids required for rapid tumour cell proliferation and invasion. Additionally, this unique molecular profile may influence key pathways such as *PI3K/AKT* and *MAPK*, which are known to mediate cellular responses to metabolic stress and growth signals (Sun & Zhao, 2022).

CARTPT

Recent study by Kast et al has highlighted that higher body mass index and significant weight gain during adulthood increased risk for postmenopausal breast cancer in *BRCA1* variant carriers (Kast et al., 2023). These finding emphasises the crucial role in lifestyle factors, like

diet and exercise, that are important in chancing cancers risks among individuals with genetic predispositions, potentially through mechanisms involving altered hormonal environment and chronic inflammation. In contrast, body mass index did not differ significantly between the BRCA1-inactivated and BRCA1-active groups in our cohort.

In parallel, the gene *CARTPT*, which encodes the cocaine- and amphetamine-regulated transcript peptide, has gained attention due to its involvement in breast cancer cell survival and resistance to endocrine therapies like tamoxifen (Brennan et al., 2012). *CARTPT* has been identified in both in primary and metastatic breast cancer tissues. In oestrogen receptor-positive, lymph node-negative tumours it has been proposed as an independent predictor of poor prognosis (Brennan et al., 2012). Mechanistically, *CARTPT* enhances transcription activity of oestrogen receptor alpha (ERα) via mitogen-activated protein kinase (MAPK) pathway in a ligand-independent fashion. In various cancer cell models *CARTPT* functions as an oncogene by activating the ERK pathway thereby stimulating pro-survival signals, inhibiting apoptosis and increasing cyclin D1 levels. Notably, this activity appears to protect tumour cells from tamoxifen-induced cell death, underscoring its potential as therapeutic target (Owe-Larsson et al., 2023).

In this study, tumours with somatic monoallelic *BRCA1* inactivation exhibited reduced *CARTPT* expression. It is possible that compromised DNA repair capacity resulting from *BRCA1* inactivation alters gene expression patterns, including that of *CARTPT*. This altered expression profile may partially explain the improved event free survival observed in the Kaplan-Meyer analysis, particularly among patients receiving hormone therapies such as tamoxifen or anastrozole.

MMP9

Matrix metalloproteinase 9 (MMP9) is a member of the matrix metalloproteinase family enzymes that are essential for degradation and remodelling of the extracellular matrix (ECM) – a complex network of proteins and carbohydrates that provides structural support to cells and regulates various cellular processes, including cell adhesion, migration, and signalling Traditionally, MMP9 is associated with cancer progression, as its proteolytic activity facilitates tumour invasion and metastasis by breaking down ECM barriers (Gonzalez-Avila et al., 2019). However, the findings in this study show the opposite association: higher *MMP9* expression correlates with a trend toward improved event free survival in somatically inactivated BRCA1 cohort. The same direction of effect was observed in the overall cohort, where higher *MMP9* expression similarly trended towards better event-free survival (but not statistically significant). This observation suggests that the role of MMP in cancer is diverse and context dependent.

Recent studies indicate that MMP9 may exert both pro-tumorigenic and anti-tumorigenic effect. While ECM degradation function can promote metastasis, MMP9 also participates in modulating the immune response, regulating angiogenesis, and facilitates tissue repair. For instance, MMP9 are capable of generating matrikines, which are bioactive ECM fragments and have anti-angiogenic properties. MMP9 also can modulate immune cell recruitment (Huang, 2018; Wells et al., 2015). Moreover, inflammatory signals, which are often upregulated in the settings of genomic instability such as that observed in *BRCA1* inactivation, can further induce *MMP9* expression, therefore suggesting a link between DNA repair deficiencies, inflammatory responses and MMP9 regulation (Zhao et al., 2023).

In the context of *BRCA1* inactivation, the inflammatory milieu of the tumour microenvironment may induce an *MMP9* expression profile that contributes to immune modulation or tissue remodelling, rather than solely facilitating invasion. Since there is lack of studies directly examining how somatic BRCA1 inactivation relates to increased MMP9 expression, further research is necessary to unravel these mechanisms and to determine if the dual roles of MMP9 can be exploited for therapeutic benefits (Wang et al., 2022).

Although direct studies connecting somatic *BRCA1* inactivation and increased MMP9 expression are limited, genomic instability inherent to homologues recombination deficiency (HDR) can create a pro-inflammatory tumour microenvironment. Inflammatory cytokines released in this context may upregulate *MMP9* expression, contributing to extracellular matrix remodelling and, paradoxically, improved clinical outcomes. This potential mechanistic connection underscores the need for further investigation into whether HRD-induced inflammation mediates MMP9 upregulation (Leifler et al., 2013; Wang et al., 2022; Zhao et al., 2023).

GPX2

GPX2 was markedly upregulated in breast cancer cases with *BRCA1* promoter inactivation and emerged as a hub gene, pointing to a possible role in tumour development under BRCA1-deficient conditions in this study. As a regulator of oxidative stress, *GPX2* may enhance cancer cell survival by mitigating reactive oxygen species that accumulate due to impaired DNA repair (An et al., 2024). The elevated expression of *GPX2* could therefore represent an adaptive mechanism to oxidative damage in the absence of functional BRCA1. These observations suggest *GPX2* may serve as a potential biomarker and therapeutic target in BRCA1-inactivated breast tumours.

ORM1

Recent study by Qiong and Yin demonstrated that ORM1 promotes resistance to epirubicin in breast cancer, particularly via upregulation of matrix metalloproteinase 2 and 9 (MMP2 and MMP9). This is of particular relevance to the present study, as increased *ORM1* expression was observed in breast cancer samples with somatic BRCA1 inactivation, accompanied by an unexpected association between elevated MMP9 levels and improved event-free survival. This study suggests that ORM1 drives chemoresistance through ECM remodelling, which is done through MMP upregulation. This in turn facilitates tumour cell survival and invasive potential. The upregulation of ORM1 in BRCA1-inactivated tumours could reflect similar mechanism, where genomic instability by inactivated BRCA1 induces an acute-phase inflammatory response that in turn increases *ORM1* expression, which then increases MMP9 activity. As previously discussed, MMP is usually associated with promoting invasion and metastasis, but due to its dual functionality it can encompass pro-tumorigenic and anti-tumorigenic effect (Leifler et al., 2013; Qiong & Yin, 2021). Thus, the result in this study together with Qiong and Yin shows potential link between *ORM1* driven *MMP9* upregulation.

TRH

The observation in this study suggests that breast cancer samples with somatic *BRCA1* inactivation show increase in thyroid releasing hormone (TRH) expression, pointing to a previously unknown connection between *BRCA1* deficiency and thyroid hormone signalling within the tumour microenvironment. Typically, TRH is known for its central role in regulating thyroid function through the hypothalamic-pituitary-thyroid axis, but it is also expressed in non-thyroid tissues, there it can affect cell growth and survival (Trubacova et al., 2022). Upregulation of TRH expression in the context of *BRCA1* inactivation suggests that these tumours are activating alternative endocrine pathways to support their growth and adapt to genomic instability. Possible explanation could be that in response to *BRCA1* loss, cancer cells might be compensating by modulating TRH-related pathways to enhanced cell proliferation and survival, in that way influencing tumour behaviour and possibly altering their response to therapies. Although this is highly speculative, it opens new research ideas to explore how endocrine factors, which are traditionally associated with thyroid functions, might be used in *BRCA1*-deficient cancers (Baldini et al., 2022; Cortellino & Longo, 2023).

IRS4

Further exploring potential endocrine alterations observed in BRCA1-inactivated tumours, another important gene from DEG analysis emerged, which is Insulin Receptor Substrate 4 (IRS4). Unlike *TRH*, which was upregulated, *IRS4* expression was significantly

decreased in the BRCA1-inactivated group. *IRS4* is critical mediator of insulin signalling and has been widely recognised for its role in promoting cellular growth and survival (Guijarro et al., 2023). Dysregulation of this pathway is a common hallmark of cancer, and elevated IRS4 levels have been reported in various breast cancer tissues, where they are thought to enhance PI3K-pathway activity. In fact, recent studies have characterised *IRS4* as an oncogene with a potential role in driving constitutive PI3K activation and even contributing to resistance against HER2-targeted therapies (Guijarro et al., 2023; Ikink et al., 2016). The decreased expression of *IRS4* in our cohort may reflect a unique regulatory pattern in *BRCA1*-deficient tumours, which might influence tumour metabolism and responsiveness to therapy. Together, the different expression patterns of *TRH* and *IRS4* highlight the complex interplay between endocrine signalling, metabolic regulation, and genomic instability in shaping breast cancer behaviour.

CGA* and *CHGB

Chromogranin A (CGA) and Chromogranin B (CHGB) are members of the granin family, traditionally associated with secretory granules in neuroendocrine cells. Their overexpression in breast cancer subtypes has been linked to increased neuroendocrine features, elevated angiogenesis, and altered cellular signalling pathways (Giovanella et al., 2001). In response to genomic stress due to *BRCA1* loss, tumor cells may adopt alternative survival pathways, sometimes linked to endocrine or neuroendocrine signalling. In this sense, increased *CGA* and *CHGB* could be part of a compensatory mechanism that modifies the tumor microenvironment. One possibility is that these granins interact with components of the immune system or extracellular matrix in a manner that restricts metastatic spread, or that they render the tumor more sensitive to DNA-damaging treatments, given the pre-existing deficiency in BRCA1-dependent DNA repair (Weisbrod et al., 2013).

4.3 Strengths and weaknesses of the study

4.3.1 Strengths of the study

This study has several notable strengths. First, high-throughput RNA-sequencing technology was used to generate comprehensive transcriptomic profiles across distinct breast cancer subtypes, particularly, triple negative breast cancer (TNBC) and tumours with monoallelic somatic *BRCA1* inactivation. This approach enabled the identification of differentially expressed genes and enriched pathways that may serve as potential biomarkers or therapeutic targets. The use on rigorous bioinformatics tools, including GO enrichment analysis and protein–protein interaction mapping using STRING database, further strengthens our

results. In addition, integration of clinical data through Kaplan-Meier survival analysis adds valuable information, therefore linking molecular alterations and patient outcomes.

4.3.2 Limitations of the study

In this study has several limitations. One of the limitations was relatively small sample size, particularly in specific subgroups such as TNBC and BRCA1-inactivated tumours. One contributing factor is that most TNBC patients undergo neoadjuvant therapy, therefore are not eligible for the study, since tissue samples are collected during surgery. This restriction further reduces the number of available samples for transcriptomics analysis. Additionally, the cohort of breast cancer with somatic monoallelic *BRCA1* inactivation was limited due to small available samples. Notable, two cases with germline *BRCA1/2* allelic variants were also excluded from analysis to mainly focus on somatic alterations. These exclusions, while necessary to maintain homologous study group, further limit the sample size. To help address this limitation, sequencing depth was increased by generating a higher read count per sample. As a result, the result should be interpreted with caution, and larger cohorts in the future studies will be essential to validate these results.

Significant limitation was the absence of detailed patient clinical information for available samples. This gap in the clinical dataset limited our ability to correlate observed transcriptomic changes with histopathological features, therapy used. Many patients were lost to follow-up. Especially limited clinical data was in first section of (TNBC vs non-TNBC). Without comprehensive clinical annotations – such as tumour grade, stage, or molecular subtypes beyond the broad TNBC and non-TNBC categories – it is challenging to fully interpret the biological significance and potential prognostic value of the identified differentially expressed genes. Moreover, while several potential biomarkers were identified, their clinical applicability remains uncertain without rigorous testing of sensitivity, specificity, and predictive accuracy in independent cohorts. Validation using well-characterised clinical datasets and functional assays will therefore be critical before these biomarkers can be translated into diagnostic or prognostic use. In future studies, the integration of robust, detailed clinical data with high-throughput transcriptomic analyses will be essential to strengthen the validity of our findings and to facilitate more personalised treatment strategies based on specific tumour phenotypes.

4.4 Future perspectives

Future perspectives for our work include many areas. Firstly, expanding patient cohort to include larger sample size to enhance the statistical power of our findings, particularly subgroups like TNBC and somatic inactivated *BRCA1*. Possible using publicly available

databases such as TCGA. Additionally, using multi-omics approaches could provide more comprehensive understanding of the molecular basis of these breast cancer subtypes, this could be done by combining transcriptomics with proteomics, epigenomics, and metabolomics.

Continued research seeks reliable, clinically validated TNBC markers, yet despite diverse gene proposals, none have consistently translated into clinical practice. Our identified hub genes, including *FOXA1, ESR1, TFF1, GATA3, TFF3, AR, SLC39A6,* and *COL9A1*, reflect known TNBC characteristics but require validation in broader patient populations, especially given the limited prognostic insight provided by current molecular subtype data regarding outcomes such as distant metastasis-free survival.

Furthermore, future work could explore the interplay between *BRCA1* inactivation, endocrine signalling, and metabolic pathways. This could help in novel therapeutic target.

Conclusions

1. RNA-sequencing identified 53 854 expressed genes, with 229 differentially expressed between TNBC and non-TNBC tumours, defining a distinct TNBC-associated transcriptomic profile enriched for extracellular matrix-related pathways, including collagen organisation and extracellular structural functions.
2. Gene Ontology enrichment confirmed that TNBC-related DEGs are functionally linked to extracellular structure organisation and ECM integrity. Protein–protein interaction analysis identified hub genes *FOXA1, ESR1, TFF1, GATA3, TFF3, AR, SLC39A6,* and *COL9A1*, highlighting their central role in regulating the tumour microenvironment and endocrine signalling pathways.
3. Transcriptome analysis of tumours with monoallelic somatic *BRCA1* inactivation identified 39 differentially expressed genes, predominantly enriched in extracellular region–related processes, indicating altered tumour-stroma interactions.
4. Functional enrichment analysis of tumours with monoallelic *BRCA1* inactivation showed significant enrichment of genes in extracellular region categories, including *TPSD1, FABP4, ORM1, ALPI, CARTPT, TRH, CSN3,* and *MMP9*. Protein–protein interaction analysis identified *GPX2* and *MMP9* as hub genes, with *GPX2* consistently ranking highest across both algorithms.
5. A tendency toward prolonged event-free survival in patients with monoallelic somatic *BRCA1* inactivation was revealed by Kaplan-Meier analysis.

Proposals

The findings of this Doctoral Thesis demonstrate the value of transcriptomic profiling for improving molecular stratification of breast cancer, particularly in clinically challenging subgroups. It is proposed that future studies validate the identified subtype-specific gene expression signatures and hub genes in larger, independent cohorts, with special emphasis on triple-negative breast cancer. Such validation is necessary to determine their reliability as prognostic or predictive biomarkers and to assess their added value beyond standard clinicopathological classification.

The results further indicate that monoallelic somatic *BRCA1* inactivation represents a biologically distinct phenotype with specific transcriptomic alterations and potential prognostic relevance. Future research should therefore move beyond a binary interpretation of *BRCA1* status and systematically investigate partial *BRCA1* loss, its association with homologous recombination related pathways, and its impact on treatment response, particularly to DNA-damaging agents and PARP inhibitors.

In addition, functional validation of selected differentially expressed genes is proposed to clarify their role in tumour biology and therapy sensitivity. Finally, integrating transcriptomic data with clinical and pathological parameters in prospective studies could support more precise patient stratification and facilitate the development of personalised treatment strategies in breast cancer care.

Publications and reports on topics of Doctoral Thesis

Publications:

1. **Kuzņecova E.,** Daneberga Z., Berga-Švītiņa E., Nakazawa-Miklaševiča M., Irmejs A., Gardovskis J., Miklaševičs E. 2023. Identification of Altered Transcripts and Pathways in Triple Negative Breast Cancer. Proceedings of the Latvian Academy of Sciences. *Section B. Natural, Exact, and Applied Sciences.*, *77*(1), 33–40. https://doi.org/10.2478/prolas-2023-0004
2. **Kuznecova E,** Nakazawa-Miklasevica M, Krike N, Satcs M, Sivina E, Irmejs A, Loza P, Gardovskis J, Miklasevics E, Daneberga Z. The Transcriptomic Profile Underlying Somatic Monoallelic *BRCA1* Inactivation: A Biomarker for Breast Cancer Prognosis. *Diagnostics*. 2025; 15(16):2037. https://doi.org/10.3390/diagnostics15162037

Reports and theses at international congresses and conferences:

1. **Kuzņecova E**., Daneberga Z., Nakazawa-Miklaševiča M., Berga-Švītiņa E., Pirsko V., Miklaševičs E., Irmejs A., Maksimenko J. HER2-positive Breast Cancer Gene Expression Influenced Pathway Analysis. Poster presentation at Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice": Abstracts, 1.–3.04.2019, 60.
2. **Kuzņecova E**., Daneberga Z., Nakazawa-Mikaševiča M., Irmejs A., Miklaševičs E. Gene expression patterns as useful biomarkers for TNBC patients. Oral presentation at Rīga Stradiņš University International Research Conference on Medical and Health Care Sciences "Knowledge for Use in Practice": Abstracts 29.-31.03.2023.

References

1. Adam, M. P., Feldman, J., & Mirzaa, G. M. (1998). Associated Hereditary Breast and Ovarian Cancer.
2. Ahlin, C., Lundgren, C., Embretsén-Varro, E., Jirström, K., Blomqvist, C., & Fjällskog, M.-L. (2017). High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER-positive but not in ER-negative breast cancers. Breast Cancer Research and Treatment, 164(3), 667–678. https://doi.org/10.1007/s10549-017-4294-5
3. Akshata Desai, K. A. (2012). Triple Negative Breast Cancer – An Overview. Hereditary Genetics. https://doi.org/10.4172/2161-1041.S2-001
4. Albergaria, A., Paredes, J., Sousa, B., Milanezi, F., Carneiro, V., Bastos, J., Costa, S., Vieira, D., Lopes, N., Lam, E. W., Lunet, N., & Schmitt, F. (2009). Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours. Breast Cancer Research, 11(3), R40. https://doi.org/10.1186/bcr2327
5. Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L., Boyault, S., Burkhardt, B., Butler, A. P., Caldas, C., Davies, H. R., Desmedt, C., Eils, R., Eyfjörd, J. E., Foekens, J. A., … Stratton, M. R. (2013). Signatures of mutational processes in human cancer. Nature, 500(7463), 415–421. https://doi.org/10.1038/nature12477
6. Al-Kabariti, A. Y., & Abbas, M. A. (2023). Progress in the Understanding of Oestrogen Receptor Alpha Signaling in Triple-Negative Breast Cancer: Reactivation of Silenced ER-α and Signaling through ER-α36. Molecular Cancer Research, 21(11), 1123–1138. https://doi.org/10.1158/1541-7786.MCR-23-0321
7. Allred, D. C. (2010). Ductal Carcinoma In Situ: Terminology, Classification, and Natural History. JNCI Monographs, 2010(41), 134–138. https://doi.org/10.1093/jncimonographs/lgq035
8. An, X., Yu, W., Liu, J., Tang, D., Yang, L., & Chen, X. (2024). Oxidative cell death in cancer: mechanisms and therapeutic opportunities. Cell Death & Disease, 15(8), 556. https://doi.org/10.1038/s41419-024-06939-5
9. Andre, F., Ismaila, N., Allison, K. H., Barlow, W. E., Collyar, D. E., Damodaran, S., Henry, N. L., Jhaveri, K., Kalinsky, K., Kuderer, N. M., Litvak, A., Mayer, E. L., Pusztai, L., Raab, R., Wolff, A. C., & Stearns, V. (2022). Biomarkers for Adjuvant Endocrine and Chemotherapy in Early-Stage Breast Cancer: ASCO Guideline Update. Journal of Clinical Oncology, 40(16), 1816–1837. https://doi.org/10.1200/JCO.22.00069
10. Angus, L., Smid, M., Wilting, S. M., van Riet, J., Van Hoeck, A., Nguyen, L., Nik-Zainal, S., Steenbruggen, T. G., Tjan-Heijnen, V. C. G., Labots, M., van Riel, J. M. G. H., Bloemendal, H. J., Steeghs, N., Lolkema, M. P., Voest, E. E., van de Werken, H. J. G., Jager, A., Cuppen, E., Sleijfer, S., & Martens, J. W. M. (2019). The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. Nature Genetics, 51(10), 1450–1458. https://doi.org/10.1038/s41588-019-0507-7
11. Arakelyan, A., Melkonyan, A., Hakobyan, S., Boyarskih, U., Simonyan, A., Nersisyan, L., Nikoghosyan, M., Filipenko, M., & Binder, H. (2021). Transcriptome Patterns of BRCA1- and BRCA2- Mutated Breast and Ovarian Cancers. International Journal of Molecular Sciences, 22(3), 1266. https://doi.org/10.3390/ijms22031266
12. Arimura, A., Sakai, K., Kaneshiro, K., Morisaki, T., Hayashi, S., Mizoguchi, K., Yamada, M., Kai, M., Ono, M., Nishio, K., Nakamura, M., & Kubo, M. (2024). TP53 and/or BRCA1 Mutations Based on CtDNA Analysis as Prognostic Biomarkers for Primary Triple-Negative Breast Cancer. Cancers, 16(6), 1184. https://doi.org/10.3390/cancers16061184
13. Arnold, M., Morgan, E., Rumgay, H., Mafra, A., Singh, D., Laversanne, M., Vignat, J., Gralow, J. R., Cardoso, F., Siesling, S., & Soerjomataram, I. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. The Breast, 66, 15–23. https://doi.org/10.1016/j.breast.2022.08.010

14. Asano, Y., Kashiwagi, S., Goto, W., Takada, K., Takahashi, K., Hatano, T., Noda, S., Takashima, T., Onoda, N., Tomita, S., Motomura, H., Ohsawa, M., Hirakawa, K., & Ohira, M. (2017). Prediction of survival after neoadjuvant chemotherapy for breast cancer by evaluation of tumor-infiltrating lymphocytes and residual cancer burden. BMC Cancer, 17(1), 888. https://doi.org/10.1186/s12885-017-3927-8

15. Babar, Q., Saeed, A., Tabish, T. A., Sarwar, M., & Thorat, N. D. (2023). Targeting the tumor microenvironment: Potential strategy for cancer therapeutics. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease, 1869(6), 166746. https://doi.org/10.1016/j.bbadis.2023.166746

16. Bacha, R., Alwisi, N., Ismail, R., Pedersen, S., & Al-Mansoori, L. (2024). Unveiling GATA3 Signaling Pathways in Health and Disease: Mechanisms, Implications, and Therapeutic Potential. Cells, 13(24), 2127. https://doi.org/10.3390/cells13242127

17. Bai, F., Zheng, C., Liu, X., Chan, H. L., Liu, S., Ma, J., Ren, S., Zhu, W.-G., & Pei, X.-H. (2022). Loss of function of GATA3 induces basal-like mammary tumors. Theranostics, 12(2), 720–733. https://doi.org/10.7150/thno.65796

18. Bailey, M. H., Tokheim, C., Porta-Pardo, E., Sengupta, S., Bertrand, D., Weerasinghe, A., Colaprico, A., Wendl, M. C., Kim, J., Reardon, B., Ng, P. K.-S., Jeong, K. J., Cao, S., Wang, Z., Gao, J., Gao, Q., Wang, F., Liu, E. M., Mularoni, L., … Mariamidze, A. (2018). Comprehensive Characterisation of Cancer Driver Genes and Mutations. Cell, 173(2), 371-385.e18. https://doi.org/10.1016/j.cell.2018.02.060

19. Baldini, E., Lauro, A., Tripodi, D., Pironi, D., Amabile, M. I., Ferent, I. C., Lori, E., Gagliardi, F., Bellini, M. I., Forte, F., Pacini, P., Cantisani, V., D'Andrea, V., Sorrenti, S., & Ulisse, S. (2022). Thyroid Diseases and Breast Cancer. Journal of Personalised Medicine, 12(2), 156. https://doi.org/10.3390/jpm12020156

20. Barker, K. T., Jackson, L. E., & Crompton, M. R. (1997). BRK tyrosine kinase expression in a high proportion of human breast carcinomas. Oncogene, 15(7), 799–805. https://doi.org/10.1038/sj.onc.1201241

21. Bayraktar, S., Gutierrez-Barrera, A. M., Liu, D., Tasbas, T., Akar, U., Litton, J. K., Lin, E., Albarracin, C. T., Meric-Bernstam, F., Gonzalez-Angulo, A. M., Hortobagyi, G. N., & Arun, B. K. (2011). Outcome of triple-negative breast cancer in patients with or without deleterious BRCA mutations. Breast Cancer Research and Treatment, 130(1), 145–153. https://doi.org/10.1007/s10549-011-1711-z

22. Bertozzi, S., Londero, A., Xholli, A., Azioni, G., Di Vora, R., Paudice, M., Bucimazza, I., Cedolini, C., & Cagnacci, A. (2023). Risk-Reducing Breast and Gynecological Surgery for BRCA Mutation Carriers: A Narrative Review. Journal of Clinical Medicine, 12(4), 1422. https://doi.org/10.3390/jcm12041422

23. Błaszczak, E., Miziak, P., Odrzywolski, A., Baran, M., Gumbarewicz, E., & Stepulak, A. (2025). Triple-Negative Breast Cancer Progression and Drug Resistance in the Context of Epithelial–Mesenchymal Transition. Cancers, 17(2), 228. https://doi.org/10.3390/cancers17020228

24. Bodily, W. R., Shirts, B. H., Walsh, T., Gulsuner, S., King, M.-C., Parker, A., Roosan, M., & Piccolo, S. R. (2020). Effects of germline and somatic events in candidate BRCA-like genes on breast-tumor signatures. PLOS ONE, 15(9), e0239197. https://doi.org/10.1371/journal.pone.0239197

25. Boyle, P. (2012). Triple-negative breast cancer: epidemiological considerations and recommendations. Annals of Oncology, 23, vi7–vi12. https://doi.org/10.1093/annonc/mds187

26. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394–424. https://doi.org/10.3322/caac.21492

27. Brennan, D. J., O'Connor, D. P., Laursen, H., McGee, S. F., McCarthy, S., Zagozdzon, R., Rexhepaj, E., Culhane, A. C., Martin, F. M., Duffy, M. J., Landberg, G., Ryden, L., Hewitt, S. M., Kuhar, M. J., Bernards, R., Millikan, R. C., Crown, J. P., Jirström, K., & Gallagher, W. M. (2012). The cocaine- and amphetamine-regulated transcript mediates ligand-independent activation of ERα, and is an independent prognostic factor in node-negative breast cancer. Oncogene, 31(30), 3483–3494. https://doi.org/10.1038/onc.2011.519

28. Burgess, A. W. (2008). EGFR family: Structure physiology signalling and therapeutic targets †. Growth Factors, 26(5), 263–274. https://doi.org/10.1080/08977190802312844

29. Byrski, T., Huzarski, T., Dent, R., Gronwald, J., Zuziak, D., Cybulski, C., Kladny, J., Gorski, B., Lubinski, J., & Narod, S. A. (2009). Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Research and Treatment, 115(2), 359–363. https://doi.org/10.1007/s10549-008-0128-9

30. Cardoso, F., Paluch-Shimon, S., Senkus, E., Curigliano, G., Aapro, M. S., André, F., Barrios, C. H., Bergh, J., Bhattacharyya, G. S., Biganzoli, L., Boyle, F., Cardoso, M.-J., Carey, L. A., Cortés, J., El Saghir, N. S., Elzayat, M., Eniu, A., Fallowfield, L., Francis, P. A., … Winer, E. P. (2020). 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5). Annals of Oncology, 31(12), 1623–1649. https://doi.org/10.1016/j.annonc.2020.09.010

31. Casimiro, M. C., Crosariol, M., Loro, E., Li, Z., & Pestell, R. G. (2012). Cyclins and Cell Cycle Control in Cancer and Disease. Genes & Cancer, 3(11–12), 649–657. https://doi.org/10.1177/1947601913479022

32. Cheang, M. C. U., Chia, S. K., Voduc, D., Gao, D., Leung, S., Snider, J., Watson, M., Davies, S., Bernard, P. S., Parker, J. S., Perou, C. M., Ellis, M. J., & Nielsen, T. O. (2009). Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer. JNCI: Journal of the National Cancer Institute, 101(10), 736–750. https://doi.org/10.1093/jnci/djp082

33. Chen, G., Yu, M., Cao, J., Zhao, H., Dai, Y., Cong, Y., & Qiao, G. (2021). Identification of candidate biomarkers correlated with poor prognosis of breast cancer based on bioinformatics analysis. Bioengineered, 12(1), 5149–5161. https://doi.org/10.1080/21655979.2021.1960775

34. Chen, X., Qian, X., Xiao, M., & Zhang, P. (2023). Survival Outcomes and Efficacy of Platinum in Early Breast Cancer Patients with Germline BRCA1 or BRCA2 Mutation: A Multicenter Retrospective Cohort Study. Breast Cancer: Targets and Therapy, Volume 15, 671–682. https://doi.org/10.2147/BCTT.S423330

35. Cheng, X. (2024). A Comprehensive Review of HER2 in Cancer Biology and Therapeutics. Genes, 15(7), 903. https://doi.org/10.3390/genes15070903

36. Cheskis, B. J., Greger, J. G., Nagpal, S., & Freedman, L. P. (2007). Signaling by oestrogens. Journal of Cellular Physiology, 213(3), 610–617. https://doi.org/10.1002/jcp.21253

37. Chin, C.-H., Chen, S.-H., Wu, H.-H., Ho, C.-W., Ko, M.-T., & Lin, C.-Y. (2014). cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Systems Biology, 8(S4), S11. https://doi.org/10.1186/1752-0509-8-S4-S11

38. Choi, E., Mun, G., Lee, J., Lee, H., Cho, J., & Lee, Y.-S. (2023). BRCA1 deficiency in triple-negative breast cancer: Protein stability as a basis for therapy. Biomedicine & Pharmacotherapy, 158, 114090. https://doi.org/10.1016/j.biopha.2022.114090

39. Cicatiello, L., Addeo, R., Sasso, A., Altucci, L., Petrizzi, V. B., Borgo, R., Cancemi, M., Caporali, S., Caristi, S., Scafoglio, C., Teti, D., Bresciani, F., Perillo, B., & Weisz, A. (2004). Oestrogens and Progesterone Promote Persistent CCND1 Gene Activation during G 1 by Inducing Transcriptional Derepression via c- Jun /c- Fos /Oestrogen Receptor (Progesterone Receptor) Complex Assembly to a Distal Regulatory Element and Recruitment of Cyclin D1 to Its Own Gene Promoter. Molecular and Cellular Biology, 24(16), 7260–7274. https://doi.org/10.1128/MCB.24.16.7260-7274.2004

40. Ciriello, G., Miller, M. L., Aksoy, B. A., Senbabaoglu, Y., Schultz, N., & Sander, C. (2013). Emerging landscape of oncogenic signatures across human cancers. Nature Genetics, 45(10), 1127–1133. https://doi.org/10.1038/ng.2762

41. Claus, E. B., Schildkraut, J. M., Thompson, W. D., & Risch, N. J. (1996). The genetic attributable risk of breast and ovarian cancer. Cancer, 77(11), 2318–2324. https://doi.org/10.1002/(SICI)1097-0142(19960601)77:11<2318::AID-CNCR21>3.0.CO;2-Z

42. Colditz, G. A., & Bohlke, K. (2014). Priorities for the primary prevention of breast cancer. CA: A Cancer Journal for Clinicians, 64(3), 186–194. https://doi.org/10.3322/caac.21225

43. Collins, L. C., Cole, K. S., Marotti, J. D., Hu, R., Schnitt, S. J., & Tamimi, R. M. (2011). Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. Modern Pathology, 24(7), 924–931. https://doi.org/10.1038/modpathol.2011.54

44. Colomer, R., González-Farré, B., Ballesteros, A. I., Peg, V., Bermejo, B., Pérez-Mies, B., de la Cruz, S., Rojo, F., Pernas, S., & Palacios, J. (2024). Biomarkers in breast cancer 2024: an updated consensus statement by the Spanish Society of Medical Oncology and the Spanish Society of Pathology. Clinical and Translational Oncology, 26(12), 2935–2951. https://doi.org/10.1007/s12094-024-03541-1

45. Cortellino, S., & Longo, V. D. (2023). Metabolites and Immune Response in Tumor Microenvironments. Cancers, 15(15), 3898. https://doi.org/10.3390/cancers15153898

46. Cuppen, E., Elemento, O., Rosenquist, R., Nikic, S., IJzerman, M., Zaleski, I. D., Frederix, G., Levin, L.-Å., Mullighan, C. G., Buettner, R., Pugh, T. J., Grimmond, S., Caldas, C., Andre, F., Custers, I., Campo, E., van Snellenberg, H., Schuh, A., Nakagawa, H., … Jobanputra, V. (2022). Implementation of Whole-Genome and Transcriptome Sequencing Into Clinical Cancer Care. JCO Precision Oncology, 6. https://doi.org/10.1200/PO.22.00245

47. Dai, X., Cheng, H., Chen, X., Li, T., Zhang, J., Jin, G., Cai, D., & Huang, Z. (2019). FOXA1 is Prognostic of Triple Negative Breast Cancers by Transcriptionally Suppressing SOD2 and IL6. International Journal of Biological Sciences, 15(5), 1030–1041. https://doi.org/10.7150/ijbs.31009

48. Dan, T., Shastri, A. A., Palagani, A., Buraschi, S., Neill, T., Savage, J. E., Kapoor, A., DeAngelis, T., Addya, S., Camphausen, K., Iozzo, R. V., & Simone, N. L. (2021). miR-21 Plays a Dual Role in Tumor Formation and Cytotoxic Response in Breast Tumors. Cancers, 13(4), 888. https://doi.org/10.3390/cancers13040888

49. Davey, M. G., Cleere, E. F., O'Donnell, J. P., Gaisor, S., Lowery, A. J., & Kerin, M. J. (2022). Value of the 21-gene expression assay in predicting locoregional recurrence rates in oestrogen receptor-positive breast cancer: a systematic review and network meta-analysis. Breast Cancer Research and Treatment, 193(3), 535–544. https://doi.org/10.1007/s10549-022-06580-w

50. De Lara, S., Nyqvist, J., Werner Rönnerman, E., Helou, K., Kenne Sarenmalm, E., Einbeigi, Z., Karlsson, P., Parris, T. Z., & Kovács, A. (2019). The prognostic relevance of FOXA1 and Nestin expression in breast cancer metastases: a retrospective study of 164 cases during a 10-year period (2004–2014). BMC Cancer, 19(1), 187. https://doi.org/10.1186/s12885-019-5373-2

51. DeGraffenried, L. A., Friedrichs, W. E., Russell, D. H., Donzis, E. J., Middleton, A. K., Silva, J. M., Roth, R. A., & Hidalgo, M. (2004). Inhibition of mTOR Activity Restores Tamoxifen Response in Breast Cancer Cells with Aberrant Akt Activity. Clinical Cancer Research, 10(23), 8059–8067. https://doi.org/10.1158/1078-0432.CCR-04-0035

52. Dent, R., Trudeau, M., Pritchard, K. I., Hanna, W. M., Kahn, H. K., Sawka, C. A., Lickley, L. A., Rawlinson, E., Sun, P., & Narod, S. A. (2007). Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence. Clinical Cancer Research, 13(15), 4429–4434. https://doi.org/10.1158/1078-0432.CCR-06-3045

53. DeSantis, C. E., Ma, J., Gaudet, M. M., Newman, L. A., Miller, K. D., Goding Sauer, A., Jemal, A., & Siegel, R. L. (2019). Breast cancer statistics, 2019. CA: A Cancer Journal for Clinicians, 69(6), 438–451. https://doi.org/10.3322/caac.21583

54. Dibitetto, D., Widmer, C. A., & Rottenberg, S. (2024). PARPi, BRCA, and gaps: controversies and future research. Trends in Cancer, 10(9), 857–869. https://doi.org/10.1016/j.trecan.2024.06.008

55. Doheny, D., Manore, S. G., Wong, G. L., & Lo, H.-W. (2020). Hedgehog Signaling and Truncated GLI1 in Cancer. Cells, 9(9), 2114. https://doi.org/10.3390/cells9092114

56. Dubsky, P., Jackisch, C., Im, S.-A., Hunt, K. K., Li, C.-F., Unger, S., & Paluch-Shimon, S. (2024). BRCA genetic testing and counseling in breast cancer: how do we meet our patients' needs? Npj Breast Cancer, 10(1), 77. https://doi.org/10.1038/s41523-024-00686-8

57. Dustin, D., Gu, G., & Fuqua, S. A. W. (2019). ESR1 mutations in breast cancer. Cancer, 125(21), 3714–3728. https://doi.org/10.1002/cncr.32345

58. Dwyer, & Mary. (2024). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines ®) Genetic/Familial High-Risk Assessment: Breast, Ovarian, Pancreatic, and Prostate. https://www.nccn.org/home/member

59. Dyba, T., Randi, G., Bray, F., Martos, C., Giusti, F., Nicholson, N., Gavin, A., Flego, M., Neamtiu, L., Dimitrova, N., Negrão Carvalho, R., Ferlay, J., & Bettio, M. (2021). The European cancer burden in 2020: Incidence and mortality estimates for 40 countries and 25 major cancers. European Journal of Cancer, 157, 308–347. https://doi.org/10.1016/j.ejca.2021.07.039

60. Elnagdy, M. H., Farouk, O., Seleem, A. K., & Nada, H. A. (2018). TFF1 and TFF3 mRNAs Are Higher in Blood from Breast Cancer Patients with Metastatic Disease than Those without. Journal of Oncology, 2018, 1–8. https://doi.org/10.1155/2018/4793498

61. Ensenyat-Mendez, M., Llinàs-Arias, P., Orozco, J. I. J., Íñiguez-Muñoz, S., Salomon, M. P., Sesé, B., DiNome, M. L., & Marzese, D. M. (2021). Current Triple-Negative Breast Cancer Subtypes: Dissecting the Most Aggressive Form of Breast Cancer. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.681476

62. Fabbri, F., Salvi, S., & Bravaccini, S. (2020). Know your enemy: Genetics, aging, exposomic and inflammation in the war against triple negative breast cancer. Seminars in Cancer Biology, 60, 285–293. https://doi.org/10.1016/j.semcancer.2019.10.015

63. Fan, K. Y., Chehade, R., Qazi, M., Moravan, V., Nofech-Mozes, S., & Jerzak, K. J. (2023). Androgen Receptor Is Expressed in the Majority of Breast Cancer Brain Metastases and Is Subtype-Dependent. Cancers, 15(10), 2748. https://doi.org/10.3390/cancers15102748

64. Fan, S., Ma, Y. X., Wang, C., Yuan, R., Meng, Q., Wang, J.-A., Erdos, M., Goldberg, I. D., Webb, P., Kushner, P. J., Pestell, R. G., & Rosen, E. M. (2001). Role of direct interaction in BRCA1 inhibition of oestrogen receptor activity. Oncogene, 20(1), 77–87. https://doi.org/10.1038/sj.onc.1204073

65. Fan, S., Wang, J.-A., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., & Rosen, E. M. (1999). BRCA1 Inhibition of Oestrogen Receptor Signaling in Transfected Cells. Science, 284(5418), 1354–1356. https://doi.org/10.1126/science.284.5418.1354

66. Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T.-C., & Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & Diseases, 5(2), 77–106. https://doi.org/10.1016/j.gendis.2018.05.001

67. Fenga, C. (2016). Occupational exposure and risk of breast cancer. Biomedical Reports, 4(3), 282–292. https://doi.org/10.3892/br.2016.575

68. Ford, D., Easton, D. F., Stratton, M., Narod, S., Goldgar, D., Devilee, P., Bishop, D. T., Weber, B., Lenoir, G., Chang-Claude, J., Sobol, H., Teare, M. D., Struewing, J., Arason, A., Scherneck, S., Peto, J., Rebbeck, T. R., Tonin, P., Neuhausen, S., … Zelada-Hedman, M. (1998). Genetic Heterogeneity and Penetrance Analysis of the BRCA1 and BRCA2 Genes in Breast Cancer Families. The American Journal of Human Genetics, 62(3), 676–689. https://doi.org/10.1086/301749

69. Fuentes, N., & Silveyra, P. (2019). Oestrogen receptor signaling mechanisms (pp. 135–170). https://doi.org/10.1016/bs.apcsb.2019.01.001

70. Furuhashi, M., Saitoh, S., Shimamoto, K., & Miura, T. (2014). Fatty Acid-Binding Protein 4 (FABP4): Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and Cardiovascular Diseases. Clinical Medicine Insights: Cardiology, 8s3, CMC.S17067. https://doi.org/10.4137/CMC.S17067

71. Garcia-Martinez, L., Zhang, Y., Nakata, Y., Chan, H. L., & Morey, L. (2021). Epigenetic mechanisms in breast cancer therapy and resistance. Nature Communications, 12(1), 1786. https://doi.org/10.1038/s41467-021-22024-3

72. Garrido-Castro, A. C., Lin, N. U., & Polyak, K. (2019). Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. Cancer Discovery, 9(2), 176–198. https://doi.org/10.1158/2159-8290.CD-18-1177

73. Garrido-Castro, A. C., Spurr, L. F., Hughes, M. E., Li, Y. Y., Cherniack, A. D., Kumari, P., Lloyd, M. R., Bychkovsky, B., Barroso-Sousa, R., Di Lascio, S., Jain, E., Files, J., Mohammed-Abreu, A., Krevalin, M., MacKichan, C., Barry, W. T., Guo, H., Xia, D., Cerami, E., Lin, N. U. (2021). Genomic Characterisation of de novo Metastatic Breast Cancer. Clinical Cancer Research, 27(4), 1105–1118. https://doi.org/10.1158/1078-0432.CCR-20-1720

74. Gerratana, L., Basile, D., Buono, G., De Placido, S., Giuliano, M., Minichillo, S., Coinu, A., Martorana, F., De Santo, I., Del Mastro, L., De Laurentiis, M., Puglisi, F., & Arpino, G. (2018). Androgen receptor in triple negative breast cancer: A potential target for the targetless subtype. Cancer Treatment Reviews, 68, 102–110. https://doi.org/10.1016/j.ctrv.2018.06.005

75. Giovanella, L., Marelli, M., Ceriani, L., Giardina, G., Garancini, S., & Colombo, L. (2001). Evaluation of Chromogranin a Expression in Serum and Tissues of Breast Cancer Patients. The International Journal of Biological Markers, 16(4), 268–272. https://doi.org/10.1177/172460080101600408

76. Gonzalez-Avila, G., Sommer, B., Mendoza-Posada, D. A., Ramos, C., Garcia-Hernandez, A. A., & Falfan-Valencia, R. (2019). Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. Critical Reviews in Oncology/Hematology, 137, 57–83. https://doi.org/10.1016/j.critrevonc.2019.02.010

77. Govindan, S., Siraganahalli Eswaraiah, M., Basavaraj, C., Adinarayan, M., Sankaran, S., & Bakre, M. (2020). Androgen Receptor mRNA levels determine the prognosis in triple-negative breast cancer patients. BMC Cancer, 20(1), 745. https://doi.org/10.1186/s12885-020-07218-0

78. Grasset, E. M., Dunworth, M., Sharma, G., Loth, M., Tandurella, J., Cimino-Mathews, A., Gentz, M., Bracht, S., Haynes, M., Fertig, E. J., & Ewald, A. J. (2022). Triple-negative breast cancer metastasis involves complex epithelial-mesenchymal transition dynamics and requires vimentin. Science Translational Medicine, 14(656). https://doi.org/10.1126/scitranslmed.abn7571

79. Guijarro, L. G., Justo Bermejo, F. J., Boaru, D. L., De Castro-Martinez, P., De Leon-Oliva, D., Fraile-Martínez, O., Garcia-Montero, C., Alvarez-Mon, M., Toledo-Lobo, M. del V., & Ortega, M. A. (2023a). Is Insulin Receptor Substrate4 (IRS4) a Platform Involved in the Activation of Several Oncogenes? Cancers, 15(18), 4651. https://doi.org/10.3390/cancers15184651

80. Gutierrez, C., & Schiff, R. (2011). HER2: Biology, Detection, and Clinical Implications. Archives of Pathology & Laboratory Medicine, 135(1), 55–62. https://doi.org/10.5858/2010-0454-RAR.1

81. Haffty, B. G., Yang, Q., Reiss, M., Kearney, T., Higgins, S. A., Weidhaas, J., Harris, L., Hait, W., & Toppmeyer, D. (2006). Locoregional Relapse and Distant Metastasis in Conservatively Managed Triple Negative Early-Stage Breast Cancer. Journal of Clinical Oncology, 24(36), 5652–5657. https://doi.org/10.1200/JCO.2006.06.5664

82. Han, Y., Rovella, V., Smirnov, A., Buonomo, O. C., Mauriello, A., Perretta, T., Shi, Y., Woodmsith, J., Bischof, J., Bove, P., Juhl, H., Scimeca, M., Sica, G., Tisone, G., Wang, Y., Giacobbi, E., Materazzo, M., Melino, G., Candi, E., & Bernassola, F. (2023). A BRCA2 germline mutation and high expression of immune checkpoints in a TNBC patient. Cell Death Discovery, 9(1), 370. https://doi.org/10.1038/s41420-023-01651-3

83. Hanahan, D. (2022). Hallmarks of Cancer: New Dimensions. Cancer Discovery, 12(1), 31–46. https://doi.org/10.1158/2159-8290.CD-21-1059

84. Hanahan, D., & Weinberg, R. A. (2000). The Hallmarks of Cancer. Cell, 100(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9

85. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. Cell, 144(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

86. Hao, J., Yan, F., Zhang, Y., Triplett, A., Zhang, Y., Schultz, D. A., Sun, Y., Zeng, J., Silverstein, K. A. T., Zheng, Q., Bernlohr, D. A., Cleary, M. P., Egilmez, N. K., Sauter, E., Liu, S., Suttles, J., & Li, B. (2018). Expression of Adipocyte/Macrophage Fatty Acid–Binding Protein in Tumor-Associated Macrophages Promotes Breast Cancer Progression. Cancer Research, 78(9), 2343–2355. https://doi.org/10.1158/0008-5472.CAN-17-2465

87. Helleday, T. (2011). The underlying mechanism for the PARP and BRCA synthetic lethality: Clearing up the misunderstandings. Molecular Oncology, 5(4), 387–393. https://doi.org/10.1016/j.molonc.2011.07.001

88. Hong, M., Tao, S., Zhang, L., Diao, L.-T., Huang, X., Huang, S., Xie, S.-J., Xiao, Z.-D., & Zhang, H. (2020). RNA sequencing: new technologies and applications in cancer research. Journal of Hematology & Oncology, 13(1), 166. https://doi.org/10.1186/s13045-020-01005-x

89. Horst, K. C., Hancock, S. L., Ognibene, G., Chen, C., Advani, R. H., Rosenberg, S. A., Donaldson, S. S., & Hoppe, R. T. (2014). Histologic subtypes of breast cancer following radiotherapy for Hodgkin lymphoma. Annals of Oncology, 25(4), 848–851. https://doi.org/10.1093/annonc/mdu017

90. Hosseini, H., Obradović, M. M. S., Hoffmann, M., Harper, K. L., Sosa, M. S., Werner-Klein, M., Nanduri, L. K., Werno, C., Ehrl, C., Maneck, M., Patwary, N., Haunschild, G., Gužvić, M., Reimelt, C., Grauvogl, M., Eichner, N., Weber, F., Hartkopf, A. D., Taran, F.-A., … Klein, C. A. (2016). Early dissemination seeds metastasis in breast cancer. Nature, 540(7634), 552–558. https://doi.org/10.1038/nature20785

91. Hoxhaj, G., & Manning, B. D. (2020). The PI3K–AKT network at the interface of oncogenic signalling and cancer metabolism. Nature Reviews Cancer, 20(2), 74–88. https://doi.org/10.1038/s41568-019-0216-7

92. Hu, H., Tong, K., Tsang, J. Y., Ko, C. W., Tam, F., Loong, T. C., & Tse, G. M. (2024). Subtyping of triple-negative breast cancers: its prognostication and implications in diagnosis of breast origin. ESMO Open, 9(4), 102993. https://doi.org/10.1016/j.esmoop.2024.102993

93. Hu, Y., Wang, C., Liang, H., Li, J., & Yang, Q. (2024). The treatment landscape of triple-negative breast cancer. Medical Oncology, 41(10), 236. https://doi.org/10.1007/s12032-024-02456-9

94. Huang, H. (2018). Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors, 18(10), 3249. https://doi.org/10.3390/s18103249

95. Hwang, K.-T., Kim, J., Jung, J., Chang, J. H., Chai, Y. J., Oh, S. W., Oh, S., Kim, Y. A., Park, S. B., & Hwang, K. R. (2019). Impact of Breast Cancer Subtypes on Prognosis of Women with Operable Invasive Breast Cancer: A Population-based Study Using SEER Database. Clinical Cancer Research, 25(6), 1970–1979. https://doi.org/10.1158/1078-0432.CCR-18-2782

96. Iamaroon, A., Pongsiriwet, S., Jittidecharaks, S., Pattanaporn, K., Prapayasatok, S., & Wanachantararak, S. (2003). Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. Journal of Oral Pathology & Medicine, 32(4), 195–199. https://doi.org/10.1034/j.1600-0714.2003.00128.x

97. Ikink, G. J., Boer, M., Bakker, E. R. M., & Hilkens, J. (2016). IRS4 induces mammary tumorigenesis and confers resistance to HER2-targeted therapy through constitutive PI3K/AKT-pathway hyperactivation. Nature Communications, 7(1), 13567. https://doi.org/10.1038/ncomms13567

98. Iqbal, N., & Iqbal, N. (2014). Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Molecular Biology International, 2014, 1–9. https://doi.org/10.1155/2014/852748

99. Isakoff, S. J., Engelman, J. A., Irie, H. Y., Luo, J., Brachmann, S. M., Pearline, R. V., Cantley, L. C., & Brugge, J. S. (2005). Breast Cancer–Associated PIK3CA Mutations Are Oncogenic in Mammary Epithelial Cells. Cancer Research, 65(23), 10992–11000. https://doi.org/10.1158/0008-5472.CAN-05-2612

100. Jackson, S. P., & Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature, 461(7267), 1071–1078. https://doi.org/10.1038/nature08467

101. Jang, M. H., Kim, H. J., Kim, E. J., Chung, Y. R., & Park, S. Y. (2015). Expression of epithelial-mesenchymal transition–related markers in triple-negative breast cancer: ZEB1 as a potential biomarker for poor clinical outcome. Human Pathology, 46(9), 1267–1274. https://doi.org/10.1016/j.humpath.2015.05.010

102. Jeng, K.S., Yu, M.C., Hsiau, H.I., Chang, F.Y., Sheen, I.S., & Jeng, W.J. (2013). High expression of Sonic Hedgehog signaling pathway genes indicates a risk of recurrence of breast carcinoma. OncoTargets and Therapy, 79. https://doi.org/10.2147/OTT.S54702

103. Jiang, J., & Hui, C. (2008). Hedgehog Signaling in Development and Cancer. Developmental Cell, 15(6), 801–812. https://doi.org/10.1016/j.devcel.2008.11.010

104. Kalavska, K., Cierna, Z., Karaba, M., Minarik, G., Benca, J., Sedlackova, T., Kolekova, D., Mrvova, I., Pindak, D., Mardiak, J., & Mego, M. (2020). Prognostic role of matrix metalloproteinase 9 in early breast cancer. Oncology Letters, 21(2), 78. https://doi.org/10.3892/ol.2020.12339

105. Kamdje, A. H. N. (2014). New targeted therapies for breast cancer: A focus on tumor microenvironmental signals and chemoresistant breast cancers. World Journal of Clinical Cases, 2(12), 769. https://doi.org/10.12998/wjcc.v2.i12.769

106. Kankkunen, J.-P., Harvima, I. T., & Naukkarinen, A. (1997). Quantitative analysis of tryptase and chymase containing mast cells in benign and malignant breast lesions. International Journal of Cancer, 72(3), 385–388. https://doi.org/10.1002/(SICI)1097-0215(19970729)72:3<385::AID-IJC1>3.0.CO;2-L

107. Kast, K., John, E. M., Hopper, J. L., Andrieu, N., Noguès, C., Mouret-Fourme, E., Lasset, C., Fricker, J.-P., Berthet, P., Mari, V., Salle, L., Schmidt, M. K., Ausems, M. G. E. M., Garcia, E. B. G., van de Beek, I., Wevers, M. R., Evans, D. G., Tischkowitz, M., Lalloo, F., … Engel, C. (2023). Associations of height, body mass index, and weight gain with breast cancer risk in carriers of a pathogenic variant in BRCA1 or BRCA2: the BRCA1 and BRCA2 Cohort Consortium. Breast Cancer Research, 25(1), 72. https://doi.org/10.1186/s13058-023-01673-w

108. Kensler, K. H., Sankar, V. N., Wang, J., Zhang, X., Rubadue, C. A., Baker, G. M., Parker, J. S., Hoadley, K. A., Stancu, A. L., Pyle, M. E., Collins, L. C., Hunter, D. J., Eliassen, A. H., Hankinson, S. E., Tamimi, R. M., & Heng, Y. J. (2019). PAM50 Molecular Intrinsic Subtypes in the Nurses' Health Study Cohorts. Cancer Epidemiology, Biomarkers & Prevention, 28(4), 798–806. https://doi.org/10.1158/1055-9965.EPI-18-0863

109. Kesson, E. M., Allardice, G. M., George, W. D., Burns, H. J. G., & Morrison, D. S. (2012). Effects of multidisciplinary team working on breast cancer survival: retrospective, comparative, interventional cohort study of 13 722 women. BMJ, 344(apr26 1), e2718–e2718. https://doi.org/10.1136/bmj.e2718

110. Kim, S., Moon, B.-I., Lim, W., Park, S., Cho, M. S., & Sung, S. H. (2016). Expression patterns of GATA3 and the androgen receptor are strongly correlated in patients with triple-negative breast cancer. Human Pathology, 55, 190–195. https://doi.org/10.1016/j.humpath.2016.04.013

111. Kruk, J., Aboul-Enein, B. H., Bernstein, J., & Gronostaj, M. (2019). Psychological Stress and Cellular Aging in Cancer: A Meta-Analysis. Oxidative Medicine and Cellular Longevity, 2019, 1–23. https://doi.org/10.1155/2019/1270397

112. Kuchenbaecker, K. B., Hopper, J. L., Barnes, D. R., Phillips, K.-A., Mooij, T. M., Roos-Blom, M.-J., Jervis, S., van Leeuwen, F. E., Milne, R. L., Andrieu, N., Goldgar, D. E., Terry, M. B., Rookus, M. A., Easton, D. F., Antoniou, A. C., McGuffog, L., Evans, D. G., Barrowdale, D., Frost, D., … Olsson, H. (2017). Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA, 317(23), 2402. https://doi.org/10.1001/jama.2017.7112

113. Kudelova, E., Smolar, M., Holubekova, V., Hornakova, A., Dvorska, D., Lucansky, V., Koklesova, L., Kudela, E., & Kubatka, P. (2022). Genetic Heterogeneity, Tumor Microenvironment and Immunotherapy in Triple-Negative Breast Cancer. International Journal of Molecular Sciences, 23(23), 14937. https://doi.org/10.3390/ijms232314937

114. Kumar, U., Ardasheva, A., Mahmud, Z., Coombes, R. C., & Yagüe, E. (2021). FOXA1 is a determinant of drug resistance in breast cancer cells. Breast Cancer Research and Treatment, 186(2), 317–326. https://doi.org/10.1007/s10549-020-06068-5

115. Kumar, V., Green, S., Stack, G., Berry, M., Jin, J.-R., & Chambon, P. (1987). Functional domains of the human oestrogen receptor. Cell, 51(6), 941–951. https://doi.org/10.1016/0092-8674(87)90581-2

116. Kwa, M. J., & Adams, S. (2018). Checkpoint inhibitors in triple-negative breast cancer (TNBC): Where to go from here. Cancer, 124(10), 2086–2103. https://doi.org/10.1002/cncr.31272

117. Lee, E. Y. H. P., & Muller, W. J. (2010). Oncogenes and Tumor Suppressor Genes. Cold Spring Harbor Perspectives in Biology, 2(10), a003236–a003236. https://doi.org/10.1101/cshperspect.a003236

118. Lehmann, B. D., Bauer, J. A., Chen, X., Sanders, M. E., Chakravarthy, A. B., Shyr, Y., & Pietenpol, J. A. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. Journal of Clinical Investigation, 121(7), 2750–2767. https://doi.org/10.1172/JCI45014

119. Lehmann, B. D., Jovanović, B., Chen, X., Estrada, M. V., Johnson, K. N., Shyr, Y., Moses, H. L., Sanders, M. E., & Pietenpol, J. A. (2016). Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLOS ONE, 11(6), e0157368. https://doi.org/10.1371/journal.pone.0157368

120. Leifler, K. S., Svensson, S., Abrahamsson, A., Bendrik, C., Robertson, J., Gauldie, J., Olsson, A.-K., & Dabrosin, C. (2013). Inflammation Induced by MMP-9 Enhances Tumor Regression of Experimental Breast Cancer. The Journal of Immunology, 190(8), 4420–4430. https://doi.org/10.4049/jimmunol.1202610

121. Lejeune, M., Reverté, L., Gallardo, N., Sauras, E., Bosch, R., Mata, D., Roso, A., Petit, A., Peg, V., Riu, F., García-Fontgivell, J., Relea, F., Vieites, B., de la Cruz-Merino, L., Arenas, M., Rodriguez, V., Galera, J., Korzynska, A., Plancoulaine, B., … López, C. (2023). Matrix Metalloproteinase-9 Expression Is Associated with the Absence of Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer Patients. International Journal of Molecular Sciences, 24(14), 11297. https://doi.org/10.3390/ijms241411297

122. Li, X., Lu, Y., Liang, K., Hsu, J.-M., Albarracin, C., Mills, G. B., Hung, M.-C., & Fan, Z. (2012). Brk/PTK6 sustains activated EGFR signaling through inhibiting EGFR degradation and transactivating EGFR. Oncogene, 31(40), 4372–4383. https://doi.org/10.1038/onc.2011.608

123. Li, Y., Zhan, Z., Yin, X., Fu, S., & Deng, X. (2021). Targeted Therapeutic Strategies for Triple-Negative Breast Cancer. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.731535

124. Litton, J. K., Hurvitz, S. A., Mina, L. A., Rugo, H. S., Lee, K.-H., Gonçalves, A., Diab, S., Woodward, N., Goodwin, A., Yerushalmi, R., Roché, H., Im, Y.-H., Eiermann, W., Quek, R. G. W., Usari, T., Lanzalone, S., Czibere, A., Blum, J. L., Martin, M., & Ettl, J. (2020). Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: final overall survival results from the EMBRACA trial. Annals of Oncology, 31(11), 1526–1535. https://doi.org/10.1016/j.annonc.2020.08.2098

125. Liu, S., Wu, D., Fan, Z., Yang, J., Li, Y., Meng, Y., Gao, C., & Zhan, H. (2022). FABP4 in obesity-associated carcinogenesis: Novel insights into mechanisms and therapeutic implications. Frontiers in Molecular Biosciences, 9. https://doi.org/10.3389/fmolb.2022.973955

126. Llop-Guevara, A., Vladimirova, V., Schneeweiss, A., Villacampa, G., Karn, T., Zahm, D.-M., Herencia-Ropero, A., Jank, P., van Mackelenbergh, M., Fasching, P. A., Marmé, F., Stickeler, E., Schem, C., Dienstmann, R., Florian, S., Nekljudova, V., Balmaña, J., Denkert, C., Loibl, S., & Serra, V. (2021). 2O Association of RAD51 with homologous recombination deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): Analysis of the GeparSixto randomised clinical trial. Annals of Oncology, 32, S22. https://doi.org/10.1016/j.annonc.2021.03.016

127. Loboda, A. P., Adonin, L. S., Zvereva, S. D., Guschin, D. Y., Korneenko, T. V., Telegina, A. V., Kondratieva, O. K., Frolova, S. E., Pestov, N. B., & Barlev, N. A. (2023). BRCA Mutations – The Achilles Heel of Breast, Ovarian and Other Epithelial Cancers. International Journal of Molecular Sciences, 24(5), 4982. https://doi.org/10.3390/ijms24054982

128. Loibl, S., André, F., Bachelot, T., Barrios, C. H., Bergh, J., Burstein, H. J., Cardoso, M. J., Carey, L. A., Dawood, S., Del Mastro, L., Denkert, C., Fallenberg, E. M., Francis, P. A., Gamal-Eldin, H., Gelmon, K., Geyer, C. E., Gnant, M., Guarneri, V., Gupta, S., … Harbeck, N. (2024). Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Annals of Oncology, 35(2), 159–182. https://doi.org/10.1016/j.annonc.2023.11.016

129. Lopez, M., Spehner, L., André, F., Viot, J., Seffar, E., Marguier, A., Curtit, E., Meynard, G., Dobi, E., Ladoire, S., Boidot, R., Loyon, R., Derangere, V., Bidard, F.-C., Borg, C., Mansi, L., & Kroemer, M. (2025). Exploring the role of ESR1 mutations in metastatic hormone receptor-positive breast cancer T cell immune surveillance disruption. Breast Cancer Research, 27(1), 19. https://doi.org/10.1186/s13058-025-01962-6

130. Lord, C. J., & Ashworth, A. (2012). The DNA damage response and cancer therapy. Nature, 481(7381), 287–294. https://doi.org/10.1038/nature10760

131. Lord, C. J., & Ashworth, A. (2017). PARP inhibitors: Synthetic lethality in the clinic. Science, 355(6330), 1152–1158. https://doi.org/10.1126/science.aam7344

132. Loza, P., Irmejs, A., Daneberga, Z., Miklasevics, E., Berga-Svitina, E., Subatniece, S., Maksimenko, J., Trofimovics, G., Tauvena, E., Ukleikins, S., & Gardovskis, J. (2021). A novel frequent BRCA1 recurrent variant c.5117G > A (p.Gly1206Glu) identified after 20 years of BRCA1/2 research in the Baltic region: cohort study and literature review. Hereditary Cancer in Clinical Practice, 19(1), 11. https://doi.org/10.1186/s13053-021-00168-z

133. Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review. Cancers, 13(17), 4287. https://doi.org/10.3390/cancers13174287

134. Mahdavi, M., Nassiri, M., Kooshyar, M. M., Vakili-Azghandi, M., Avan, A., Sandry, R., Pillai, S., Lam, A. K., & Gopalan, V. (2019). Hereditary breast cancer; Genetic penetrance and current status with BRCA. Journal of Cellular Physiology, 234(5), 5741–5750. https://doi.org/10.1002/jcp.27464

135. Makki, J. (2015). Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. Clinical Medicine Insights: Pathology, 8. https://doi.org/10.4137/CPath.S31563

136. Maksimenko, J., Irmejs, A., Trofimovičs, G., Bērziņa, D., Skuja, E., Purkalne, G., Miklaševičs, E., & Gardovskis, J. (2018). High frequency of pathogenic non-founder germline mutations in BRCA1 and BRCA2 in families with breast and ovarian cancer in a founder population. Hereditary Cancer in Clinical Practice, 16(1), 12. https://doi.org/10.1186/s13053-018-0094-0

137. Manié, E., Vincent-Salomon, A., Lehmann-Che, J., Pierron, G., Turpin, E., Warcoin, M., Gruel, N., Lebigot, I., Sastre-Garau, X., Lidereau, R., Remenieras, A., Feunteun, J., Delattre, O., de Thé, H., Stoppa-Lyonnet, D., & Stern, M.-H. (2009). High Frequency of TP53 Mutation in BRCA1 and Sporadic Basal-like Carcinomas but not in BRCA1 Luminal Breast Tumors. Cancer Research, 69(2), 663–671. https://doi.org/10.1158/0008-5472.CAN-08-1560

138. Mars, N., Kerminen, S., Tamlander, M., Pirinen, M., Jakkula, E., Aaltonen, K., Meretoja, T., Heinävaara, S., Widén, E., Ripatti, S., Palotie, A., Daly, M., Riley-Gills, B., Jacob, H., Paul, D., Matakidou, A., Platt, A., Runz, H., John, S., … Southerington, T. (2024). Comprehensive Inherited Risk Estimation for Risk-Based Breast Cancer Screening in Women. Journal of Clinical Oncology, 42(13), 1477–1487. https://doi.org/10.1200/JCO.23.00295

139. Maturi, V., Enroth, S., Heldin, C., & Moustakas, A. (2018). Genome–wide binding of transcription factor ZEB1 in triple-negative breast cancer cells. Journal of Cellular Physiology, 233(10), 7113–7127. https://doi.org/10.1002/jcp.26634

140. Mavaddat, N., Barrowdale, D., Andrulis, I. L., Domchek, S. M., Eccles, D., Nevanlinna, H., Ramus, S. J., Spurdle, A., Robson, M., Sherman, M., Mulligan, A. M., Couch, F. J., Engel, C., McGuffog, L., Healey, S., Sinilnikova, O. M., Southey, M. C., Terry, M. B., Goldgar, D., … Antoniou, A. C. (2012). Pathology of Breast and Ovarian Cancers among BRCA1 and BRCA2 Mutation Carriers: Results from the Consortium of Investigators of Modifiers of BRCA1 / 2 (CIMBA). Cancer Epidemiology, Biomarkers & Prevention, 21(1), 134–147. https://doi.org/10.1158/1055-9965.EPI-11-0775

141. Mayer, I. A., & Arteaga, C. L. (2016). The PI3K/AKT Pathway as a Target for Cancer Treatment. Annual Review of Medicine, 67(1), 11–28. https://doi.org/10.1146/annurev-med-062913-051343

142. McDevitt, T., Durkie, M., Arnold, N., Burghel, G. J., Butler, S., Claes, K. B. M., Logan, P., Robinson, R., Sheils, K., Wolstenholme, N., Hanson, H., Turnbull, C., & Hume, S. (2024). EMQN best practice guidelines for genetic testing in hereditary breast and ovarian cancer. In European Journal of Human Genetics (Vol. 32, Issue 5, pp. 479–488). Springer Nature. https://doi.org/10.1038/s41431-023-01507-5

143. Mercogliano, M. F., Bruni, S., Mauro, F. L., & Schillaci, R. (2023). Emerging Targeted Therapies for HER2-Positive Breast Cancer. Cancers, 15(7), 1987. https://doi.org/10.3390/cancers15071987

144. Metovic, J., Borella, F., D'Alonzo, M., Biglia, N., Mangherini, L., Tampieri, C., Bertero, L., Cassoni, P., & Castellano, I. (2022a). FOXA1 in Breast Cancer: A Luminal Marker with Promising Prognostic and Predictive Impact. Cancers, 14(19), 4699. https://doi.org/10.3390/cancers14194699

145. Metovic, J., Borella, F., D'Alonzo, M., Biglia, N., Mangherini, L., Tampieri, C., Bertero, L., Cassoni, P., & Castellano, I. (2022b). FOXA1 in Breast Cancer: A Luminal Marker with Promising Prognostic and Predictive Impact. Cancers, 14(19), 4699. https://doi.org/10.3390/cancers14194699

146. Miah, S., Martin, A., & Lukong, K. E. (2012). Constitutive activation of breast tumor kinase accelerates cell migration and tumor growth in vivo. Oncogenesis, 1(5), e11–e11. https://doi.org/10.1038/oncsis.2012.11

147. Michailidou, K., Lindström, S., Dennis, J., Beesley, J., Hui, S., Kar, S., Lemaçon, A., Soucy, P., Glubb, D., Rostamianfar, A., Bolla, M. K., Wang, Q., Tyrer, J., Dicks, E., Lee, A., Wang, Z., Allen, J., Keeman, R., Eilber, U., … Easton, D. F. (2017). Association analysis identifies 65 new breast cancer risk loci. Nature, 551(7678), 92–94. https://doi.org/10.1038/nature24284

148. Mittal, S., Subramanyam, D., Dey, D., Kumar, R. V, & Rangarajan, A. (2009). Cooperation of Notch and Ras/MAPK signaling pathways in human breast carcinogenesis. Molecular Cancer, 8(1), 128. https://doi.org/10.1186/1476-4598-8-128

149. Miziak, P., Baran, M., Błaszczak, E., Przybyszewska-Podstawka, A., Kałafut, J., Smok-Kalwat, J., Dmoszyńska-Graniczka, M., Kiełbus, M., & Stepulak, A. (2023). Oestrogen Receptor Signaling in Breast Cancer. Cancers, 15(19), 4689. https://doi.org/10.3390/cancers15194689

150. Mohammed, A. A. (2021). Prognostic parameter differences in breast cancer patients between luminal A and luminal B types after application of the new classification according to Ki67 score. International Journal of Surgery Open, 34, 100357. https://doi.org/10.1016/j.ijso.2021.100357

151. Mohammed, A. A., Elsayed, F. M., Algazar, M., Rashed, H. E., & Anter, A. H. (2020). Neoadjuvant Chemotherapy in Triple Negative Breast Cancer: Correlation between Androgen Receptor Expression and Pathological Response. Asian Pacific Journal of Cancer Prevention, 21(2), 563–568. https://doi.org/10.31557/APJCP.2020.21.2.563

152. Moo, T.-A., Sanford, R., Dang, C., & Morrow, M. (2018). Overview of Breast Cancer Therapy. PET Clinics, 13(3), 339–354. https://doi.org/10.1016/j.cpet.2018.02.006

153. Murphy, C. G., & Dickler, M. N. (2015). The Role of CDK4/6 Inhibition in Breast Cancer. The Oncologist, 20(5), 483–490. https://doi.org/10.1634/theoncologist.2014-0443

154. Nik-Zainal, S., Davies, H., Staaf, J., Ramakrishna, M., Glodzik, D., Zou, X., Martincorena, I., Alexandrov, L. B., Martin, S., Wedge, D. C., Van Loo, P., Ju, Y. S., Smid, M., Brinkman, A. B., Morganella, S., Aure, M. R., Lingjærde, O. C., Langerød, A., Ringnér, M., … Stratton, M. R. (2016). Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature, 534(7605), 47–54. https://doi.org/10.1038/nature17676

155. Nolan, E., Lindeman, G. J., & Visvader, J. E. (2023). Deciphering breast cancer: from biology to the clinic. Cell, 186(8), 1708–1728. https://doi.org/10.1016/j.cell.2023.01.040

156. Nwabo Kamdje, A. H., Seke Etet, P. F., Vecchio, L., Muller, J. M., Krampera, M., & Lukong, K. E. (2014). Signaling pathways in breast cancer: Therapeutic targeting of the microenvironment. Cellular Signalling, 26(12), 2843–2856. https://doi.org/10.1016/j.cellsig.2014.07.034

157. Obidiro, O., Battogtokh, G., & Akala, E. O. (2023). Triple Negative Breast Cancer Treatment Options and Limitations: Future Outlook. Pharmaceutics, 15(7), 1796. https://doi.org/10.3390/pharmaceutics15071796

158. Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Buján, J., García-Honduvilla, N., & Coca, S. (2020). Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/mTOR. Journal of Oncology, 2020, 1–11. https://doi.org/10.1155/2020/9258396

159. Oshi, M., Gandhi, S., Wu, R., Asaoka, M., Yan, L., Yamada, A., Yamamoto, S., Narui, K., Chishima, T., Ishikawa, T., Endo, I., & Takabe, K. (2022). Development of a novel BRCAness score that predicts response to PARP inhibitors. Biomarker Research, 10(1), 80. https://doi.org/10.1186/s40364-022-00427-8

160. Owe-Larsson, M., Pawłasek, J., Piecha, T., Sztokfisz-Ignasiak, A., Pater, M., & Janiuk, I. R. (2023). The Role of Cocaine- and Amphetamine-Regulated Transcript (CART) in Cancer: A Systematic Review. International Journal of Molecular Sciences, 24(12), 9986. https://doi.org/10.3390/ijms24129986

161. Pal, S. K., Childs, B. H., & Pegram, M. (2011). Triple negative breast cancer: unmet medical needs. Breast Cancer Research and Treatment, 125(3), 627–636. https://doi.org/10.1007/s10549-010-1293-1

162. Pérez-Tenorio, G., & Stål, O. (2002). Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. British Journal of Cancer, 86(4), 540–545. https://doi.org/10.1038/sj.bjc.6600126

163. Perou, C. M., Sørlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., Pollack, J. R., Ross, D. T., Johnsen, H., Akslen, L. A., Fluge, Ø., Pergamenschikov, A., Williams, C., Zhu, S. X., Lønning, P. E., Børresen-Dale, A.-L., Brown, P. O., & Botstein, D. (2000). Molecular portraits of human breast tumours. Nature, 406(6797), 747–752. https://doi.org/10.1038/35021093

164. Perry, N., Broeders, M., de Wolf, C., Törnberg, S., Holland, R., & von Karsa, L. (2008). European guidelines for quality assurance in breast cancer screening and diagnosis. Fourth edition – summary document. Annals of Oncology, 19(4), 614–622. https://doi.org/10.1093/annonc/mdm481

165. Peters, J. M., & Gonzalez, F. J. (2018). The Evolution of Carcinogenesis. Toxicological Sciences, 165(2), 272–276. https://doi.org/10.1093/toxsci/kfy184

166. Poggio, F., Bruzzone, M., Ceppi, M., Pondé, N. F., La Valle, G., Del Mastro, L., de Azambuja, E., & Lambertini, M. (2018). Platinum-based neoadjuvant chemotherapy in triple-negative breast cancer: a systematic review and meta-analysis. Annals of Oncology, 29(7), 1497–1508. https://doi.org/10.1093/annonc/mdy127

167. Polyak, K. (2007). Breast cancer: origins and evolution. Journal of Clinical Investigation, 117(11), 3155–3163. https://doi.org/10.1172/JCI33295

168. Popova, N. V., & Jücker, M. (2022). The Functional Role of Extracellular Matrix Proteins in Cancer. Cancers, 14(1), 238. https://doi.org/10.3390/cancers14010238

169. Prakash, R., Zhang, Y., Feng, W., & Jasin, M. (2015). Homologous Recombination and Human Health: The Roles of BRCA1, BRCA2, and Associated Proteins. Cold Spring Harbor Perspectives in Biology, 7(4), a016600. https://doi.org/10.1101/cshperspect.a016600

170. Prat, A., Fan, C., Fernández, A., Hoadley, K. A., Martinello, R., Vidal, M., Viladot, M., Pineda, E., Arance, A., Muñoz, M., Paré, L., Cheang, M. C. U., Adamo, B., & Perou, C. M. (2015). Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy. BMC Medicine, 13(1), 303. https://doi.org/10.1186/s12916-015-0540-z

171. Prat, A., Pineda, E., Adamo, B., Galván, P., Fernández, A., Gaba, L., Díez, M., Viladot, M., Arance, A., & Muñoz, M. (2015). Clinical implications of the intrinsic molecular subtypes of breast cancer. The Breast, 24, S26–S35. https://doi.org/10.1016/j.breast.2015.07.008

172. Puppe, J., Seifert, T., Eichler, C., Pilch, H., Mallmann, P., & Malter, W. (2020). Genomic Signatures in Luminal Breast Cancer. Breast Care, 15(4), 355–365. https://doi.org/10.1159/000509846

173. Qiong, L., & Yin, J. (2021). Orosomucoid 1 promotes epirubicin resistance in breast cancer by upregulating the expression of matrix metalloproteinases 2 and 9. Bioengineered, 12(1), 8822–8832. https://doi.org/10.1080/21655979.2021.1987067

174. Querzoli, P., Pedriali, M., Rinaldi, R., Secchiero, P., Rossi, P. G., & Kuhn, E. (2021). GATA3 as an Adjunct Prognostic Factor in Breast Cancer Patients with Less Aggressive Disease: A Study with a Review of the Literature. Diagnostics, 11(4), 604. https://doi.org/10.3390/diagnostics11040604

175. Rebbeck, T. R., Kauff, N. D., & Domchek, S. M. (2009). Meta-analysis of Risk Reduction Estimates Associated With Risk-Reducing Salpingo-oophorectomy in BRCA1 or BRCA2 Mutation Carriers. JNCI Journal of the National Cancer Institute, 101(2), 80–87. https://doi.org/10.1093/jnci/djn442

176. Redig, A. J., & McAllister, S. S. (2013). Breast cancer as a systemic disease: a view of metastasis. Journal of Internal Medicine, 274(2), 113–126. https://doi.org/10.1111/joim.12084

177. Reedijk, M., Odorcic, S., Chang, L., Zhang, H., Miller, N., McCready, D. R., Lockwood, G., & Egan, S. E. (2005). High-level Coexpression of JAG1 and NOTCH1 Is Observed in Human Breast Cancer and Is Associated with Poor Overall Survival. Cancer Research, 65(18), 8530–8537. https://doi.org/10.1158/0008-5472.CAN-05-1069

178. Renoir, J.-M., Marsaud, V., & Lazennec, G. (2013). Oestrogen receptor signaling as a target for novel breast cancer therapeutics. Biochemical Pharmacology, 85(4), 449–465. https://doi.org/10.1016/j.bcp.2012.10.018

179. Rodríguez-Bejarano, O. H., Parra-López, C., & Patarroyo, M. A. (2024). A review concerning the breast cancer-related tumour microenvironment. Critical Reviews in Oncology/Hematology, 199, 104389. https://doi.org/10.1016/j.critrevonc.2024.104389

180. Romond, E. H., Perez, E. A., Bryant, J., Suman, V. J., Geyer, C. E., Davidson, N. E., Tan-Chiu, E., Martino, S., Paik, S., Kaufman, P. A., Swain, S. M., Pisansky, T. M., Fehrenbacher, L., Kutteh, L. A., Vogel, V. G., Visscher, D. W., Yothers, G., Jenkins, R. B., Brown, A. M., … Wolmark, N. (2005). Trastuzumab plus Adjuvant Chemotherapy for Operable HER2-Positive Breast Cancer. New England Journal of Medicine, 353(16), 1673–1684. https://doi.org/10.1056/NEJMoa052122

181. Rosati, D., Palmieri, M., Brunelli, G., Morrione, A., Iannelli, F., Frullanti, E., & Giordano, A. (2024). Differential gene expression analysis pipelines and bioinformatic tools for the identification of specific biomarkers: A review. Computational and Structural Biotechnology Journal, 23, 1154–1168. https://doi.org/10.1016/j.csbj.2024.02.018

182. Roskoski, R. (2014). The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacological Research, 79, 34–74. https://doi.org/10.1016/j.phrs.2013.11.002

183. Sadida, H. Q., Abdulla, A., Marzooqi, S. Al, Hashem, S., Macha, M. A., Akil, A. S. A.-S., & Bhat, A. A. (2024). Epigenetic modifications: Key players in cancer heterogeneity and drug resistance. Translational Oncology, 39, 101821. https://doi.org/10.1016/j.tranon.2023.101821

184. Said, T. K., Conneely, O. M., Medina, D., O'Malley, B. W., & Lydon, J. P. (1997). Progesterone, in Addition to Oestrogen, Induces Cyclin D1 Expression in the Murine Mammary Epithelial Cell, in Vivo*. Endocrinology, 138(9), 3933–3939. https://doi.org/10.1210/endo.138.9.5436

185. Schmid, P., Cortes, J., Dent, R., Pusztai, L., McArthur, H., Kümmel, S., Bergh, J., Denkert, C., Park, Y. H., Hui, R., Harbeck, N., Takahashi, M., Untch, M., Fasching, P. A., Cardoso, F., Andersen, J., Patt, D., Danso, M., Ferreira, M., … O'Shaughnessy, J. (2022). Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. New England Journal of Medicine, 386(6), 556–567. https://doi.org/10.1056/NEJMoa2112651

186. Schwartz, R. S., & Erban, J. K. (2017). Timing of Metastasis in Breast Cancer. New England Journal of Medicine, 376(25), 2486–2488. https://doi.org/10.1056/NEJMcibr1701388

187. Sergina, N. V., & Moasser, M. M. (2007). The HER family and cancer: emerging molecular mechanisms and therapeutic targets. Trends in Molecular Medicine, 13(12), 527–534. https://doi.org/10.1016/j.molmed.2007.10.002

188. Serrano García, L., Jávega, B., Llombart Cussac, A., Gión, M., Pérez-García, J. M., Cortés, J., & Fernández-Murga, M. L. (2024). Patterns of immune evasion in triple-negative breast cancer and new potential therapeutic targets: a review. Frontiers in Immunology, 15, 1513421. https://doi.org/10.3389/fimmu.2024.1513421

189. Sever, R., & Brugge, J. S. (2015). Signal Transduction in Cancer. Cold Spring Harbor Perspectives in Medicine, 5(4), a006098–a006098. https://doi.org/10.1101/cshperspect.a006098

190. Shah, S. P., Roth, A., Goya, R., Oloumi, A., Ha, G., Zhao, Y., Turashvili, G., Ding, J., Tse, K., Haffari, G., Bashashati, A., Prentice, L. M., Khattra, J., Burleigh, A., Yap, D., Bernard, V., McPherson, A., Shumansky, K., Crisan, A., … Aparicio, S. (2012). The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature, 486(7403), 395–399. https://doi.org/10.1038/nature10933

191. Shan, R., Dai, L.-J., Shao, Z.-M., & Jiang, Y.-Z. (2024). Evolving molecular subtyping of breast cancer advances precision treatment. Cancer Biology & Medicine, 21(9), 731–739. https://doi.org/10.20892/j.issn.2095-3941.2024.0222

192. Sharma, S., Kelly, T. K., & Jones, P. A. (2010). Epigenetics in cancer. Carcinogenesis, 31(1), 27–36. https://doi.org/10.1093/carcin/bgp220

193. Shen, M., Pan, H., Chen, Y., Xu, Y. H., Yang, W., & Wu, Z. (2020). A review of current progress in triple-negative breast cancer therapy. Open Medicine, 15(1), 1143–1149. https://doi.org/10.1515/med-2020-0138

194. Shi, X., Wang, X., Yao, W., Shi, D., Shao, X., Lu, Z., Chai, Y., Song, J., Tang, W., & Wang, X. (2024). Mechanism insights and therapeutic intervention of tumor metastasis: latest developments and perspectives. Signal Transduction and Targeted Therapy, 9(1), 192. https://doi.org/10.1038/s41392-024-01885-2

195. Sideris, N., Dama, P., Bayraktar, S., Stiff, T., & Castellano, L. (2022). LncRNAs in breast cancer: a link to future approaches. Cancer Gene Therapy, 29(12), 1866–1877. https://doi.org/10.1038/s41417-022-00487-w

196. Singla, H., Ludhiadch, A., Kaur, R. P., Chander, H., Kumar, V., & Munshi, A. (2017). Recent advances in HER2 positive breast cancer epigenetics: Susceptibility and therapeutic strategies. European Journal of Medicinal Chemistry, 142, 316–327. https://doi.org/10.1016/j.ejmech.2017.07.075

197. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., & McGuire, W. L. (1987). Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/ neu Oncogene. Science, 235(4785), 177–182. https://doi.org/10.1126/science.3798106

198. Sobiepanek, A., Kuryk, Ł., Garofalo, M., Kumar, S., Baran, J., Musolf, P., Siebenhaar, F., Fluhr, J. W., Kobiela, T., Plasenzotti, R., Kuchler, K., & Staniszewska, M. (2022). The Multifaceted Roles of Mast Cells in Immune Homeostasis, Infections and Cancers. International Journal of Molecular Sciences, 23(4), 2249. https://doi.org/10.3390/ijms23042249

199. SPKC. Statistikas Dati. Slimību Profilakses un Kontroles Centrs. (2020). Slimību profilakses kontroles centrs. Https://Www.Spkc.Gov.Lv/Lv/Statistikas-Dati.

200. SPKC. Statistikas Dati. Slimību Profilakses un Kontroles Centrs. (2025). https://www.spkc.gov.lv/lv/statistikas-dati

201. Sporikova, Z., Koudelakova, V., Trojanec, R., & Hajduch, M. (2018). Genetic Markers in Triple-Negative Breast Cancer. Clinical Breast Cancer, 18(5), e841–e850. https://doi.org/10.1016/j.clbc.2018.07.023

202. Sridhar, N., Iwase, T., Xie, X., Lee, J., Damodaran, S., & Ueno, N. T. (2025). Paving the path ForwARd: Advances and challenges in androgen receptor targeting in breast cancer. Cancer Treatment Reviews, 138, 102958. https://doi.org/10.1016/j.ctrv.2025.102958

203. Stylianou, S., Clarke, R. B., & Brennan, K. (2006). Aberrant Activation of Notch Signaling in Human Breast Cancer. Cancer Research, 66(3), 1517–1525. https://doi.org/10.1158/0008-5472.CAN-05-3054

204. Suba, Z. (2014). Triple-negative breast cancer risk in women is defined by the defect of oestrogen signaling: preventive and therapeutic implications. OncoTargets and Therapy, 147. https://doi.org/10.2147/OTT.S52600

205. Sun, N., & Zhao, X. (2022). Therapeutic Implications of FABP4 in Cancer: An Emerging Target to Tackle Cancer. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.948610

206. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

207. Symington, L. S., & Gautier, J. (2011). Double-Strand Break End Resection and Repair Pathway Choice. Annual Review of Genetics, 45(1), 247–271. https://doi.org/10.1146/annurev-genet-110410-132435

208. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. von. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research, 47(D1), D607–D613. https://doi.org/10.1093/nar/gky1131

209. Tang, D.-D., Ye, Z.-J., Liu, W.-W., Wu, J., Tan, J.-Y., Zhang, Y., Xu, Q., & Xiang, Y.-B. (2025). Survival feature and trend of female breast cancer: A comprehensive review of survival analysis from cancer registration data. The Breast, 79, 103862. https://doi.org/10.1016/j.breast.2024.103862

210. Thiebaut, C., Konan, H.-P., Guerquin, M.-J., Chesnel, A., Livera, G., Le Romancer, M., & Dumond, H. (2020). The Role of ERα36 in Development and Tumor Malignancy. International Journal of Molecular Sciences, 21(11), 4116. https://doi.org/10.3390/ijms21114116

211. Torgovnick, A., & Schumacher, B. (2015). DNA repair mechanisms in cancer development and therapy. Frontiers in Genetics, 6. https://doi.org/10.3389/fgene.2015.00157

212. Trubacova, R., Drastichova, Z., & Novotny, J. (2022). Biochemical and physiological insights into TRH receptor-mediated signaling. Frontiers in Cell and Developmental Biology, 10. https://doi.org/10.3389/fcell.2022.981452

213. Tsimberidou, A. M., Fountzilas, E., Bleris, L., & Kurzrock, R. (2022). Transcriptomics and solid tumors: The next frontier in precision cancer medicine. Seminars in Cancer Biology, 84, 50–59. https://doi.org/10.1016/j.semcancer.2020.09.007

214. Tuly, K., Hossen, Md., Islam, Md., Kibria, Md., Alam, Md., Harun-Or-Roshid, Md., Begum, A., Hasan, S., Mahumud, R., & Mollah, Md. (2023). Robust Identification of Differential Gene Expression Patterns from Multiple Transcriptomics Datasets for Early Diagnosis, Prognosis, and Therapies for Breast Cancer. Medicina, 59(10), 1705. https://doi.org/10.3390/medicina59101705

215. Tutt, A., Robson, M., Garber, J. E., Domchek, S. M., Audeh, M. W., Weitzel, J. N., Friedlander, M., Arun, B., Loman, N., Schmutzler, R. K., Wardley, A., Mitchell, G., Earl, H., Wickens, M., & Carmichael, J. (2010). Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. The Lancet, 376(9737), 235–244. https://doi.org/10.1016/S0140-6736(10)60892-6

216. Untch, M., von Minckwitz, G., Gerber, B., Schem, C., Rezai, M., Fasching, P. A., Tesch, H., Eggemann, H., Hanusch, C., Huober, J., Solbach, C., Jackisch, C., Kunz, G., Blohmer, J.-U., Hauschild, M., Fehm, T., Nekljudova, V., & Loibl, S. (2018). Survival Analysis After Neoadjuvant Chemotherapy With Trastuzumab or Lapatinib in Patients With Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer in the GeparQuinto (G5) Study (GBG 44). Journal of Clinical Oncology, 36(13), 1308–1316. https://doi.org/10.1200/JCO.2017.75.9175

217. Van Vugt, M. A. T. M., & Parkes, E. E. (2022). When breaks get hot: inflammatory signaling in BRCA1/2-mutant cancers. Trends in Cancer, 8(3), 174–189. https://doi.org/10.1016/j.trecan.2021.12.003

218. Vera-Badillo, F. E., Templeton, A. J., de Gouveia, P., Diaz-Padilla, I., Bedard, P. L., Al-Mubarak, M., Seruga, B., Tannock, I. F., Ocana, A., & Amir, E. (2014). Androgen Receptor Expression and Outcomes in Early Breast Cancer: A Systematic Review and Meta-Analysis. JNCI Journal of the National Cancer Institute, 106(1), djt319–djt319. https://doi.org/10.1093/jnci/djt319

219. Veronesi, U., Orecchia, R., Zurrida, S., Galimberti, V., Luini, A., Veronesi, P., Gatti, G., D'Aiuto, G., Cataliotti, L., Paolucci, R., Piccolo, P., Massaioli, N., Sismondi, P., Rulli, A., Lo Sardo, F., Recalcati, A., Terribile, D., Acerbi, A., Rotmensz, N., & Maisonneuve, P. (2005). Avoiding axillary dissection in breast cancer surgery: a randomised trial to assess the role of axillary radiotherapy. Annals of Oncology, 16(3), 383–388. https://doi.org/10.1093/annonc/mdi089

220. Vidarsdottir, L., Olafsdottir, E. J., Barkardottir, R. B., Bjarnadottir, O., Jonasson, J. G., Sigurdsson, S., & Tryggvadottir, L. (2023). Oestrogen receptor-positive breast cancer and adverse outcome in BRCA2 mutation carriers and young non-carrier patients. Npj Breast Cancer, 9(1), 95. https://doi.org/10.1038/s41523-023-00600-8

221. Virnig, B. A., Tuttle, T. M., Shamliyan, T., & Kane, R. L. (2010). Ductal Carcinoma In Situ of the Breast: A Systematic Review of Incidence, Treatment, and Outcomes. JNCI Journal of the National Cancer Institute, 102(3), 170–178. https://doi.org/10.1093/jnci/djp482

222. Vtorushin, S., Dulesova, A., & Krakhmal, N. (2022). Luminal androgen receptor (LAR) subtype of triple-negative breast cancer: molecular, morphological, and clinical features. Journal of Zhejiang University-SCIENCE B, 23(8), 617–624. https://doi.org/10.1631/jzus.B2200113

223. Wang, J., Wei, X., Dou, X., Huang, W., Du, C., & Zhang, G. (2018). The association between Notch4 expression, and clinicopathological characteristics and clinical outcomes in patients with breast cancer. Oncology Letters. https://doi.org/10.3892/ol.2018.8442

224. Wang, Q., Wang, K., Tan, X., Li, Z., & Wang, H. (2022). Immunomodulatory role of metalloproteases in cancers: Current progress and future trends. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.1064033

225. Wang, X., Zhao, S., Xin, Q., Zhang, Y., Wang, K., & Li, M. (2024). Recent progress of CDK4/6 inhibitors' current practice in breast cancer. Cancer Gene Therapy. https://doi.org/10.1038/s41417-024-00747-x

226. Weijzen, S., Rizzo, P., Braid, M., Vaishnav, R., Jonkheer, S. M., Zlobin, A., Osborne, B. A., Gottipati, S., Aster, J. C., Hahn, W. C., Rudolf, M., Siziopikou, K., Kast, W. M., & Miele, L. (2002). Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. Nature Medicine, 8(9), 979–986. https://doi.org/10.1038/nm754

227. Weisbrod, A. B., Zhang, L., Jain, M., Barak, S., Quezado, M. M., & Kebebew, E. (2013). Altered PTEN, ATRX, CHGA, CHGB, and TP53 Expression Are Associated with Aggressive VHL-Associated Pancreatic Neuroendocrine Tumors. Hormones and Cancer, 4(3), 165–175. https://doi.org/10.1007/s12672-013-0134-1

228. Wells, J. M., Gaggar, A., & Blalock, J. E. (2015). MMP generated matrikines. Matrix Biology, 44–46, 122–129. https://doi.org/10.1016/j.matbio.2015.01.016

229. Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J., & Werb, Z. (2020). Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nature Communications, 11(1), 5120. https://doi.org/10.1038/s41467-020-18794-x

230. Wroblewski, M., Bauer, R., Cubas Córdova, M., Udonta, F., Ben-Batalla, I., Legler, K., Hauser, C., Egberts, J., Janning, M., Velthaus, J., Schulze, C., Pantel, K., Bokemeyer, C., & Loges, S. (2017). Mast cells decrease efficacy of anti-angiogenic therapy by secreting matrix-degrading granzyme B. Nature Communications, 8(1), 269. https://doi.org/10.1038/s41467-017-00327-8

231. Yang, Y., Lu, S., Zeng, W., Xie, S., & Xiao, S. (2017). GATA3 expression in clinically useful groups of breast carcinoma: a comparison with GCDFP15 and mammaglobin for identifying paired primary and metastatic tumors. Annals of Diagnostic Pathology, 26, 1–5. https://doi.org/10.1016/j.anndiagpath.2016.09.011

232. Yersal, O. (2014). Biological subtypes of breast cancer: Prognostic and therapeutic implications. World Journal of Clinical Oncology, 5(3), 412. https://doi.org/10.5306/wjco.v5.i3.412

233. Yi, J., Ren, L., Li, D., Wu, J., Li, W., Du, G., & Wang, J. (2020). Trefoil factor 1 (TFF1) is a potential prognostic biomarker with functional significance in breast cancers. Biomedicine & Pharmacotherapy, 124, 109827. https://doi.org/10.1016/j.biopha.2020.109827

234. Yoon, E. C., Wang, G., Parkinson, B., Huo, L., Peng, Y., Wang, J., Salisbury, T., Wu, Y., Chen, H., Albarracin, C. T., Resetkova, E., Middleton, L. P., Krishnamurthy, S., Gan, Q., Sun, H., Huang, X., Shen, T., Chen, W., Parwani, A. V., … Ding, Q. (2022). TRPS1, GATA3, and SOX10 expression in triple-negative breast carcinoma. Human Pathology, 125, 97–107. https://doi.org/10.1016/j.humpath.2022.04.006

235. Yu, T., & Di, G. (2017). Role of tumor microenvironment in triple-negative breast cancer and its prognostic significance. Chinese Journal of Cancer Research, 29(3), 237–252. https://doi.org/10.21147/j.issn.1000-9604.2017.03.10

236. Zaha, D. C. (2014). Significance of immunohistochemistry in breast cancer. World Journal of Clinical Oncology, 5(3), 382–392. https://doi.org/10.5306/wjco.v5.i3.382

237. Zhao, Y., Simon, M., Seluanov, A., & Gorbunova, V. (2023). DNA damage and repair in age-related inflammation. Nature Reviews Immunology, 23(2), 75–89. https://doi.org/10.1038/s41577-022-00751-y

Acknowledgments

I would like to begin by expressing my heartfelt appreciation to my scientific supervisors, **Zanda Daneberga** and **Miki Nakazawa-Miklaševiča**, whose guidance and support were essential throughout my doctoral studies. I am grateful to **E. Miklaševičs** for offering me the opportunity to initiate PhD studies and for proposing the initial research concept that laid the foundation for this work. I owe particular thanks to **Z. Daneberga** for her openness, trust, and continuous mentorship, as well as for patiently guiding me through each stage of this research. Without your expertise and encouragement, the completion of both the analyses and this Thesis would not have been possible.

I would also like to acknowledge the Breast Surgery Unit of Pauls Stradiņš Clinical University Hospital for their valuable assistance with patient material collection and sample selection, which was crucial for the success of this study.

My sincere thanks go to the reviewer of this Thesis, **Dace Pjanova**, for her careful evaluation of the work and for her insightful comments and constructive suggestions, which significantly enhanced its quality. I am likewise grateful to all members of the Doctoral Thesis Discussion Committee for their time, assessment, and thoughtful recommendations.

I extend my appreciation to Rīga Stradiņš University, Institute of Oncology and Molecular Genetics, for their support, collaboration, and professional advice during my doctoral studies.

Finally, I am deeply thankful to my friends and family for their enduring support and belief in me. I am especially grateful to my husband for his patience, understanding, and constant encouragement throughout this demanding journey. I thank everyone who has supported me along the way, these contributions have played an important role in both my academic development and personal growth.

Annexes

Approval of the Genome Research Council of Latvia

Genoma izpētes padome

Rātsupītes iela 1 k-1, Rīga, LV-1067 • Tālr. 67473083 • E-pasts: genoma.padome@biomed.lu.lv

Rīgā

19.10.2018. Nr.A-2/18-10-19

RSU Onkoloģijas Institūtam

Atzinums par pētījumu: *"Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"*

Genoma izpētes padome izskatīja RSU Onkoloģijas Institūta iesniegumu par pētījumu *"Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"*.

Pamatojoties uz Genoma izpētes padomes locekļu balsojumu, tiek izsniegts atzinums, ka Genoma izpētes padome atbalsta RSU Onkoloģijas Institūta pētījuma *"Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"* īstenošanu.

Genoma izpētes padomes
Priekšsēdētājs J.Kloviņš

Rovite, 67473083
vita.rovite@biomed.lu.lv

ŠIS DOKUMENTS IR ELEKTRONISKI PARAKSTĪTS AR DROŠU ELEKTRONISKO PARAKSTU UN SATUR LAIKA ZĪMOGU

Template of informed consent for study participant

Pacienta piekrišana dalībai pētījumā

1. Esmu saņēmis un iepazinies ar rakstisku informāciju par daudzpusīgu vēža izpētes projekta mērķi, saturu un iespējamiem riskiem. Uz visiem maniem jautājumiem esmu saņēmis izsmeļošas atbildes. Man bija pietiekami daudz laika, lai pārdomātu savu lēmumu piekrist nodot bioloģisko materiālu vēža izpētei.

2. Es piekrītu bez atlīdzības nodot asinis un/vai audu paraugus (bioloģisko materiālu) šūnu iegūšanai ar mērķi tās identificēt, kā arī nukleīnskābju un/vai proteīnu izdalīšanai molekulāri – ģenētiskiem pētījumiem. Es saprotu, ka asins vai audu paraugu nodošana nerada risku manai veselībai, kas man ir izskaidrots.

3. Asins un/vai audu paraugi, ko nododu izpētei, ir nepieciešami, lai noskaidrotu labākās šūnu izdalīšanas metodes, identificētu vēža šūnas, kā arī izdalītu nukleīnskābes (DNS; RNS) un/vai proteīnus, ar ko veikt molekulāri ģenētiskās analīzes.

4. Es saprotu, ka jebkura mani identificējoša informācija būs konfidenciāla, un ka visi mani paraugi būs kodēti. Es apzinos, ka es jebkurā brīdī bez paskaidrojumiem varu pārtraukt piedalīšanos pētījumā, zinot, ka tas neietekmēs manu turpmāko ārstēšanos. Zinu, ka šādā gadījumā mani nodotie asins un/vai audu paraugi, izdalītās nukleīnskābes un/vai proteīni, veselības stāvokļa apraksts un jebkura mani identificējoša informācija tiks iznīcināta.

5. Veselības stāvokļa apraksti:

☐ Es ATĻAUJU sava veselības stāvokļa apraksta papildināšanu, atjaunošanu vai pārbaudi:

☐ Es AIZLIEDZU sava veselības stāvokļa apraksta papildināšanu, atjaunošanu vai pārbaudi:

6. Gadījumā, ja mana bioloģiskā materiāla izpētē tiks atklāta informācija par kādu man līdz šim nezināmu apdraudējumu manai un / vai manu radinieku veselībai (vajadzīgo atzīmēt):

☐ Es PIEKRĪTU, ka man tiek paziņota šī informācija;

☐ Es PIEKRĪTU, ka man tiek paziņota šāda informācija tikai tādā gadījumā, ja risks veselībai ir novēršams;

☐ Es NEVĒLOS saņemt nekādu papildus informāciju.

7. Bioloģiskā materiāla uzglabāšana:

☐ Es PIEKRĪTU, ka mans bioloģiskais materiāls turpmāk glabāsies RSU Onkoloģijas institūta Molekulārās ģenētikas laboratorijā un tiks izmantots pētījumos, kas saistīti ar vēža ģenētisko izpēti bez ierobežojuma;

☐ Es PIEKRĪTU mana bioloģiskā materiāla un veselības stāvokļa apraksta nosūtīšanai izpētei ārpus Latvijas;

☐ Es NEPIEKRĪTU mana bioloģiskā materiāla un veselības stāvokļa apraksta nosūtīšanai izpētei ārpus Latvijas;

Bioloģiskā materiāla donors (nepieciešamības gadījumā viņa aizbildnis vai aizgādnis):

Vārds un uzvārds (drukātiem burtiem):

_____

Personas kods: | | | | | | | — | | | | | |

Paraksts: _____

Adrese: _____

Datums: __ __ / __ __ / 20__ (DD/MM/GGG)

Ārsts/klīnicists:

Vārds un uzvārds, amats (drukātiem burtiem vai spiedogs):

_____

Datums: __ __ / __ __ / 20__ (DD/MM/GGG)

Apstiprinu, ka esmu informējis pacientu par šo pētījumu (paraksts):

_____

Pacientam piešķirtais kods | | | | | | | |

Ja Jums rodas kādas komplikācijas bioloģisko paraugu noņemšanas dēl, lūdzu nekavējoties sazināties ar ārstu, kurš šos paraugus noņēma vai Urzulu Lakuču (tālr. 67704028, e-pasts: urzula.lakuca@rsu.lv).

2 no 2

Approval of the Central Medical Ethics committee of Latvia

Centrālā medicīnas ētikas komiteja

Brīvības iela 72, Rīga, LV-1011 • Tālr. 67876182 • Fakss 67876071 • E-pasts: vm@vm.gov.lv

Rīgā

19.09.2018. Nr.1/18-09-19

Rīgas Stradiņa universitātes
Onkoloģijas institūtam

Atzinums par pētījumu
„Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"

Centrālā medicīnas ētikas komiteja 2018.gada 5.septembrī ir izskatījusi Rīgas Stradiņa Universitātes Onkoloģijas institūta iesniegto pētījumu *„Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"*.

Pamatojoties uz Centrālās medicīnas ētikas komitejas 2018.gada 5.septembra sēdes protokola Nr.2018-4 punktu Nr.2 un iesniegtajiem labojumiem, tiek izsniegts atzinums, ka Rīgas Stradiņa Universitātes Onkoloģijas institūta iesniegtais pētījums *„Audzēja transkriptoms kā prognostisks faktors trīskārši negatīva krūts audzēja terapijas efektivitātei"* nav pretrunā ar bioētikas normām.

Centrālās medicīnas ētikas
komitejas priekšsēdētājs V.Sīlis

Strautiņš, 67876190
Edgars.Strautins@vm.gov.lv

Table 1. MLPA results for *BRCA1* CNV and methylation status

		CNV Analysis			Methylation Analysis		
ID	**Group**	**FR**	**SD**	**Interpretation**	**FR**	**SD**	**Interpretation**
1110	BRCA−	0.69	0.08	Hz deletion *	0.00	0.00	No methylation detected
903	BRCA−	0.61	0.09	Hz deletion *	Hz	0.00	No methylation detected
1005	BRCA−	0.53	0.08	Hz deletion *	0.00	0.00	No methylation detected
1007	BRCA−	0.65	0.09	Hz deletion *	0.00	0.00	No methylation detected
1043	BRCA−	0.66	0.08	Hz deletion *	0.00	0.00	No methylation detected
1047	BRCA−	0.67	0.09	Hz deletion *	0.00	0.00	No methylation detected
1136	BRCA−	0.68	0.11	Hz deletion *	0.00	0.00	No methylation detected
1122	BRCA−	0.67	0.10	Hz deletion *	0.00	0.00	No methylation detected
1074	BRCA−	0.63	0.09	Hz deletion *	0.04	0.00	Very low signal
1209	BRCA−	0.68	0.11	Hz deletion *	0.00	0.00	No methylation detected
1088	BRCA−	0.55	0.08	Hz deletion *	0.00	0.00	No methylation detected
1078	BRCA−	0.60	0.06	Hz deletion *	0.00	0.00	No methylation detected
1155	BRCA−	0.64	0.07	Hz deletion *	0.00	0.00	No methylation detected
1107	BRCA−	0.59	0.06	Hz deletion *	0.00	0.00	No methylation detected
1046	BRCA−	0.86	0.09	Likely normal	0.00	0.00	No methylation detected
1171	BRCA−	0.51	0.12	Hz deletion	0.69	0.05	High methylation
1109	BRCA−	0.72	0.12	Hz deletion	0.42	0.04	Partial methylation
1000	BRCA+	0.82	0.08	Normal	0.00	0.00	No methylation detected
1003	BRCA+	0.89	0.05	Normal	0.00	0.00	No methylation detected
1004	BRCA+	0.73	0.09	Hz deletion	0.00	0.00	No methylation detected
1006	BRCA+	0.82	0.10	Normal	0.00	0.00	No methylation detected
1008	BRCA+	0.72	0.06	Hz deletion	0.00	0.00	No methylation detected
1009	BRCA+	0.81	0.06	Normal	0.00	0.00	No methylation detected
1010	BRCA+	1.02	0.11	Normal	0.00	0.00	No methylation detected
1011	BRCA+	1.02	0.14	Normal	0.00	0.00	No methylation detected
1013	BRCA+	0.76	0.09	Borderline	0.00	0.00	No methylation detected
1018	BRCA+	0.75	0.18	Borderline	0.34	0.06	Partial methylation
1019	BRCA+	0.84	0.08	Normal	0.00	0.00	No methylation detected
1030	BRCA+	0.85	0.04	Normal	0.00	0.00	No methylation detected
1036	BRCA+	0.79	0.07	Normal	0.00	0.00	No methylation detected
1039	BRCA+	0.76	0.08	Borderline	0.27	0.04	Low methylation
1050	BRCA+	0.70	0.07	Hz deletion	0.00	0.00	No methylation detected
1051	BRCA+	0.74	0.09	Borderline	0.00	0.00	No methylation detected
1065	BRCA+	0.76	0.07	Borderline	0.00	0.00	No methylation detected
1068	BRCA+	0.88	0.09	Normal	0.00	0.00	No methylation detected
1112	BRCA+	0.79	0.08	Normal	0.00	0.00	No methylation detected

Table 2. ME001-D1 MS-MLPA *BRCA1* probes according to chromosomal location

SALSA MLPA probe	5162-L04543
Gene	*BRCA1*
Ligation site	NM_007294.4; 59-60
Location	17-038,531
Complete sequence	TTCTCAGATAACTGGGCCCCTGC-GCTCAG GAGGCCTTCACCCTCTGCTCTGGGTAAAGGT

Table 3. TaqMan probes used for transcript validation, with corresponding assay details

Gene Symbol	Interrogated Sequence	Translated Protein	Exon Boundary	Assay Location	IMAGE Clone ID	Amplicon Length
TPSD1	NM_012217.2	NP_036349.1	3-4	533		76
TPSD1	AF206664.1	-	2-3	370		76
TPSD1	BC069143.1	-	3-4	524	7217003	76
FABP4	NM_001442.2	NP_001433.1	2-3	315		96
FABP4	BC003672.1	-	2-3	310	3683235	96
FABP4	BQ880795.1	-	2-3	247		96
FABP4	BT006809.1	-	2-3	244		96
FABP4	CD000452.1	-	2-3	316		96
FABP4	CR456903.1	-	2-3	244		96
FABP4	FJ224319.1	-	2-3	306		96
FABP4	J02874.1	-	2-3	306		96
CARTPT	NM_004291.3	NP_004282.1	1-2	295		77
CARTPT	BC029882.1	-	1-2	291	5171665	77
CARTPT	CR542216.1	-	1-2	164		77
CARTPT	U16826.1	-	1-2	183		77
MMP9	NM_004994.2	NP_004985.2	8-9	1354		67
MMP9	AK298246.1	-	8-9	1277		67
MMP9	AK301446.1	-	6-7	1015		67
MMP9	AK302530.1	-	3-4	506		67
MMP9	AK303080.1	-	6-7	1074		67
MMP9	AK313137.1	-	8-9	1354		67
MMP9	AK316145.1	-	9-10	1257		67
MMP9	BC006093.1	-	8-9	1340	4054882	67
MMP9	CN288579.1	-	3-4	542		67
MMP9	J05070.1	-	8-9	1354		67
GPX2	NM_002083.3	NP_002074.2	1-2	400		169
GPX2	BC005277.1	-	1-2	240	3996732	169
GPX2	BC016756.1	-	1-2	254	3681457	169
GPX2	BC022820.1	-	1-2	223	5429926	169
GPX2	BC067221.1	-	1-2	244	6668339	169
GPX2	X68314.1	-	1-2	255		169
RPLP1	NM_001003.2	NP_000994.1	3-4	394		68
RPLP1	NM_213725.1	NP_998890.1	2-3	319		68
RPLP1	AK026579.1	-	3-4	394		68
RPLP1	AK130958.1	-	3-4	397		68

Gene Symbol	Interrogated Sequence	Translated Protein	Exon Boundary	Assay Location	IMAGE Clone ID	Amplicon Length
RPLP1	AY303789.1	-	3-4	395		68
RPLP1	BC003369.1	-	3-4	377	2900846	68
RPLP1	BC007590.1	-	3-4	365	3343021	68
RPLP1	CR450339.1	-	3-4	265		68
RPLP1	CR542209.1	-	3-4	265		68
RPLP1	M17886.1	-	3-4	394		68
RPL13A	NM_001270491.1	NP_001257420.1	7	929		113
RPL13A	NM_0123.3	NP_036555.1	8	1005		113
RPL13A	NR_073024.1		8	995		113
RPL13A	AB082924.1	-	5	784		113
RPL13A	AK056837.1	-	3	2142		113
RPL13A	AK130605.1	-	8	952		113
RPL13A	AK291120.1	-	8	951		113
RPL13A	AK297859.1	-	6	755		113
RPL13A	BC000514.2	-	8	934	2822802	113
RPL13A	BC000847.1	-	1	184		113
RPL13A	BC004900.2	-	7	925		113
RPL13A	BC032107.2	-	8	940		113
RPL13A	BC062537.1	-	7	931	4908022	113
RPL13A	BC070223.1	-	8	951	6736442	113
RPL13A	BC071929.1	-	7	935	5808487	113