EGAS MONIZ SCHOOL of HEALTH & SCIENCE | INSTITUTO UNIVERSITÁRIO EGAS MONIZ

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MESTRADO INTEGRADO EM CIÊNCIAS FARMACÊUTICAS

ESTUDO DAS MOLÉCULAS BIOATIVAS DA SOJA E SEU IMPACTO NA PREVENÇÃO E NO SUPORTE AO TRATAMENTO DO CANCRO DA MAMA

Trabalho submetido por
Lison Marie Gisèle Geneviève FORESTIER-ROCHE
para a obtenção do grau de Mestre em Ciências Farmacêuticas

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Trabalho orientado por
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Resumo

O cancro da mama continua a ser um dos principais desafios globais de saúde pública, representando uma causa importante de morbilidade e mortalidade entre as mulheres. As limitações dos tratamentos convencionais têm suscitado um interesse crescente em estratégias complementares, nas quais a nutrição desempenha um papel cada vez mais relevante. A soja, rica em moléculas bioativas como as isoflavonas, surgiu como um candidato promissor devido às suas propriedades estrogénicas seletivas, efeitos antioxidantes e capacidade de regular a proliferação celular

Dados experimentais, epidemiológicos e clínicos sugerem que estes compostos podem contribuir para a prevenção e para o apoio terapêutico no cancro da mama. Contudo, persistem controvérsias particularmente no que respeita à sua segurança em doentes com tumores hormono-dependentes e às suas potenciais interações com as terapias convencionais.

A evidência atual destaca simultaneamente o potencial promissor das isoflavonas da soja e a necessidade de abordagens clínicas mais rigorosas e personalizadas, de modo a definir melhor o seu papel na gestão integrativa do cancro da mama

Soja – Cancro da mama– isoflavonas – Fitoestrogénios

Abstract

Breast cancer remains one of the leading global public health challenges, representing a major cause of morbidity and mortality among women. The limitation of conventional treatments have prompted going interest in complementary strategies, with nutrition playing an increasingly important role. Soy rich in bioactive molecule such as isoflavones, has emerged as a promising candidate due to its selective estrogenic properties, antioxidant effects, and regulation of cell proliferation.

Experimental, epidemiological, and clinical data suggest that these compounds may contribute to the prevention and supportive management of breast cancer. However, controversies persist, particularly regarding their safety in patients with hormone-dependant tumors and their potential interactions with conventional therapies.

Current evidence highlights both the promising potential of soy isoflavones and the need for more rigorous and personalized clinical approaches to better define their role in the integrative management of breast cancer.

Soy – Breast cancer – isoflavones – Phytoestrogens

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List of Abbreviations

ABL1 : Abelson murine leukemia viral oncogene homolog 1
AF-2 : Activation function 2
AHRQ : Agency for Healthcare Research and Quality
AICR : American Institute for Cancer Research
AP-1 Activator Protein-1
Apaf-1 : Apoptotic protease activating factor 1
AR : androgen receptor
ASCO: American society of clinical Oncology
Bad : Bcl-2-associated agonist of cell death
BAK : BCL-2 Antagonist/Killer
BARD1 : BRCA1 Associated RING Domain 1
BAX : BCL-2-Associated X protein
BC : Breast Cancer
BCL2: B-cell lymphoma 2
BL-1 : Basal-like 1
BL-2 : Basal-like 2
BRCA : Breast cancer gene
BRIP1 : BRCA1 Interacting Protein C-terminal Helicase 1
CAFs : cancer-associated fibroblasts
CFIA : Canadian Food Inspection Agency
CGH : Comparative genomic hybridization
CHEK2 : Checkpoint Kinase 2
Cmax : Maximum plasma concentration
CNS : Central Nervous System
COX-2 cyclooxygenase-2
c-SRC : Cellular Sarcoma gene
DIAAS : Digestible Indispensable Amino Acid Score
EFSA : European Food Safety Authority
EGFR : Epithelial Growth Factor Receptor
EMT : Epithelial-to-Mesenchymal Transition
ER : Estrogen Receptor
ESMO: European Society for medical oncology
ET : Endometrial Thickness
E2 : 17β-estradiol
FAO : Food and Agriculture Organization of the United Nations
FDA : U.S. Food and Drug Administration
FFQs : Food Frequency Questionnaires
FGV : fibroglandular volume
FISH : Fluoresence In Situ Hybridization
FOXO3a : Forkhead box O3
FSH: Follicle-Stimulating hormone
GDP : Guanosine Diphosphate
GnRH: Gonadotropin-Realeasing Hormone
GLOBOCAN : Global Cancer Observatory
GOF: Gain Of Function
GSK-3β : Glycogen Synthase Kinase 3 beta
GTP : guanosine triphosphate
HER2 : Human Epidermal Receptor 2

HIF-1α: Hypoxia-inducible factor 1-alpha
HPPA: 2-(4-hydroxyphenyl)-propanoic acid
HR : Hormone Recepteur
HRT : hormone replacement therapy
IAPs : inhibitors of apoptosis proteins
IGF-1 : Insulin-like Growth Factor-1
IHC: Immunohistochemistry
IKK : IκB kinase
IκBα : Inhibitor of kappa B alpha
IKWG : The International Ki67 in Breast Cancer Working Group
IM : Immunomodulatory
LAR : Luminal Androgen Receptor
LBD : ligand-binding domain
LH: Luteinizing Hormone
LOF: Loss of Function
M : Mesenchymal
MAPK : Mitogen-Activated Protein Kinase
MD : mammographyc density
MET : Tyrosine-protein kinase Met
MRI : Magnetic Resonance Imaging
MSL : Mesenchymal Stem cell-Like
MYC : Myelocytomatosis oncogen
NCI : National Cancer Institute
NF-κB : nuclear factor-kappa B
NSABP : National Surgical Adjuvant Breast and Bowel Project
O-DMA : O-desmethylangolensin
ODS : Office of Dietary Supplements
OR : Odds Ratio
PAM50: Prediction Analysis of Microarray 50
PALB2 : Partner And Localizer of BRCA2
PDCAAS : Protein Digestibility Corrected Amino Acid Score
PI3K/AKT : Phosphoinositide 3-kinase/protein kinase B
PPAR : Peroxisome Proliferator-Activated Receptors
PR : Progesterone Receptor
PSA : prostate specific antigen, a growth-related gene
PTEN : Phosphatase and Tensin Homolog
p21WAF1 :Cyclin-dependent kinase inhibitor 1
Ras : Rat sarcoma virus
RBA : relative binding affinity
RCA : Relative Coactivator Affinity
RNA: Ribonucleic acid
RSV : Rous Sarcoma Virus
SBCSS : Shanghai Breast Cancer Survival Study
SEER : Surveillance, Epidemiology, and End Results
SERM : Selective Estrogen Receptor Modulator
SRC : Sarcoma gene
SULT : sulfotransferases
TAMs : tumor-associated macrophages
TAILORx : Trial Assigning Individualized Options for Treatment
THB: trihydroxybenzene

Tmax : Time to Maximum Concentration
TME: Tumor microenvironment
TNBC : Triple Negative Breast Cancer
TNF-α : Tumor necrosis factor
TP53 : Tumor protein 53
Tregs : regulatory T cells
TR-FRET : Time-Resolved Fluorescence Resonance Energy Transfer
UGT : UDP-glucuronosyltransferases
VEGF: Vascular endothelial growth factor
VEGFR : vascular Endothelial Growth Factor
VHL : Von Hippel-Lindau tumor suppressor
VMI : Vaginal Maturation Index
v-SRC : Viral Sarcoma gene
WCRF : World Cancer Research Fund

Introduction

Breast cancer remains, worldwide, the most common malignancy in women and a major public health challenge. Despite substantial progress in screening, early diagnosis, and adjuvant therapies, its incidence continues to rise in many regions, partly driven by lifestyle changes and environmental factors. In this context, prevention and the optimisation of therapeutic strategies are essential priorities to lessen the burden of the disease.

At the same time, interest in the influence of diet on breast cancer risk and outcomes has steadily increased. Among plant-derived compounds, soy occupies a prominent position. Cultivated for millennia in Asia, it is a rich source of protein and bioactive molecules, particularly isoflavones. These belong to the family of phytoestrogens: their chemical structure, similar to that of estradiol, allows them to interact selectively with estrogen receptors. This property has fuelled both scientific enthusiasm and concerns about their safety, especially in the setting of hormone-dependent tumours.

Over recent decades, a large body of experimental, epidemiological, and clinical research has explored the potential effects of soy isoflavones on breast cancer prevention and prognosis. Available data sometimes suggest a protective effect, especially when consumption is regular and begins early in life, but findings remain affected by methodological heterogeneity and occasional inconsistencies. Concerns about a possible detrimental "estrogenic" action, particularly in patients treated for breast cancer, have strengthened the need for a critical and balanced appraisal.

In this context, assessing the true role of isoflavones between prevention and therapeutic support is of major scientific and clinical interest. Understanding their mechanisms of action, evaluating the level of evidence from observational and interventional studies, identifying areas of controversy, and translating this knowledge into practical recommendations are essential steps for guiding healthcare professionals and informing patients. This thesis aims to provide a comprehensive and critical analysis of the impact of soy bioactive compounds, particularly isoflavones, on the prevention and treatment of breast cancer. Specifically, it seeks to describe breast cancer, its characteristics, and risk factors, as well as to identify which soy compounds, and particularly which isoflavones, may have a potential effect on this disease. It also aims to

understand and analyze the mechanisms of action of isoflavones in relation to breast cancer, while integrating fundamental, experimental, and clinical data on their role in the prevention and management of the disease. Furthermore, the thesis aims to evaluate existing controversies regarding their safety and efficacy and to propose practical perspectives for their use within a nutritional approach supporting oncological care. To achieve these objectives, a systematic literature review was conducted. Studies were identified through databases such as PubMed, Scopus, and Web of Science, using keywords including “soy isoflavones,” “breast cancer,” “prevention,” and “therapy.” Inclusion criteria comprised original research articles, including experimental, epidemiological, and clinical studies, as well as meta-analyses, published in peer-reviewed journals and evaluating the effects of soy or its isoflavones on breast cancer risk, progression, or clinical outcomes. Publications consisting of abstracts only or without full text were excluded. Selected articles were critically appraised according to study type, population, intervention or exposure, outcomes, and methodological quality.

1. Breast cancer: classification and biology

1.1. Subtypes of breast cancer

Breast cancer constitutes a heterogeneous entity, both histologically and molecularly. While histological classification retains descriptive value, it is now the molecular approach that prevails as the reference for prognosis and therapeutic decisions [1,2].

The PAM50 (Prediction Analysis of Microarray 50) profiling test is a tool based on the expression of 50 breast cancer–specific genes. It enabled the current classifications, which are distinguished mainly by their intrinsic biology rather than only by classical receptor profiles. It thus defined four subtypes: Luminal A, Luminal B, HER2-enriched (Human Epidermal Receptor 2), and Basal-like, the latter corresponding largely to so-called TNBC (triple-negative breast cancers) [2]. This stratification is based on the expression of HR (hormone receptors): estrogen and progesterone, the HER2 receptor, as well as proliferation markers such as Ki-67,a nuclear protein expressed only in dividing cells. It is used as a proliferation marker to assess tumor aggressiveness.

The Luminal A and B subtypes include breast cancers that express hormone receptors, namely the ER (estrogen receptor) and/or the PR **(**progesterone receptor). Expression of these receptors indicates that tumor growth is partly dependent on hormonal stimulation, which opens the way to specific treatments such as hormone therapy. The Luminal A subtype is the most frequent (about 70%) and is characterized by ER+ and/or PR > 20%, low expression of the HER2 receptor, and low expression of Ki67, a histological marker reflecting the rate of cell proliferation. These features are associated with slower tumor growth, a better prognosis, and an improved response to hormone therapy.

The Luminal B subtype also expresses hormone receptors. It may be associated with either underexpression or overexpression of HER2, a membrane protein involved in cell growth signaling, but it is characterized by a higher proliferation index : Ki67. This confers a more aggressive behavior and a less favorable prognosis compared with the Luminal A subtype, although it remains more favorable than the HER2-enriched subtype. For Luminal B tumors, hormone therapy combined with chemotherapy is generally recommended [2,3].

The HER2-enriched subtype is defined by HER2 gene overexpression or amplification, with no expression of ER/PR and high Ki67. It represents a smaller but

significant proportion of breast cancers. Before the introduction of anti-HER2 therapies, this subtype was associated with poor prognosis. Today, the use of monoclonal antibodies such as trastuzumab**,** which specifically target tumor cells expressing HER2, has profoundly changed its clinical outcome [2,4].

Finally, the Basal-like subtype, referring to a genetic signature similar to basal cells of the mammary gland and largely overlapping with triple-negative cancers (ER−, PR−, HER2−), constitutes the most aggressive and least differentiated form. These cells have a completely altered appearance and behavior compared with normal tissue cells in which they first developed. It affects 10–20% of patients with breast cancer and is characterized by the absence of conventional targeted therapeutic options, with an overall unfavorable prognosis. Its frequency is higher in certain populations, particularly in young women and those of African ancestry. Chemotherapy remains the standard treatment, sometimes combined with agents targeting angiogenesis or immunotherapy strategies aimed at stimulating the immune system against the tumor [1,2,4].

This four-subtype classification should, however, be viewed alongside a simpler approach, still widely used in clinical practice, which is based on three major groups: cancers expressing hormone receptors (ER+ and/or PR+), HER2+ cancers, and triple-negative cancers [1]. This distinction has the advantage of directly guiding therapeutic strategies but does not account for all the biological specificities revealed by genomic profiling. This may lead to clinical decisions that are not fully optimal for the patient. Given the severity of breast cancer, the most precise possible approach can save a patient's life by avoiding wasted time on therapies that will not fully match the patient's profile.

Triple-negative breast cancer has a particularity: it has prompted extensive work aimed at refining its internal classification. Based on gene expression analyses, six molecular subcategories have been identified: BL-1 (Basal-like 1), characterized by strong activity of genes linked to DNA repair and cell proliferation; BL-2 (Basal-like 2), defined by activation of growth signaling pathways (EGFR (Epithelial Growth Factor Receptor) and MET (Tyrosine-protein kinase Met) as well as specific metabolic mechanisms; IM (Immunomodulatory), the most promising profile for immunotherapies, marked by high expression of genes related to immune response and cytokines; M (Mesenchymal), showing aggressive properties with high metastatic potential as it is associated with cell differentiation, migration, and invasion; MSL (Mesenchymal Stem Like), which shares invasion-related characteristics with (M) but also shows expression

of genes linked to stem cells and tumor plasticity—meaning the ability of cancer cells to change their characteristics to adapt to their environment or escape treatments, making this subtype particularly resistant. Finally, LAR (Luminal Androgen Receptor) is defined by strong expression of the androgen receptor. These hormones are also produced in women by the ovaries and adrenal glands, albeit in smaller amounts, which means this subtype has a profile closer to hormone-dependent cancers and could respond to treatments targeting the androgen pathway [1]. This heterogeneity partly explains the variability in treatment response in TNBC and opens the way to personalized management strategies [1].

Epidemiological data confirm these trends. According to the GLOBOCAN (Global Cancer Observatory, an international database providing global estimates of cancer incidence and mortality) 2020 analysis, breast cancer is the most diagnosed cancer in women, with a high overall incidence and a predominance of Luminal subtypes [3]. In the United States, SEER (Surveillance, Epidemiology, and End Results) registry data indicate that HR+/HER2− cancers (Luminal A) represent about 70% of cases, TNBC 11%, HR+/HER2+ (Luminal B) about 9%, and HR−/HER2+ (HER2-enriched) about 4% [4]. These proportions highlight the clear predominance of hormone-dependent forms in industrialized countries.

Beyond subtype distribution, marked prognostic differences exist. Survival data from the SEER registry show that patients with Luminal A cancers have the best overall and disease-specific survival, followed by Luminal B and HER2+. Triple-negative cancers are associated with the lowest survival, particularly within the first five years after diagnosis [4]. These differences illustrate the importance of molecular stratification not only to guide therapy but also to inform patients about prognosis.

Finally, the distribution of subtypes varies across populations. GLOBOCAN data indicate a higher incidence of triple-negative cancers in certain regions of Africa and Latin America, often associated with earlier diagnosis and limited therapeutic resources [3]. Conversely, industrialized countries show a predominance of hormone-dependent cancers, probably reflecting not only genetic, environmental, and lifestyle differences but also wider access to screening and treatments.

Thus, the molecular classification of breast cancer illustrates the biological complexity of this pathology. It not only guides treatment but also helps to better understand prognostic and epidemiological variations according to world regions and patient profiles. The development of finer classifications, such as TNBC subtypes,

reflects the evolution toward increasingly personalized oncology. However, to better understand the origin of these differences and the mechanisms driving tumor progression, it is essential to focus on the underlying biology of breast cancer. The following section will therefore address the main processes involved, such as genetic mutations, deregulation of apoptosis, angiogenesis, and the activation of specific signaling pathways, which constitute the biological foundations of these subtypes.

1.2.Tumor biology

The development of cancer relies on a set of biological mechanisms that disrupt the normal balance between cell proliferation, differentiation, and programmed cell death. It is important to recall that a mutated cell is not necessarily a tumor cell. Cells constantly accumulate mutations through replication errors and environmental factors such as UV radiation, tobacco, and others. Most of these mutations are silent, repaired, or else the cell undergoes apoptosis. To become tumorigenic, a sequence of mutations and deregulations affecting several categories of genes and mechanisms is required [5,6]. These mechanisms are common to many cancers but display well-documented specificities in breast cancer [1,2]. Among them are oncogenes, tumor suppressor genes, deregulation of apoptosis, angiogenesis, and genetic mutations. A single mechanism is not sufficient to initiate cancer; multiple types of genetic and cellular alterations are usually needed, along with co-mechanisms that may support or sometimes even replace these genetic mutations [7,8]. These principles form the essential basis for understanding the pathophysiology of cancer in general, including breast cancer, as well as the differences observed among its subtypes [5,6,7].

1.2.1. Oncogenes

The first key step in cancer development involves the activation of oncogenes. Before addressing them, it is necessary to consider proto-oncogenes. These genes are present in all cells and code for proteins that play a role in regulating the cell cycle or signaling [5,6]. Examples include the surface receptors EGFR and HER2, which are crucial in the development of HER2-positive breast cancer. When they spontaneously activate each other, the cell begins to multiply much faster, leading to the emergence of an aggressive cancer [1,2,6].

Other examples include intracellular signaling molecules, transcription factors, or fusion proteins with enzymatic activity. Proto-oncogenes therefore support normal cell growth and division. However, they can undergo what is called “activation” and become oncogenes, a mutated version. This phenomenon is caused by a GOF (gain-of-function) mutations, which increase or confer a new activity to a protein, making the mutated gene hyperactive or abnormally active. The cell then divides continuously, without control and without waiting for external signals. Unlike tumor suppressor genes, proto-oncogenes require only one allele to be mutated in order to be activated and trigger carcinogenesis. Since they act as accelerators, a single one is enough to send uncontrolled growth signals. These GOF mutations, the moment when a proto-oncogene ceases to be one and becomes activated as an oncogene, arise through different mechanisms: some cause mutations, others deregulate proto-oncogenes [5,7].

The first mechanism is transduction, a transfer of genetic material by a virus that can insert sequences activating a proto-oncogene. A well-known example is the proto-oncogene c-SRC (Cellular Sarcoma gene), which codes for the protein SRC (Sarcoma gene), an intracellular tyrosine kinase that modulates the signaling of multiple receptors. This gene is transferred into the viral genome of the retrovirus RSV (Rous Sarcoma Virus), becoming its viral version v-SRC (Viral Sarcoma gene). In this process, v-SRC loses its phosphorylation site that normally acts as a safety brake. It then becomes a mutated version, overexpressed and bearing a GOF mutation, transforming into an oncogene. Without this regulatory brake, the tyrosine kinase enzyme encoded by the gene is permanently active. The cell receives constant growth and division signals, which enhances its oncogenic potential. Infected cells are transformed into cancerous cells, leading to tumor formation. HER2 receptors themselves are tyrosine kinase receptors, which explains their role in breast cancer development [5].

The second mechanism of oncogene activation is insertional mutagenesis. Similar to transduction, a retrovirus integrates into the host cell’s DNA and places its own promoter next to a proto-oncogene. A promoter is a DNA sequence located upstream of a gene, serving as the binding site for transcription factors and RNA (Ribonucleic acid)polymerase, enabling gene transcription into messenger RNA. Without a promoter, the gene remains silent. However, if a virus places its own promoter—which is often extremely powerful—the gene becomes overexpressed. This leads to an overproduction of the corresponding protein, deregulation, oncogenic activation, and ultimately an increased cancer risk. In this case, it is not a mutation in the gene sequence itself but

deregulation of its expression that provokes a GOF event and the activation into an oncogene [5].

The third mechanism is amplification, an abnormal increase in the number of copies of a gene. Instead of one or two, the cell produces dozens or more. More copies mean more messenger RNA and therefore more proteins. Their excess results in the overexpression of the corresponding signals. Here again, the proto-oncogene does not change its sequence; it is the excess that activates it. In aggressive breast cancers, amplification of the HER2 gene results in far too many copies. The cell then produces an enormous number of HER2 receptors on its surface. These receptors spontaneously activate one another, even in the absence of external signals, due to the transformation of the proto-oncogene into an oncogene. As a result, the cell receives constant signals for cell proliferation, which becomes entirely uncontrolled and contributes to the recognized aggressiveness of this cancer type [1,2,6].

Point mutations also play a role. They involve the alteration of a single DNA base, changing one nucleotide in the proto-oncogene. An example is the Ras (Rat sarcoma virus) proto-oncogene family, which codes for GTPase (guanosine triphosphate) proteins acting as molecular switches. When Ras is bound to GTP, it is active and sends a proliferation signal. When Ras hydrolyzes GTP to GDP (Guanosine Diphosphate) through its GTPase activity, it is turned off and no proliferation signal is sent. Usually, Ras alternates according to the cell's needs. Mutations in codons 12, 13, or 61 destroy or reduce its GTPase activity. As a result, Ras can no longer stop the proliferation signal, leading to the activation of the proto-oncogene into an oncogene, uncontrolled cell growth, and an increased cancer risk [6].

The final mechanism is chromosomal translocation, which causes an exchange of segments between chromosomes. This can result in an abnormal gene fusion, where a proto-oncogene merges with another gene to form a hybrid protein, or in the uncontrolled activation of a proto-oncogene by a new promoter. A well-known example of a hybrid protein is the tyrosine kinase BCR-ABL1 (Abelson murine leukemia viral oncogene homolog 1), produced by an exchange of genetic material between chromosomes 9 and 22. This creates the so-called Philadelphia chromosome, an abnormally small chromosome 22. Normally, ABL1 codes for a regulated tyrosine kinase. When fused with BCR, it becomes much more active, driving uncontrolled proliferation of myeloid cells and leading to leukemia [5].

In summary, all these mechanisms activating proto-oncogenes into oncogenes encouraged primarily by environmental factors such as infections, tobacco, alcohol, asbestos, UV radiation, infectious agents, and radioactivity. All carcinogens cause DNA damage or disrupt cellular regulation, which provokes mutations, amplifications, and other alterations. Genetic factors also exist, though rarer, but with major consequences in high-risk individuals. These involve germline mutations inherited from a parent and therefore present in all cells from birth. Known cancer predisposition syndromes for breast cancer include BRCA (Breast cancer gene)1/2 gene mutations and TP53 (Tumor protein 53) mutations, as in Li-Fraumeni syndrome [9]. Oncogenes are therefore genes whose activation or overexpression confers a growth advantage to cells, fostering the development of tumor cells.

1.2.2. Suppressor genes

The second mechanism involved in cancer development, when mutated, concerns tumor suppressor genes. Under normal conditions, they exert a protective effect by slowing cell division and inducing apoptosis, or controlled cell death [5,6]. Unlike oncogenes, which when mutated become hyperactive through GOF mutations, tumor suppressor genes are inactivated when mutated, leading to LOF (loss-of-function). This results in uncontrolled cell growth and an increased risk of cancer, since these genes lose their protective activity [5].

The mechanism by which tumor suppressor genes lose control of their function is explained by Alfred G. Knudson's "two-hit" hypothesis. According to this model, both copies of a tumor suppressor gene must be inactivated to trigger a tumorigenic effect, unlike proto-oncogenes, which require alteration of only one allele to be activated. Knudson showed that two mutagenic events are necessary for a tumor suppressor gene to be fully inactivated [5]. In the hereditary form, the first mutation is inherited and therefore present in all cells of the body, while the second is acquired later in a somatic cell, at random. In the non-hereditary form, both mutations occur randomly in somatic cells. In both cases, the tumor suppressor gene ceases to function, leading to loss of control over cell proliferation. However, the hereditary form carries a higher probability of developing cancer, since the mutated allele is already present in all cells from birth [9].

A typical example of a tumor suppressor gene frequently mutated is TP53**,** which is altered in more than 50% of human cancers and around 30% of breast neoplasms,

particularly in basal-like and triple-negative tumors [1,6]. TP53 may be directly mutated or indirectly neutralized, rendering it inactive or dysfunctional. Under normal circumstances, it encodes the protein p53, a transcription factor that protects the cell. When DNA is damaged, p53 blocks cell division to allow repair. If the damage is irreparable, it triggers apoptosis. It is often referred to as “the guardian of the genome.” Inactivation of this gene therefore directly enables cancer development.

1.2.3. Mechanism against apoptosis

The term *apoptosis* was first described by Kerr et al. in the 1970s. It was introduced in contrast to necrosis. Apoptosis refers to a programmed cell death process that may take several hours, depending on the cell type, the stimuli, and the apoptotic pathway involved [10]. It is a normal biological process that allows the organism to eliminate unnecessary, damaged, or potentially dangerous cells, such as those that have undergone DNA mutations, before they become tumorigenic. Under normal conditions, when a cell receives excessive stress signals or irreparable damage, it self-destructs without causing inflammation [10].

Cancer cells, however, acquire mutations that enable them to evade this programmed cell death. They achieve this by inhibiting, inactivating, or amplifying apoptotic stimuli and regulators. Apoptosis can be initiated through two main pathways. The first is the intrinsic or mitochondrial pathway, in which the cell itself detects stress and initiates its own death. The second is the extrinsic or death receptor pathway, in which an external signal, often of immune origin, triggers apoptosis.

In the intrinsic pathway, for example in response to hypoxia, DNA damage, or excessive oncogenic signaling, the cell releases molecules belonging to the BCL2 (B-cell lymphoma 2) protein family, which includes both pro-apoptotic and anti-apoptotic members. The balance between these proteins determines whether cytochrome C is released. Once released, cytochrome C activates caspases, a family of cysteine proteases that function as the main executioners of apoptosis by cleaving essential cellular proteins, thus initiating the so-called apoptotic cascade. Without caspases, apoptosis cannot proceed, leaving the cell effectively immortal [10].

Cancer cells may develop strategies to prevent apoptosis through genetic alterations they accumulate. For instance, chromosomal translocation between chromosomes 14 and 18 or amplification of the BCL-2 gene—mechanisms that

correspond to proto-oncogene activation—lead to BCL-2 overexpression. This results in an excess of anti-apoptotic proteins that block cytochrome C release, thereby preventing apoptosis. In breast cancer, overexpression of anti-apoptotic proteins such as BCL-2 is frequently observed, particularly in luminal A subtypes [1]. In addition, apoptosis inhibition may also arise from reduced expression of BAX (BCL-2-Associated X protein) and BAK (BCL-2 Antagonist/Killer), which belong to the same BCL2 family but are pro-apoptotic proteins.

Even when cytochrome C is successfully released, caspases are required to execute apoptosis by dismantling the cell in a controlled manner. Cancer cells may escape this step as well by producing excessive amounts of IAPs (inhibitors of apoptosis proteins). In such cases, apoptosis is initiated but caspases are blocked, and without caspases the cell becomes immortal [10].

Another strategy involves mutations in the TP53 gene. The p53 protein normally triggers apoptosis when DNA damage occurs. However, when TP53 is mutated, p53 loses this function. Even with severely damaged DNA, the cell continues to proliferate. As mentioned earlier, TP53 is considered a tumor suppressor gene, and its inactivation plays a central role in driving cancer cell proliferation [2,6].

With regard to the extrinsic pathway, apoptosis is triggered when death receptors on the cell surface bind to their ligands, which recruit adaptor proteins that initiate the signaling cascade. Some tumor cells, however, express defective receptors, preventing them from binding to death ligands and blocking activation of the extrinsic pathway [10].

In summary, the genetic alterations accumulated by cancer cells block, inhibit, or reduce the key signals and actors of apoptosis. Cancer cells thus develop a true arsenal against cellular mortality, making resistance to apoptosis one of the fundamental mechanisms that provides a major advantage for tumor cells to survive and proliferate despite lethal signals [7].

1.2.4. Angiogenesis

In addition to being a mutated cell, cancer also represents a constant dialogue with its environment. One of its strategies is to create a TME (tumor microenvironment). The tumor, this cluster of cancer cells, evolves within the patient's body, and its growth requires the formation of new blood vessels from pre-existing capillaries in order to

establish a complete vascular network. This process, known as angiogenesis, illustrates the communication between the tumor and its TME [11].

In normal adult tissues, angiogenesis is rare and strictly limited to physiological events such as wound healing, the menstrual cycle, or organ regeneration, and it is always short-lived. It involves degradation of the basement membrane, activation, proliferation, and migration of endothelial cells, all finely regulated by pro- and anti-angiogenic factors. Under physiological conditions, endothelial mitotic activity is extremely low, around 0.5% [11].

In cancer, angiogenesis contributes directly to the progression of malignant tumors such as breast cancer [1]. Initially, small tumors can survive through passive diffusion of oxygen and nutrients from surrounding tissue. However, tumor cells are hyperactive at every level and therefore require far more oxygen and nutrients than normal cells once the tumor reaches 2–3 mm^3. To trigger tumor angiogenesis, cancer cells exploit two major mechanisms.

The first mechanism is hypoxia. In a growing tumor, the transcription factor HIF-1α (Hypoxia-inducible factor 1-alpha) accumulates in response to the lack of oxygen. It activates transcription of VEGF (Vascular endothelial growth factor) and other pro-angiogenic factors [11]. The second mechanism is excessive production of VEGF, which can result either from oncogenic activation of Ras or MYC (Myelocytomatosis oncogene) (—genes that activate signaling pathways, including those driving VEGF transcription—or from inactivation of tumor suppressor genes such as p53 or VHL (Von Hippel-Lindau tumor suppressor). Under normal conditions, p53 limits angiogenesis by stimulating production of its natural inhibitor, thrombospondin-1. Similarly, VHL normally degrades HIF-1α under normoxia; its inactivation allows HIF-1α accumulation even when the tumor cell is not oxygen-deprived [1,11].

Through these strategies, cancer cells secrete pro-angiogenic factors, of which VEGF is the most critical. These signals recruit and activate endothelial cells from nearby vessels and capillaries. Normally quiescent, endothelial cells exposed to these tumor-derived signals begin to proliferate, migrate, and degrade their basement membrane to form new vascular sprouts. New capillaries develop and infiltrate the tumor.

However, unlike the well-regulated angiogenesis of wound healing, tumor angiogenesis is chaotic. The resulting vessels are tortuous, leaky, immature, and poorly organized, creating areas of hypoxia. Chronic hypoxia in turn stimulates further VEGF

secretion, establishing a vicious cycle. Tumor cells may also hijack pre-existing vessels to secure an essentially unlimited supply of oxygen and nutrients.

Tumor angiogenesis therefore allows cancer to construct its own microenvironment: it sustains unlimited growth, facilitates the entry of tumor cells into the blood and lymphatic circulation due to vessel permeability, thereby increasing the risk of metastasis, and enables reprogramming of surrounding structures and immune cells such as Tregs (regulatory T cells), TAMs (tumor-associated macrophages), and CAFs (cancer-associated fibroblasts), which are co-opted to defend the tumor instead of eliminating it [11].

In breast cancer, VEGF overexpression correlates with faster metastatic progression and poor prognosis, making it a major therapeutic target [1]. Development of anti-VEGF antibodies includes bevacizumab, although clinical benefits remain variable among patients [2].

1.2.5. Genetic mutations

Ultimately, genetic mutations are the primary driving force of cancer transformation. These may be germline mutations, present from birth, inherited from the parents, and found in every cell of the body [9], or somatic mutations, which arise during life and are increased by environmental factors such as tobacco, UV radiation, or asbestos [5,6]. They underlie the activation of oncogenes, the inactivation of tumor suppressor genes, indirectly contribute to apoptosis evasion, and stimulate angiogenesis. It is the progressive accumulation of somatic mutations and chromosomal rearrangements that forms the foundation of breast carcinogenesis [5,7]. These genetic alterations generate genomic instability, which produces substantial intratumoral heterogeneity. This heterogeneity partly explains the variability of therapeutic responses and the difficulty in completely eradicating the disease [6].

However, tumor progression does not depend solely on modifications of the DNA sequence. Epigenetic mechanisms, such as hypermethylation of tumor suppressor gene promoters like BRCA1, histone modifications, or post-transcriptional regulation by microRNAs, can also repress the expression of key genes without involving direct mutations. These constitute co-mechanisms of carcinogenesis. Although reversible, such alterations promote tumor plasticity and contribute to therapeutic resistance [8]. In breast cancer, they particularly affect hormonal signaling. ERs may be silenced through

promoter methylation or histone modifications, thereby altering the tumor's response to hormones. Epigenetics has thus become a growing field of interest in oncology, especially as certain dietary molecules, including soy isoflavones, appear capable of modulating these epigenetic regulations, opening new perspectives in both prevention and therapeutic support [8].

Genetic and epigenetic alterations affecting key genes such as BRCA1/2**,** TP53, or hormone receptors contribute to the biological heterogeneity of breast cancer. This variability has major clinical implications: it drives disease progression, influences treatment response, and explains the diversity of prognoses. Identifying and characterizing these abnormalities has therefore become a crucial objective, which justifies the importance of biomarkers and molecular tests specific to breast cancer.

1.3 Breast cancer biomarkers and molecular testing

1.3.1. Conventional Biomarkers

The most important biomarker currently available for predicting breast cancer is the ER [1]. Estrogens, mainly estradiol, and progesterone are two female sex hormones produced by the ovaries under the influence of FSH (follicle-stimulating hormone) and LH (luteinizing hormone) released by the pituitary gland, which itself is stimulated by GnRH (gonadotropin-releasing hormone). In the case of estradiol, it is secreted by developing follicles prior to ovulation. After ovulation, the ruptured follicle becomes the corpus luteum, which secretes mainly progesterone and a smaller amount of estrogen. Once released, these two hormones bind to their respective receptors, ER and PR in target tissues such as the uterus, bone, brain, and of course breast tissue. These nuclear receptors modulate the expression of genes involved in pregnancy preparation, mammary stimulation, and endometrial proliferation. When estrogen or progesterone levels rise, a negative feedback signal is sent to the brain, reducing GnRH secretion and consequently lowering FSH and LH release, thus regulating the ovarian cycle.

Physiologically, both hormones play complementary roles in breast development: estradiol stimulates growth and induces PR expression, while progesterone directly drives epithelial proliferation in the adult mammary gland [12]. This normal mitogenic activity becomes problematic when it occurs for prolonged periods, as in early menarche—the

first menstrual cycle—or late menopause, both of which extend hormonal exposure and increase breast cancer risk [12].

In breast cancer, this circuit is hijacked. Tumor cells often overexpress ER and/or PR, meaning that the presence of estradiol directly stimulates their proliferation. Although estradiol was long considered the main driver of this process, recent data suggest that progesterone and progestins are in fact the major oncogenic actors, with estradiol serving more as an amplifier by increasing PR expression [12]. This mechanism explains why prolonged exposure to contraceptives or hormone replacement therapy combining estrogens and progestins may slightly increase breast cancer risk.

This biological reality is reflected in the molecular classification of breast neoplasia discussed earlier. ER+ and/or PR+ tumors correspond to Luminal A and Luminal B subtypes. More precisely, two distinct estrogen receptors have been described: ERα and ERβ [13]. ERα is the main receptor studied in breast cancer and defines hormone-dependent cancers, If overactivated, it is associated with strong cell proliferation. Its prognostic and predictive value is well established, and it directly guides the use of hormone therapy. ERβ, discovered later, is also expressed in mammary tissue but plays a modulatory role: it can limit the proliferative action of ERα and influence the response to hormone therapy. In ERα+/PR+ cancers (Luminal A), high ERβ levels are associated with better overall survival and reduced risk of death compared with tumors with low ERβ expression. It may therefore represent an independent prognostic marker, but only in the context of Luminal cancers [13].

From a therapeutic perspective, the estrogen pathway is the primary target. Anti-estrogens such as tamoxifen, which specifically targets ERα, or aromatase inhibitors, enzymes that convert steroid hormones into estrogens, are highly effective. By blocking estradiol production, they interrupt the ovarian cycle, prevent corpus luteum formation, and thus reduce progesterone release. Estradiol therefore represents the central hormonal target to block in hormone-dependent breast cancers [12].

Among other classical biomarkers of breast cancer is HER2 overexpression, whose evaluation is mandatory in combination with ER [1]. HER2 positivity occurs in about 15–20% of breast cancers [14]. The HER2 gene is a proto-oncogene that, when overactivated through GOF mutations, becomes an oncogene. In many cases of breast cancer, HER2 undergoes amplification, producing too many copies of the gene. The HER2 protein, a membrane tyrosine kinase receptor that regulates cell growth and survival, is then overexpressed. As a result, the number of HER2 receptors on the cell

surface increases massively. Since HER2 tends to activate through dimerization—receptors becoming functional when paired—excess receptors greatly increase the probability of permanent activation. Consequently, the MAPK (Mitogen-Activated Protein Kinase) and PI3K/AKT (Phosphoinositide 3-kinase*)* signaling pathways are constantly active, driving cell proliferation, survival, and inhibition of apoptosis [14]. In patients with HER2 overexpression, anti-HER2 therapies have been developed. Trastuzumab and pertuzumab are monoclonal antibodies that bind to different HER2 domains and inhibit activation of downstream signaling pathways. Their activity is preferentially exerted on tumor cells overexpressing HER2 compared with cells lacking this overexpression [14].

Since the early 1980s, the protein Ki-67 has attracted considerable interest as a proliferation marker in breast cancer subtypes, for prognosis and especially for predicting therapeutic response [15,16]. Ki-67 activity is detectable throughout all active phases of the cell cycle but absent in resting cells (G0 phase) [15]. In healthy tissues, Ki-67 levels are below 3%. When markedly increased, they indicate rapid cell division and aggressive tumor behavior. In hormone-dependent cancers without HER2 expression, Ki-67 helps distinguish Luminal A from Luminal B tumors, as low Ki-67 levels are associated with a better prognosis, while high levels are common in Luminal B. Therapeutically, high Ki-67 expression predicts greater sensitivity to chemotherapy. However, paradoxically, it also indicates a worse baseline prognosis because of the tumor's high growth rate.

In a study conducted by the IKWG (International Ki67 Working Group)**,** the possibility of using neoadjuvant endocrine therapy was explored. Nevertheless, the research group emphasized that Ki-67 assessment is not yet fully standardized, with results varying across laboratories. Its clinical use therefore remains limited despite its promise, although it has already proven valuable as a marker for helping to classify breast cancer subtypes [16].

1.3.2. Genetic and Molecular Biomarkers

The tumor markers previously discussed—such as estrogen receptors, progesterone receptors, and HER2—are routinely used to classify breast cancer subtypes. However, next-generation analyses of breast tumors and multigene germline panels performed in women with breast cancer have revealed molecular heterogeneity of predisposition genes even within hormonal subtypes. Approximately 5–10% of breast

cancers are hereditary, and 25% of hereditary breast cancers are linked to mutations in highly specific genes [17].

It has been shown that TNBCs are the most strongly associated with germline mutations, particularly BRCA1/BRCA2, which are DNA repair genes involved in homologous recombination. Around 11% of patients with TNBC carry this predisposition [7]. Women carrying BRCA mutations face a lifetime risk of developing breast cancer ranging from 45% up to 70%. Overall, 68% of patients with TNBC present genetic predispositions. Mutations are also found in other DNA repair genes such as BARD1(BRCA1 Associated RING Domain 1)**,** BRIP1 (BRCA1 Interacting Protein C-terminal Helicase 1), PALB2 (Partner And Localizer of BRCA2), among others. These data confirm that TNBC is often a disease of genomic instability, which opens the way to potential targeted therapies.

In HER2+ cancers, mutations in the TP53 gene are frequently observed, with 1.4% being germline mutations. In contrast, mutations in the CHEK2 (Checkpoint Kinase 2) gene are more often associated with ER+ subtypes [7]. Finally, the tumor suppressor gene PTEN (Phosphatase and Tensin Homolog) is inactivated in about 40% of breast cancers. A recent analysis of more than 10,000 patients also showed that PTEN loss is associated with a more aggressive course of breast neoplasia [18].

1.3.3. Evaluation Methods and Tests

Evaluation methods and tests are essential for cancer diagnosis. The first step involves standard histological staining, which is used in virtually all pathologies. Hematoxylin, a dye that binds to acids, DNA, and RNA, stains nuclei blue-violet. Eosin, an acidic dye, binds to cytoplasmic and extracellular proteins, coloring them pink. This provides a simple contrast view of cells and tissues, allowing visualization of their architecture and cellular abnormalities, such as whether a lesion is benign or malignant, the presence of nuclear atypia, mitoses, necrosis, and so forth. While this guides the initial diagnosis, it does not reveal the precise cellular nature or whether the tumor expresses ER, PR, or HER2 in breast neoplasia. This is why IHC (immunohistochemistry**)** is performed. By adding antibodies directed against specific proteins (ER, PR, HER2, Ki-67), a molecular signature of the tumor can be obtained, based on nuclear or membrane staining [19]. Once validated, this test can predict which patients will benefit from hormone therapy.

According to the ASCO (American Society of Clinical Oncology), the official guideline states that if at least 1% of tumor cell nuclei in a breast cancer sample are stained for ER, the tumor is considered ER+. When more than 80% are positive, there is no doubt. However, in cases between 1% and 10%, the interpretation is uncertain, and the success of hormone therapy cannot be guaranteed. Clinical data suggest that these partially ER+ tumors often behave like ER– tumors, meaning treatment decisions regarding hormone therapy must be made on a case-by-case basis [20].

Another technique, FISH (fluorescence in situ hybridization), is a molecular cytogenetic method that enables direct visualization of genes, chromosomal segments, or genetic abnormalities using fluorescent probes hybridized to DNA. In cancer, FISH is used to detect mutations, deletions, amplifications, and more. It is thus crucial for mapping oncogenes or altered tumor suppressor genes. Variants such as CGH (comparative genomic hybridization) and multicolor-FISH allow, respectively, a global view of the tumor genome by comparing normal and mutated DNA to identify amplifications (oncogenes) or losses (tumor suppressor genes, such as TP53 or HER2), and simultaneous labeling of multiple chromosomes in different colors to visualize complex translocations. FISH is therefore a highly versatile tool for identifying genetic abnormalities in breast cancer [21].

Beyond immunohistochemistry and FISH, which assess key protein expression and genetic alterations, new transcriptomic tools have been developed to refine prognosis and therapeutic decision-making. Among these, Oncotype DX has become a validated clinical reference [22]. This multigene panel analyzes the expression of 21 genes (16 cancer-related and 5 reference genes) in tumor DNA extracted from paraffin-fixed tissue samples. These genes cover several key pathways: proliferation, estrogen signaling, invasion, HER2, apoptosis, and detoxification. The algorithm provides a Recurrence Score ranging from 0 to 100, which estimates the risk of distant recurrence and, more importantly, the expected benefit of adding chemotherapy to hormone therapy in patients with early-stage ER+/HER2– breast cancer [22].

A low score suggests that hormone therapy alone is sufficient, sparing patients unnecessary exposure to chemotherapy toxicity. A high score (>31) justifies combined therapeutic strategies including chemotherapy, due to a high 10-year recurrence risk. For intermediate scores, the decision is individualized by the physician, based on patient profile and additional clinical factors. The NSABP (National Surgical Adjuvant Breast and Bowel Project) B-20 trial (USA, 1997–2006) was the founding study of Oncotype

DX. This study, along with the large TAILORx (Trial Assigning Individualized Options for Treatment) trial (2015–2018, >10,000 patients), definitively validated its clinical utility in avoiding unnecessary chemotherapy. Integration of this tool into ASCO, ESMO, and other international guidelines has reduced chemotherapy indications by nearly 50% in clinical registries, strengthening confidence in therapeutic decision-making.

Oncotype DX thus illustrates the transition toward precision oncology, where simple morphological and immunohistochemical evaluation is complemented by genomic approaches, allowing highly personalized treatment [22].

1.3.4. Perspectives and the Connection with Soy

The combination of breast cancer biomarkers and molecular tests provides an essential foundation for translational research. A detailed understanding of ER and PR hormone receptor expression makes it possible to explore the modulatory effects of certain dietary compounds, such as soy isoflavones. Studying isoflavones in this context could therefore offer innovative perspectives, both in elucidating molecular mechanisms and in evaluating complementary approaches for prevention or therapeutic support.

2. Soy: botanical and biochemical aspects

2.1 Discovery, composition and main bioactive molecule

2.1.1. Historical discovery of soy and isoflavones

Soybeans, officially named *Glycine max* (L.) Merrill, are among the most widely consumed foods in the world and represent the richest source of isoflavones. Historically, Asian populations have been the greatest consumers of soy, as it is a central ingredient in their traditional diet. Through specific processing and fermentation methods, they developed a wide variety of culinary preparations such as soy milk, natto, tempeh, soy sauce, and tofu, which constitute the foundation of their cuisine [23].

Archaeological research suggests that soybean domestication dates back approximately 6,000 to 9,000 years in East Asia. More precisely, archaeologists propose that domestication occurred in northeastern China around 2510 BC, based on the discovery of numerous soybean ecotypes with great genetic diversity, along with charred soybean seed remains. Its introduction into the West is much more recent: the first records date to the late 19th century, but it was mainly during the 20th century that soy became established as both a crop and a food, particularly with the rise of meat substitutes and vegetarian diets [24].

According to historian Ines Prodöhl in her book "Globalizing the Soybean", northeastern China was the primary production center before World War II, while Germany became the largest importer during the interwar period. This was the pathway through which soy spread into the West. During and after World War II, the United States replaced Germany as the leading importer and simultaneously became the world's largest producer, as access to Chinese soybeans was interrupted during the war, leading to massive domestic production [25].

Soybeans were initially named *Phaseolus max* and *Dolichos soja* by Carl von Linné, the father of modern taxonomy in the 18th century (Figure 1). Later, the scientific name and classification of soy were standardized under the International Botanical Rules as Glycine Willd. comprising two subgroups: Glycine and soy. Isoflavones are abundant in soy; they are phytoestrogens, meaning plant-derived molecules capable of weakly interacting with estrogen receptors by mimicking or modulating the action of endogenous hormones.

From the 1940s, scientific interest in soy grew due to suspicions of negative effects on reproduction. Numerous studies followed, eventually confirming the estrogenic effect of soy. After decades of research, in the 1970s, three major types of isoflavones were identified in soy: daidzein**,** genistein (the most abundant), and glycitein (the least represented) [26]. Since the 1990s, extensive research has focused on the health effects of soy, particularly through initiatives from the U.S. National Cancer Institute, aiming to determine whether soy could contribute to the prevention of diseases such as cancer, especially hormone-dependent cancers like breast cancer [27].

Figure 1 : Carl von Linné (1707–1778). Engraving from Gallery of Portraits, published in 1833 (public domain).

2.1.2. Botanical identity of soy

Soy is a major legume of the Fabaceae family, an important source of dietary protein and oil for animal feed, as well as a staple food for human consumption. It is widely accepted that modern cultivated soybean originates from the domestication of wild soybean thousands of years ago [24]. According to the CFIA (Canadian Food Inspection

Agency)**,** soy belongs to the kingdom *Plantae*, the genus *Glycine*, and the species *Glycine max* (L.) Merr, last revised by Hermann.

Domesticated soybean is an annual herbaceous plant that completes its life cycle—from germination to seed production—within a single season before drying out. It has an erect, bushy form and can reach up to 1.5 meters in height. Its root system consists of a deep taproot complemented by lateral roots. These roots host numerous symbiotic nodules formed in association with the bacterium *Bradyrhizobium japonicum*. This symbiosis enables biological nitrogen fixation, a process by which atmospheric nitrogen is converted into forms assimilable by the plant, thereby reducing the need for nitrogen fertilizers.

The stems and pods are covered with trichomes, protective hairs that help limit water loss, protect against certain insects, and regulate the temperature of aerial organs. During its development, the plant displays different types of leaves: cotyledons, or embryonic leaves, which appear at germination; two primary unifoliate leaves, each with a single blade located just above the cotyledons; and, finally, the majority of the foliage, which consists of alternate trifoliate leaves composed of three leaflets arranged alternately along the stem (Figure 2).

Soy flowers are small and range in color from white to violet. The fruits are pubescent pods (covered with fine hairs) typically containing 2 to 4 seeds. The seeds themselves display wide variability in size, shape, and color. Varieties include yellow, green, brown, black, and even bicolored seeds. This morphological diversity is related both to genetics and to environmental conditions and varietal selection.

Soy exhibits three types of growth. In determinate growth, the plant ceases to grow in height and produce leaves once flowering begins, directing its energy into flower and pod production. This results in compact, uniformly sized plants, often cultivated in warmer regions. In indeterminate growth, the plant continues to produce stems and leaves after flowering begins, leading to taller, more branched plants with extended flowering and maturation periods. This growth type is typical of temperate regions, such as Canada, where it adapts better to short seasons. Semi-determinate growth represents an intermediate form between the two.

Maturity groups constitute a classification system to organize soybean varieties according to their development speed and climatic adaptation. There are 13 groups, ranging from 000 (the earliest-maturing varieties, which complete their cycle very quickly) to X (the latest, requiring a long growing season). This classification depends on

three key factors: latitude, photoperiod, and temperature. In northern regions, early-maturing varieties are cultivated due to shorter seasons, while in warmer regions, later-maturing groups are preferred. In summary, maturity groups serve to "calibrate" soybeans to their environment [28].

While its botanical identity situates soybean among the major legumes, it is above all its biochemical composition that explains its unique role in nutrition. Studying its macronutrient profile is therefore an essential step to understanding its dietary importance and health implications.

Figure 2 : Growth stages of soybean (Glycine max)

2.1.3. Macronutrient profile

Soy stands out not only for its botanical characteristics but also for a nutritional composition that makes it a legume of major importance. Its macronutrient profile—dominated by a high content of high-quality protein, lipids rich in unsaturated fatty acids, and a notable proportion of complex carbohydrates and fiber—explains its use in human food, animal feed, and the agri-food industry.

Today, soy is present in more than 60% of processed foods. Textured soy protein, representing 50–70% protein content, is widely used in vegetarian diets as a meat substitute**.** Soy protein isolate, with about 90% protein content, is found in energy bars, sports drinks, infant formulas, dairy products, and more. Thus, soy is used not only in vegetarian diets but also as a supplement within mainstream food products [29]. This versatility is due not only to its protein content (35–45%) but also to its protein quality [30].

Soy protein has numerous advantages over animal protein: it contains no cholesterol, is rich in complex carbohydrates and unsaturated fats, provides dietary fiber, is lactose-free, and contains over one hundred phytoestrogens [29]. It also has a relatively complete amino acid profile. Protein quality is assessed using international indices such as the PDCAAS (Protein Digestibility-Corrected Amino Acid Score)**,** which accounts for both digestibility and amino acid composition. For soy, this score typically ranges from 0.9 to 1.0, corresponding to a high-quality protein comparable to animal protein standards. A more recent method, the DIAAS (Digestible Indispensable Amino Acid Score)**,** confirms this quality with an estimated value around 0.9, well above the minimum threshold set by the FAO (Food and Agriculture Organization of the United Nations) for a protein to be considered of high nutritional value [30].

Unlike its protein fraction, soy is relatively low in carbohydrates. Most of its carbohydrates are oligosaccharides, which escape digestion in the small intestine and reach the colon, where they promote the growth of beneficial gut microbiota, thus conferring prebiotic properties. Its low carbohydrate content may also be of interest in the management of diabetes. The only potential drawback of oligosaccharides is their association with gas and bloating, although modern processing methods tend to reduce their presence in many soy-based products [30].

Soy lipid content consists of about 10–15% saturated fatty acids, 19–41% monounsaturated fatty acids, and 46–62% polyunsaturated fatty acids. It is therefore rich in unsaturated fats, making it a high-quality source of dietary fat. The main fatty acids are linoleic acid (omega-6) and α-linolenic acid (omega-3)**,** both of which are essential, as they cannot be synthesized by the human body. Soy oil is therefore highly popular.

A study compared boiled soybeans with nine other legumes—lentils, pinto beans, chickpeas, red beans, black beans, azuki beans, great northern beans, and lima beans. Soy clearly stood out in several categories: it was the most energy-dense, the highest in protein and lipid content, and the lowest in carbohydrate content. In terms of micronutrients, soy

was also remarkable, being particularly rich in minerals (especially iron) and vitamin B2 compared with other legumes [30].

Glycine max thus represents a highly valuable alternative for diverse populations, including vegetarians, diabetics, and individuals needing to monitor cholesterol or iron intake [30]. In 1999, the U.S. FDA (Food and Drug Administration) officially approved the claim that daily soy consumption reduces the risk of coronary heart disease. Since then, soy sales and consumption have soared, and soy is now also available as a dietary supplement at concentrations higher than those found in traditional soy-based foods [29].

Beyond its macronutrient composition, which explains its nutritional and agronomic value, soy is distinguished by a wide array of bioactive molecules. Among these, isoflavones have attracted considerable scientific interest due to their phytoestrogenic properties and potential effects on human health. Studying these compounds is therefore an essential step toward understanding the role of soy not only as a source of energy and nutrients but also as a functional food with physiological and therapeutic implications.

2.1.4. Main bioactive compounds of soy

Soy contains several chemical families, including phenolic compounds—the major group—which comprise isoflavones and phenolic acids. In the lipid fraction of soy, phytosterols and saponins are found. Within the protein fraction, bioactive peptides are present.

The main bioactive molecules of soy are isoflavones, a class of phenolic compounds belonging to phytoestrogens. Phenolic compounds are organic molecules characterized by the presence of at least one hydroxyl group (-OH) directly attached to an aromatic ring (benzene cycle). All derivatives retaining this core motif are considered phenolic compounds, forming a vast family of plant secondary metabolites. Among the most studied groups are isoflavones [23].

Isoflavone intake varies widely across regions: in Japan, the average adult intake is estimated at 30–50 mg per day, while in Europe and North America it is generally below 3 mg per day [30]. Isoflavones are present in berries, wine, cereals, and particularly in soy and other legumes. Daidzein and genistein are the predominant soy isoflavones, representing about 40% and 50% of the total isoflavone content, while glycitein accounts for the remaining ~10% (30). Humans are exposed to them when consuming soy in its

various forms [23]. These three molecules are of particular interest to the scientific community for their potentially beneficial effects in hormone-dependent cancers [30].

It is important to note, however, that the health effects of soy isoflavones depend on their concentration in the plant, which in turn is influenced by multiple factors. Studies have shown that genotype, environment, variety origin, seed coat color, and maturity cycle can significantly affect isoflavone content, and consequently, their physiological effects. Reported concentrations range from as low as 677 µg/g in some plants to over 5800 µg/g in others. On average, American soy contains more isoflavones, followed by Japanese varieties. Black seed coats are associated with higher levels, and late-maturing varieties accumulate more isoflavones than early ones [31].

Within soy phenolic compounds, besides isoflavones, phenolic acids are also present, though in much smaller amounts. Eight have been identified, including vanillic acid, gentisic acid, salicylic acid, cinnamic acid, chlorogenic acid, and caffeic acid. The latter two are typically responsible for an undesirable effect known as browning, which reduces nutrient quality and alters the color and flavor of soy as a result of hydrolysis. Nevertheless, phenolic acids are primarily recognized for their antioxidant activity and their ability to help prevent DNA damage caused by reactive oxygen species [32].

Among the bioactive compounds associated with the lipid fraction of soy are phytosterols and saponins**.** Soy is one of the legumes richest in saponins, particularly triterpenoid saponins, derived from triterpene biosynthesis, a large family of lipid-derived compounds. These molecules are amphiphilic, consisting of a lipophilic aglycone (triterpene backbone) and hydrophilic sugar chains. Depending on their structure and the number of attached sugar residues, they are classified into groups A and B. They thus belong to the family of glycosidic secondary metabolites. Soy saponins may contain arabinose, rhamnose, xylose, glucuronic acid, galactose, or glucose. Once considered nutritionally unimportant, saponins have recently been shown to exert beneficial health effects. Reported activities include immune stimulation, cholesterol reduction, and anticancer properties in sarcomas, by reducing DNA synthesis in tumor cells and enhancing natural killer cell activity [32].

Phytosterols, also found in soy alongside saponins, are lipids whose chemical structure closely resembles that of cholesterol. Soy is among the legumes with the highest phytosterol content, particularly in soy oil. The most common phytosterols are sitosterol, campesterol, and stigmasterol. While their precise mechanisms remain incompletely understood, their cholesterol-lowering effect has been well established since the 1960s.

More recent studies also suggest anticancer properties and benefits in chronic disease management.

Within the protein fraction, bioactive peptides are released during digestion by enzymatic hydrolysis of major soy proteins. Unlike simple amino acids, they exert biological functions essential to health, with properties including hypocholesterolemic, antidiabetic, antihypertensive, immunomodulatory, anti-inflammatory, chemopreventive, and antioxidant activities. One example is lactostatin, a soy-derived bioactive peptide known to reduce blood cholesterol. It binds to a specific membrane receptor that facilitates calcium entry into the cell. Calcium then activates an intracellular signaling cascade leading to expression of the CYP7A1 gene, which encodes cholesterol 7α-hydroxylase, a key enzyme catalyzing the first step in cholesterol conversion into bile acids. This process lowers blood cholesterol and contributes to cardiovascular protection. Many studies therefore suggest that a balanced intake of both plant and animal proteins is preferable [32].

Although the entire set of bioactive compounds contributes to the nutritional and functional value of soy, isoflavones have attracted the greatest scientific attention. Their effects on the human body depend closely on their structure, metabolism, and bioavailability—key parameters for understanding their potential role in cancer prevention and treatment.

2.2. Isoflavones: Structure, metabolism and bioavailability

2.2.1. Structure

Isoflavones share a structural similarity with 17β-estradiol (Figure 3 and 4), the biological most active form of estrogen. Thus, isoflavones can act either as agonists or antagonist of estrogen receptors, depending on endogenous estrogen levels in the body when they are bound to the receptors [33].

From the perspective of isoflavone biosynthesis, particularly the best-known ones, daidzein and genistein, soy attracts rhizobial bacteria through the release of isoflavonoids. Isoflavonoids are a subclass of flavonoids, themselves part of the polyphenol family, which is highly abundant in the plant kingdom [34]. Their biosynthesis follows the phenylpropanoid pathway, which is common to all flavonoids [33].

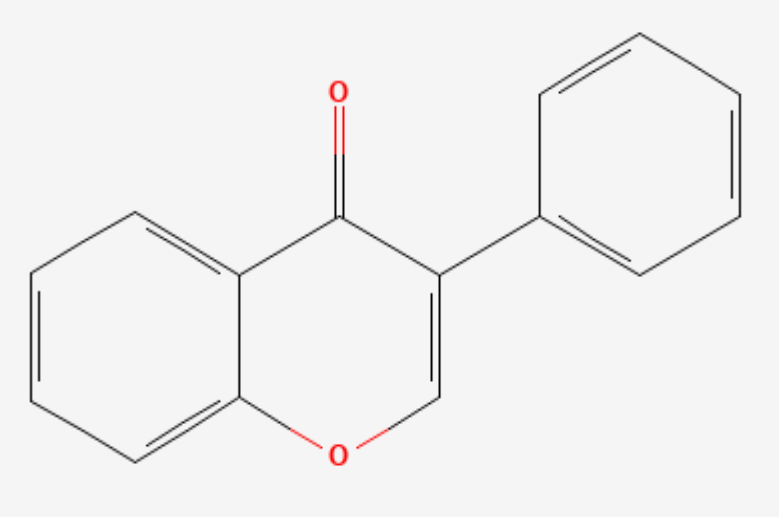

Figure 3 : Isoflavone core structure (retrieved from PubChem, CID 72304, public domain).

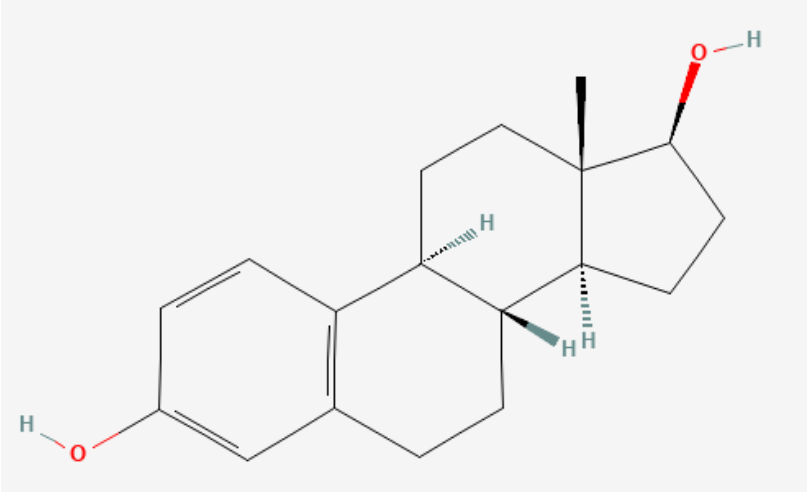

Figure 4: β-Estradiol (retrieved from PubChem, CID 5757, public domain).

It begins with the conversion of phenylalanine into 4-hydroxycinnamoyl-CoA, in the presence of malonyl-CoA. Chalcone synthase then catalyzes the formation of naringenin chalcone, which can be reduced into isoliquiritigenin through the combined action of chalcone synthase and chalcone reductase. Subsequently, chalcone isomerase catalyzes the cyclization of the intermediate, leading to the formation of flavanones such as liquiritigenin and naringenin**.** Isoflavone synthase then shifts the B-ring of the flavonoid skeleton from position 2 to position 3. Finally, isoflavone dehydratase generates a double bond between carbons 2 and 3, resulting in the formation

of the major soybean isoflavones: daidzein (4′,7-dihydroxyisoflavone) and genistein (4′,5,7-trihydroxyisoflavone).

In parallel with this main pathway, a metabolic branch allows the formation of glycitein (7,4′-dihydroxy-6-methoxyisoflavone), another soybean isoflavone, though in smaller proportion (about 10% of total isoflavones). This biosynthesis involves specific methylation reactions starting from the same chalcone precursors. In summary, soybean isoflavone biosynthesis leads to three principal compounds: daidzein, genistein, and, through a parallel pathway, glycitein (Figure 5) [33,34].

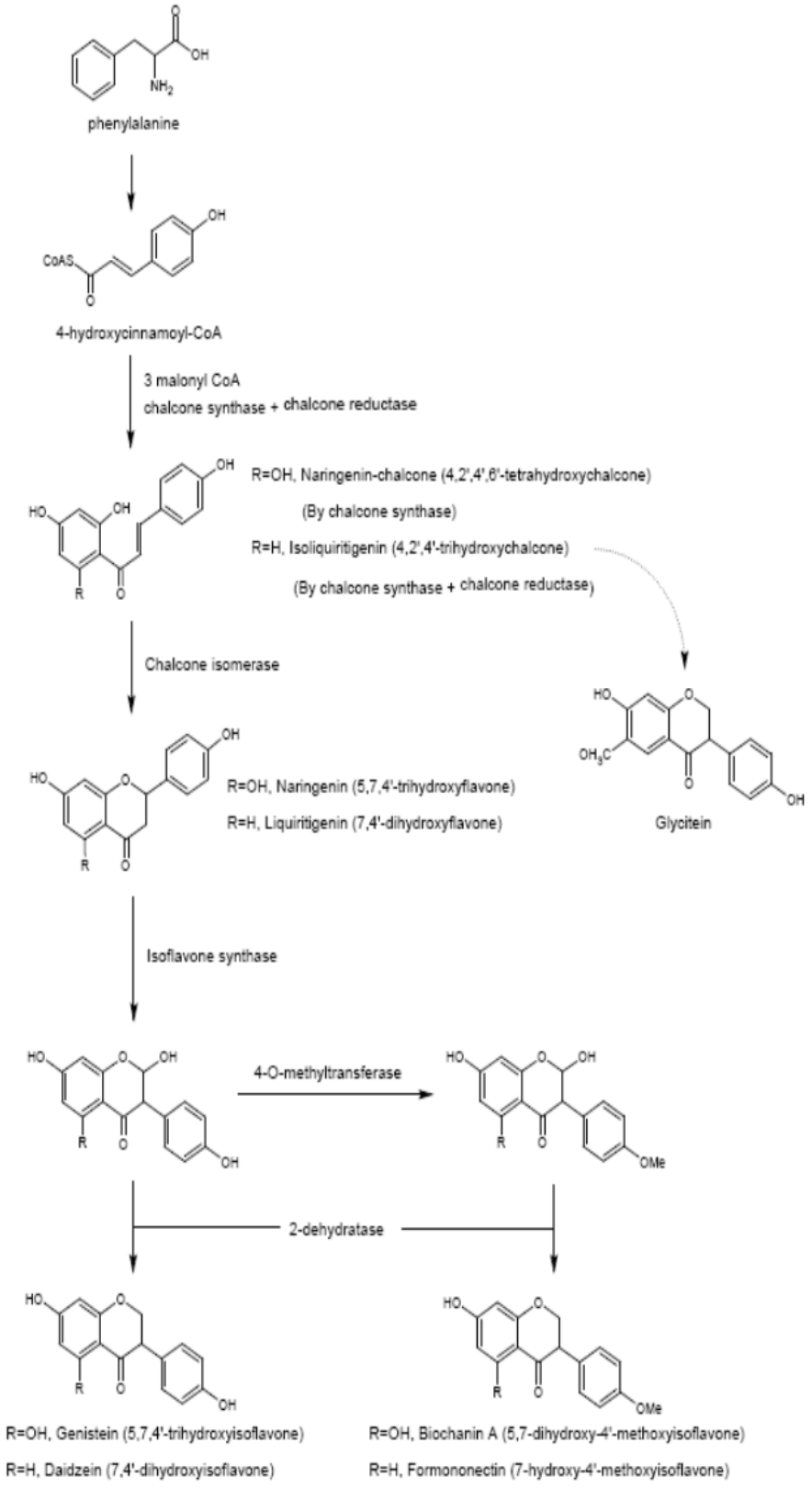

Figure 5 : Biosynthetic pathway of soybean isoflavones, from phenylalanine to the main isoflavones (genistein, daidzein, glycitein, biochanin A, formononetin). [34]

2.2.2 : Isoflavone metabolism

The three major soy isoflavones can exist in different forms depending on their localization or the transformations they undergo. In the plant, after the formation of genistein, daidzein, and glycitein, these molecules are rapidly glycosylated, most often by the addition of a glucose moiety. This results in genistin, daidzin, and glycitin. These glycosylated forms, which are more stable but biologically inactive, constitute the main storage form of isoflavones in the plant [35,36]

Upon ingestion, two of them (genistin and daidzin) are converted back into aglycones—the free, non-sugar-bound form—through the action of the intestinal microbiota. In the small intestine and later in the colon, bacterial β-glucosidases hydrolyze these glycosylated forms; brush-border glucosidases also contribute, though to a lesser extent. This conversion may also occur upstream, during the fermentation of certain soy-based foods such as tempeh or tofu, where microorganisms already transform part of the glycosides into aglycones [37]. Glycitin, however, is only minimally hydrolyzed, since microbiota enzymes show limited affinity for it; as a result, it largely remains in a non-absorbable form [38]. The free aglycones are therefore the biologically active forms: they cross the intestinal epithelium, interact with estrogen receptors, and enter the circulation [35]. The intestinal microbiota thus enables the release of primary metabolites derived from daidzin, genistin, and, to a lesser extent, glycitin.

After absorption, these aglycones enter the portal vein and reach the liver. At the hepatic level, they undergo reactions first catalyzed by certain phase I enzymes**,** such as cytochrome P450s or P81E (minor hydroxylations), and especially by phase II enzymes**,** including SULT (sulfotransferases) and UGT (UDP-glucuronosyltransferases). These reactions generate glucuronidated and sulfated metabolites, which are hydrophilic and therefore more easily excreted in urine (Figure 6) [39]. At this stage, isoflavones circulate mainly in conjugated, biologically inactive forms (90–99%), while a minor fraction (<10%) escapes conjugation and persists as free, active aglycones. Most isoflavones thus enter systemic circulation before being eliminated in urine (Figure 6) [38].

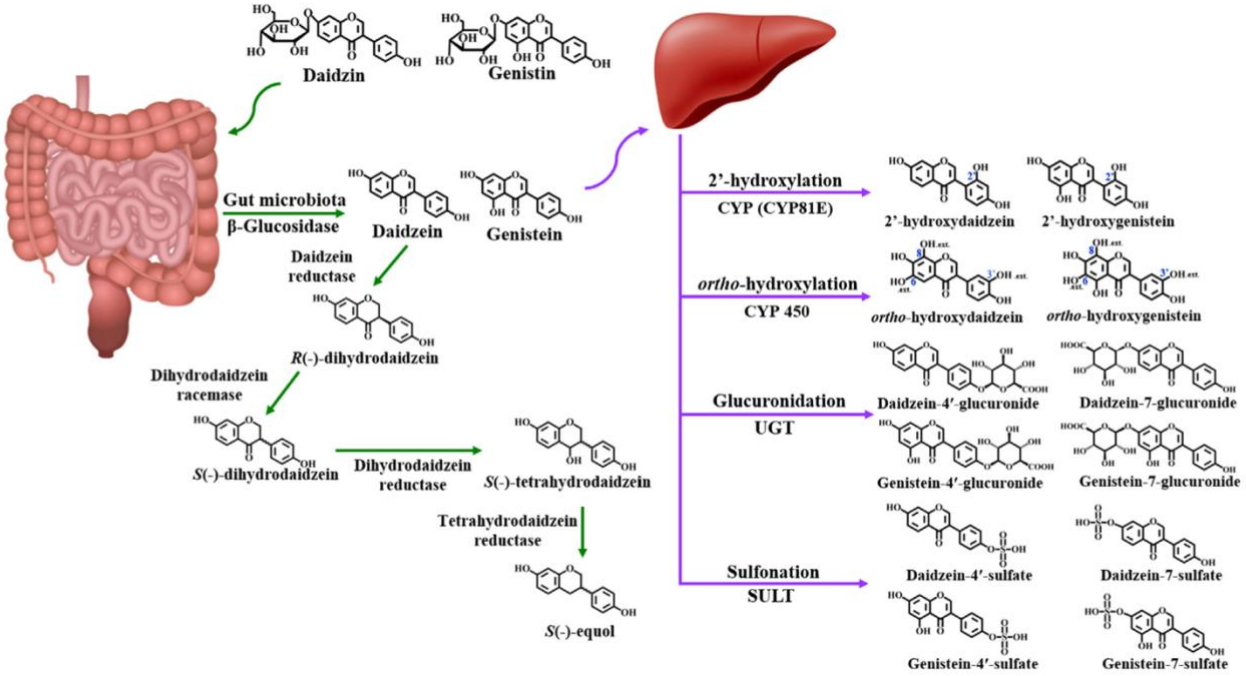

Figure 6:Isoflavone metabolism in the human body: formation of aglycones (daidzein, genistein) and equol by intestinal and gut microbiota enzymes, and subsequent hydroxylation, glucuronidation, and sulfonation by hepatic phase I/II enzymes [39].

A fraction of hepatic conjugates, however, may be excreted into bile, return to the intestine, reach the colon, and be re-exposed to the gut microbiota. There, bacterial β-glucuronidases deconjugate these metabolites, releasing absorbable aglycones again. Daidzein, in particular, can then be biotransformed by colonic flora into secondary metabolites, or reintroduced into the liver–intestine cycle known as enterohepatic circulation, a mechanism in which genistein also participates [34,38,39].

Microbiota enzymes therefore play a central role in modulating the pharmacokinetic distribution of isoflavones. This mechanism contributes to prolonging their plasma half-life and maintaining the availability of active forms. Yet the colonic flora does more than release aglycones: it can also transform primary metabolites into secondary compounds, some of which display stronger estrogenic activity than their precursors, while others are less active. The proportion and nature of these metabolites depend heavily on the composition of each individual's microbiota [35,38].

A well-documented example is daidzin: its primary metabolite, daidzein, can be further metabolized into O-DMA **(**O-desmethylangolensin**)**, which is weakly estrogenic, or into equol (4′,7-dihydroxyisoflavan**)**, which is much more active and structurally

similar to estradiol. About 30% of the Western population and 60% of the Asian population harbor a colonic flora capable of converting daidzein into equol. Individuals unable to produce equol therefore produce O-DMA instead [36,38]. The chemical structure of equol comprises two phenolic rings with reactive hydroxyl groups and a central furan ring containing an inert oxygen atom. Equol is water-insoluble and unstable in acidic environments. It is specifically S-equol that is naturally produced in equol-producing individuals. Its high affinity for both ERα and ERβ receptors makes it one of the most bioactive isoflavone metabolites. Conversion of daidzein into equol is ensured by specialized intestinal bacteria: *Eggerthella* and *Adlercreutzia* were among the first genera identified in humans. Other genera such as *Slackia* and *Bifidobacterium* also contribute, while *Lactococcus garvieae* has been shown to exhibit strong equol-producing capacity, with a complete metabolic pathway from daidzein to S-equol. It is thought that an individual's ability to produce equol depends on both genetic and environmental factors, including lifelong dietary habits [38]. The higher proportion of equol producers in Asia correlates with a lower incidence of hormone-dependent diseases in these populations, where soy consumption is higher. Thus, at least part of the health benefits associated with soy consumption depends on the individual capacity to convert isoflavones into key metabolites, particularly equol(Figure 7) [35,38].

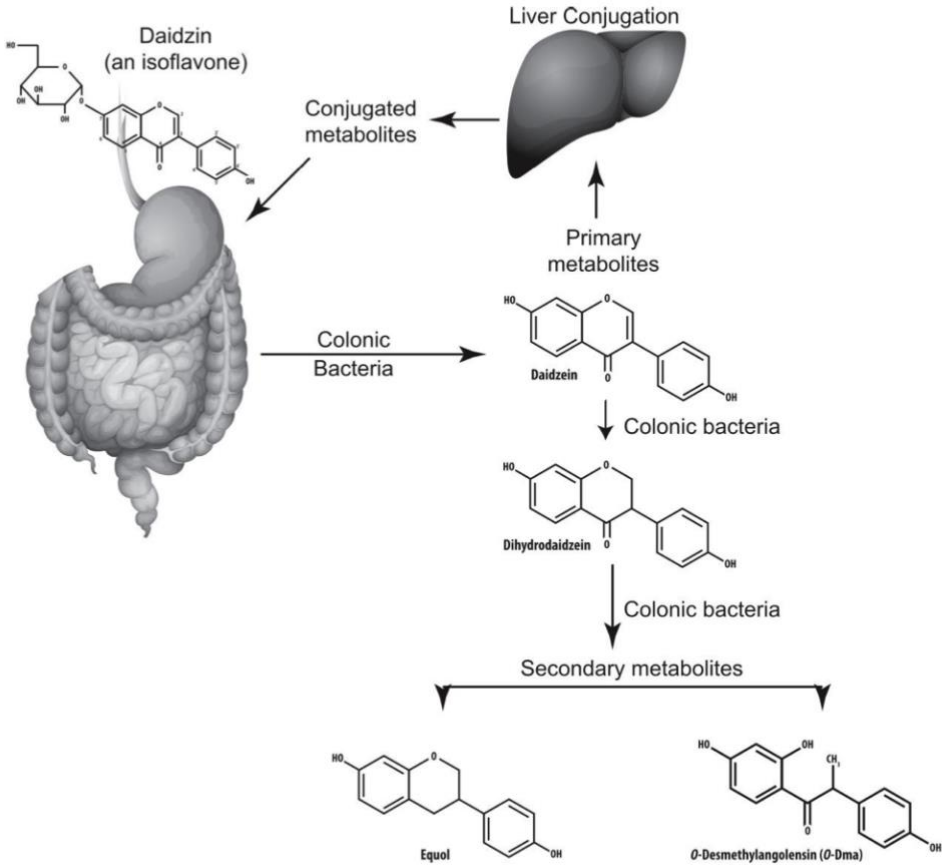

Figure 7: Role of colonic bacteria in the production of primary (dihydrodaidzein) and secondary (equol, O-desmethylangolensin) metabolites from daidzin, and in the metabolism/circulation of daidzein liver conjugates [38].

Although daidzein is not the most abundant soybean isoflavone, it has emerged as the true "star" compound, due to its potential to be converted into equol, a highly active metabolite [38]. Genistein, although more abundant, follows a different fate: it is mainly subject to hepatic conjugation reactions and therefore circulates predominantly in inactive forms. Its secondary pathways (such as dihydrogenistein, HPPA (2-(4-hydroxyphenyl)-propanoic acid), and THB (trihydroxybenzene), derived from ring fission) remain marginal and poorly characterized [37]. Glycitein is the least prominent: it generates only small amounts of metabolites (dihydro-glycitein and demethylated products), which explains why its biological role remains poorly documented [34].

Overall, the three major soy isoflavones do not share the same metabolic fate. Genistein is by far the most extensively studied soy isoflavone, owing to its wide range of biological activities, including tyrosine kinase inhibition and estrogen receptor modulation. Although more abundant in soy, it is mainly subject to hepatic conjugation

(glucuronidation and sulfation) and therefore circulates predominantly as inactive forms, while its secondary metabolites remain of limited importance and poorly defined. Daidzein, in contrast, is less abundant but of particular interest because it can be converted by the colonic microbiota into secondary metabolites such as equol who is characterized by a strong affinity for estrogen receptors, or O-DMA, which is much less active. Glycitein appears as the least significant, producing only small quantities of metabolites, often through demethylation reactions, and its biological role remains largely unclear.

Thus, while the metabolism of isoflavones directly conditions their physiological effects, it also underscores a central issue: their actual bioavailability in humans, which depends as much on hepatic metabolic capacity as on the individual composition of the intestinal microbiota [35].

2.2.3. Bioavailibility

The bioavailability of isoflavones is a central element for understanding their physiological effects in humans. It corresponds to the proportion of these compounds that becomes available for distribution in tissues where they can exert their biological activity [40]. Indeed, their anticancer, antioxidant, and anti-atherosclerotic activities, as well as their ability to interact with estrogen receptors, depend directly on their absorption, distribution, and excretion. To optimize beneficial effects in target tissues and minimize the risk of adverse events related to their estrogenic properties, it is therefore essential to identify the factors influencing isoflavone absorption and excretion [41].

First, intrinsic factors include the ingested chemical form, individual metabolism, and the personal capacity to produce specific metabolites such as equol [42]. The relative proportions of daidzein, genistein, and glycitein largely determine bioavailability. Each isoflavone has a distinct structure, which alters its absorption rate, secondary metabolites, and thus its physiological effects. For example, genistein is generally found at higher serum concentrations, whereas daidzein may give rise to secondary metabolites such as equol, which has a much higher estrogenic activity. Glycitein, on the other hand, undergoes limited transformation and contributes only marginally to overall effects.

A second major determinant of bioavailability is intestinal fermentation. The ability of the gut microbiota to hydrolyze and metabolize isoflavones is critical. Colonic fermentation increases the production of short-chain fatty acids, which improves

absorption and contributes to systemic effects. The liver also plays a central role: its conjugation capacity and its role in enterohepatic circulation allow reintroduction of these compounds into the intestine, where the microbiota can further transform them into secondary metabolites. Finally, the equol-producer phenotype directly influences the bioavailability of this metabolite, considered the most bioactive.[40,42,43]

Extrinsic factors also play a role, such as dietary habits, food matrix, intestinal fermentation, and transit time. Faster intestinal transit increases bioavailability: women with short transit (<40 h) absorb 2–3 times more isoflavones than those with long transit (>65 h). The longer isoflavones remain in the intestine, the greater the opportunity for microbial degradation, reducing the amount available for absorption. The food matrix also influences bioavailability: plasma concentrations of genistein appear more rapidly when consumed in a liquid matrix, though elimination is also faster. In contrast, solid matrices release isoflavones more slowly, prolonging their circulation and increasing urinary excretion. A high-fiber diet (40 g/day, wheat) reduces genistein bioavailability by about one-third compared to a low-fiber diet (15 g/day), while daidzein is not significantly affected [40,42]. Finally, dietary patterns can modulate microbiota composition, potentially promoting the presence of equol-producing bacteria [36].

Conversely, several supposed factors are not actually linked to isoflavone bioavailability. Lipid quantity and source do not influence absorption or metabolism. Human intervention studies have shown higher serum genistein concentrations compared with daidzein, regardless of age or sex, with no difference between pre- and postmenopausal women. Likewise, although fermentation of soy foods increases the proportion of aglycones—which are theoretically more absorbable—the actual impact remains debated. Some studies report faster absorption and higher plasma concentrations after consuming fermented products (miso, tempeh, natto) [40] while others found no significant differences, suggesting that intestinal enzymatic hydrolysis largely compensates for this step [41,42]. Thus, fermentation mainly produces a sharper and earlier plasma peak, but overall exposure remains similar whether soy is consumed in fermented or non-fermented forms. Therefore, ingestion of soy already in aglycone form does not constitute a significant advantage for bioavailability [43].

Kinetic studies of absorption have shown that isoflavones can be detected in plasma as early as 30 minutes after soy ingestion, probably due to the small proportion of aglycones naturally present in the food, which, being already in a free form, are directly absorbed. A first plasma peak occurs about 1 hour after the meal, reflecting hydrolysis of

glycosides into aglycones by small-intestinal β-glucosidases and their rapid absorption in the duodenum and proximal jejunum [36,40]. A second, major peak occurs between 5 and 8 hours post-ingestion. This corresponds to the activity of colonic bacterial β-glucosidases, which release aglycones from unhydrolyzed glycosides, together with the contribution of enterohepatic recycling of conjugated isoflavones [36,41].

Pharmacokinetic studies confirm that the Tmax (Time to Maximum Concentration) of aglycones is generally between 5 and 7 hours (6.6 h for daidzein and 5.2 h for genistein), while for their glycosidic forms (daidzin and genistin) Tmax is delayed to about 9 hours (9.0 h and 9.3 h, respectively) [39]. Direct comparisons show that the Cmax (Maximum plasma concentration) of genistin is about 1.6 times higher than that of genistein, and that of daidzin about 1.8 times higher than that of daidzein, with no notable differences in half-life between aglycone and glycosidic forms [41].

In systemic circulation, approximately 90% of isoflavones are present as conjugates (glucuronides, sulfates), which are biologically inactive, while less than 10% circulate as free aglycones, the only bioactive fraction [38]. Most urinary excretion of daidzein and genistein occurs within 24 hours of ingestion [40]. Finally, among secondary metabolites, equol appears in plasma about 8 hours post-ingestion, corresponding to the transit of daidzein to the colon and its transformation by intestinal microbiota [36].

3. Mechanisms of action of soy bioactive molecules in breast cancer

The estrogenic potential of isoflavones was first suspected long before the 1960s. In 1932, equol, a metabolite of daidzein, was isolated from the urine of pregnant mares and later recognized as a major contributor to the estrogenic activity of soy isoflavones [26]. In 1946, Bennetts et al. [26] reported the so-called “clover disease” in sheep grazing subterranean clover pastures, where infertility was caused by the estrogenic effects of the isoflavone formononetin. At that time, isoflavones were considered anti-nutritional factors because of their adverse reproductive effects in animals. Over the following decades, however, they gradually gained attention as phytoestrogens with potential beneficial effects in humans [26].

In the 1950s, researchers discovered that genistein stimulated uterine growth in immature mice, which confirmed the estrogenic activity of this isoflavone. However, at that time, the link between isoflavones and estrogen receptors had not yet been established [44].

The discovery of the affinity between isoflavones and estrogen receptors dates back to the 1960s. Their binding to ERα receptors was demonstrated, leading to their official classification as phytoestrogens. This was the first time their potential role in hormone-dependent cancers was suggested [45, 44]. In 1982, fifty years after equol had first been identified in mare’s urine, it was also detected in human urine, where it is synthesized from daidzein. It was not until 1987 that the implication of isoflavones was clearly demonstrated: in that year, it was discovered that genistein is capable of inhibiting tyrosine kinase. As previously discussed, overexpression of this enzyme is a major feature of cancer, particularly breast cancer, driving increased cell proliferation and tumor growth. In 1990, the NCI (National Cancer Institute) launched large-scale research programs, investing millions of dollars to investigate the anticancer effects of soy. A year later, epidemiological studies conducted in Asia revealed a correlation between soy consumption and a reduced risk of breast cancer [45, 44].

The year 1995 marked a turning point: Wake Forest University popularized the idea that isoflavones act as natural SERMs (Selective Estrogen Receptor Modulators), meaning that depending on the tissue, they may behave either as agonists or antagonists. This led to the suggestion that they could represent a potential alternative to conventional hormone therapy. Another major development in 1995 was the publication of Lamartiniere’s work, Toxicologist and specialist in hormonal breast cancer prevention,

which demonstrated in animal models that early exposure to isoflavones reduced the risk of developing breast cancer in adulthood. These discoveries were reinforced by epidemiological studies that had already noted the lower prevalence of breast cancer in Asian populations whose diets are based largely on soy [45]. It was discovered in 1996 the Erβ and then 2 years later that isoflavones display greater affinity for ERβ receptors [44]. As noted earlier, activation of ERβ is associated with modulation of cell proliferation, whereas ERα activation stimulates it.

By the late 1990s, clinical trials multiplied, aiming to test and develop soy isoflavone extracts and supplements. The concept of "equol producers" also emerged: certain individuals were thought to benefit more from soy due to their ability to metabolize daidzein into equol. This hypothesis was formalized by Setchell, a pioneering researcher in the field. Today, equol remains an active area of investigation [45].

In the United States, public institutional support played a decisive role in advancing soy research. Beyond funding, several structural initiatives were launched: the creation in 1999 of a database on isoflavone content in foods, followed by dedicated workshops on phytoestrogens and their effects on aging. In 2005, a systematic review of the clinical literature was commissioned, and in 2009, a consensus workshop was organized to establish methodological recommendations for future studies.[45]

However, the early 2000s marked a shift: following the wave of enthusiasm of the 1990s, soy came under scrutiny as a potential safety concern. Alongside positive findings, a counter-discourse emerged, focusing on risk. Isoflavones, in addition to being phytoestrogens and mixed agonists/antagonists of estrogen receptors, were now classified as endocrine disruptors. Their ability to bind to both ERα and ERβ raised concerns that chronic exposure might stimulate estrogen-sensitive tissues such as the breast. Worries were particularly directed at possible proliferative effects in patients with estrogen-sensitive breast cancer or at high risk, in cases of ERα stimulation. Similarly, in infants, massive and early exposure raised questions about potential long-term effects on reproductive development.[45]

Several international agencies (Europe, Japan, Israel, EFSA (European Food Safety Authority)) assessed these risks, primarily using in vitro and animal studies with very high doses. However, evaluations conducted by the National Toxicology Program concluded that the level of concern was low [45].

Between 2005 and 2009, several institutional initiatives marked a turning point in soy and isoflavone research. The AHRQ (Agency for Healthcare Research and

Quality) tasked the Tufts–New England Medical Center with conducting a systematic review of the clinical literature on soy, commissioned by the ODS (Office of Dietary Supplements) [45]. At the same time, AFSSA (Agence française de sécurité sanitaire des aliments), now ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail), concluded in 2005 that soy consumption significantly reduces the risk of breast cancer among Asian women. This reduction was associated with an intake of 30–40 mg/day of isoflavones beginning in adolescence, within a favorable dietary context rich in vegetables and low in saturated fats. However, this protective effect was not confirmed in Western women. Nonetheless, AFSSA concluded that soy consumption does not increase the risk of breast cancer [46].

In the same year, the ODS (Office of Dietary Supplements)„ a U.S. agency funding and supervising dietary supplement research, requested that the AHRQ (Agency for Healthcare Research and Quality) and Tufts University carry out a systematic review on soy. In 2009, the ODS organized a consensus workshop to establish methodological recommendations for future clinical studies, such as standardizing extracts, specifying dosages, and considering hormonal status [45].

In short, in the 2000s and until now, institutions from different countries sought to bring order to soy research, which had until then been marked by scattered and contradictory results [26,45,44, 46]. Table 1 exhibits the key milestones in the discovery and development of scientific knowledge on soy isoflavones, from their identification as phytoestrogens in the 1960s to research on their anticancer potential and official recommendations.

Table 1 : Key milestones in the discovery and development of scientific knowledge on soy isoflavones [45, 46].

Year	1960s	1987	1990s	2005	2009
Event	Discovery of the affinity between isoflavones and estrogen receptors (Erα), leading to their first classification as phytoestrogens.	Genistein identified as a tyrosine kinase inhibitor, revealing a clear link to cell proliferation and tumor growth	The U.S. National Cancer Institute launches large-scale research programs on the anticancer effects of soy. Epidemiological studies in Asia report an association between soy consumption and a reduced risk of breast cancer. Wake Forest University promotes the concept of isoflavones as natural SERMs; stronger affinity for ERβ receptors demonstrated. Lamartiniere shows in animal models that early exposure to isoflavones lowers the risk of breast cancer later in life. Clinical trials on soy isoflavone extracts and supplements expand; Setchell introduces the concept of "equol producers". A U.S. database on isoflavone content in foods is created.	AFSSA (France) concludes soy intake significantly reduces breast cancer risk in Asian women (30-40mg/day since adolescence.) The AHRQ and Tufts University are commissioned by ODS to conduct a systematic review on soy.	The U.S. Office of Dietary Supplements holds a consensus workshop to establish methodological recommendations for future clinical studies.

Recently, meta-analyses have confirmed that high soy consumption and, consequently, increased isoflavone intake, is associated with improved breast cancer prognosis and a reduced risk of recurrence, particularly among postmenopausal patients. The American Cancer Society concluded that post-diagnosis soy intake may reduce recurrence and improve survival in 2022 too. In 2023 the World Cancer Research Fund International (Global Cancer Update Programme) identified soy consumption as a decisive factor in improving survival in patients with breast cancer [44].

Thus, despite the debates and uncertainties of previous decades, clinical data increasingly support the hypothesis of a protective effect of soy. In this context, it becomes essential to examine in detail the mechanisms through which isoflavones exert their effects, beginning with their interaction with estrogen receptors [47].

3.1 Estradiol as the reference ligand of estrogen receptors

To understand how phytoestrogens bind, the first step is to examine how the reference ligand interacts and how these receptors function. The work of Jensen and Jacobsen demonstrated that estrogens exert their effects via a protein receptor. The

cloning of ERα in 1986, followed by the discovery of ERβ in 1995, revolutionized the understanding of this signaling pathway. Since then, numerous isoforms of ERβ have been identified, arising from structural variations in either the N-terminal domain or the LBD (ligand-binding domain)**.** Some isoforms, such as ERβ-503 or ERβcx**,** differ in their affinity for estradiol, as well as in their ability to bind DNA, dimerize, and activate transcription. [48]

Estrogen receptors belong to the steroid/thyroid nuclear receptor superfamily and display a modular organization: an N-terminal A/B domain containing the AF1 activation function (ligand-independent transcriptional activity), a highly conserved C domain responsible for DNA binding, a hinge D domain, and finally the LBD carrying the AF2 activation function (ligand-dependent). ERα and ERβ share high homology within the DNA-binding domain, conferring a similar affinity for estrogen response elements. In contrast, their amino-terminal regions differ markedly: the AF1 region of ERα contributes to estradiol agonism and the partial agonism of tamoxifen, whereas ERβ lacks this property, helping to explain divergent responses to selective modulators. Within the LBD, helix H12 plays a pivotal role: depending on the ligand, its positioning dictates the recruitment of coactivators and the intensity of transcriptional activation. Estradiol promotes an agonist positioning of H12, while compounds such as raloxifene or genistein induce antagonistic or partial conformations. Thus, although ERα and ERβ share a broadly similar architecture, their differences in AF1 and AF2 dynamics underpin tissue selectivity and the differential modulation of gene networks by estrogens and their therapeutic derivatives (Figure 8) [48,49].

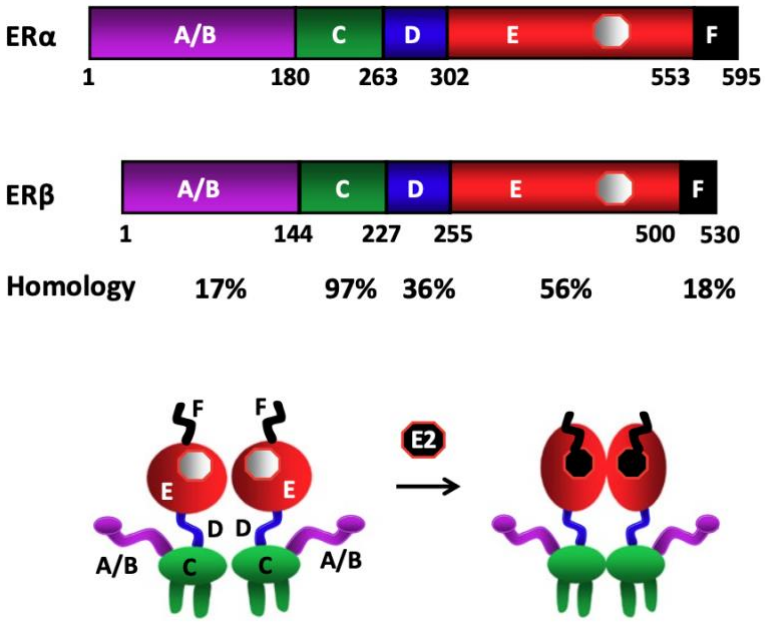

Figure 8 : Structural domains of estrogen receptors α and β, showing the DNA-binding domain, hinge, ligand-binding domain and carboxyl-terminal region [49].

The LBD of ERα contains a hydrophobic pocket in which estradiol is stabilized by a precise network of hydrogen bonds and van der Waals contacts. The A-ring of the steroid is anchored through a hydrogen bond between its 3-hydroxyl group and the side chain of Glutamine353, an interaction that confers receptor specificity toward hydroxylated rather than 3-keto ligands. This glutamate is oriented and rigidified by Arginine394 via a polarized network, sometimes mediated by a water molecule, while Arginine394 itself is stabilized by a hydrogen bond with the carbonyl preceding Phenylalanine404. At the opposite end, the 17-hydroxyl group of estradiol forms a hydrogen bond with the imidazole nitrogen of Histidine524. The interaction is further reinforced by hydrophobic contacts along the polycyclic backbone, shaped by residues such as Leucine387, which fit the contours of the ligand. This cooperative framework, where specific hydrogen bonds combine with van der Waals forces, turns estradiol into the structural core of the LBD, ensuring stability and the proper arrangement of the lower half of the domain around the ligand (Figure 9 and 10) [50].

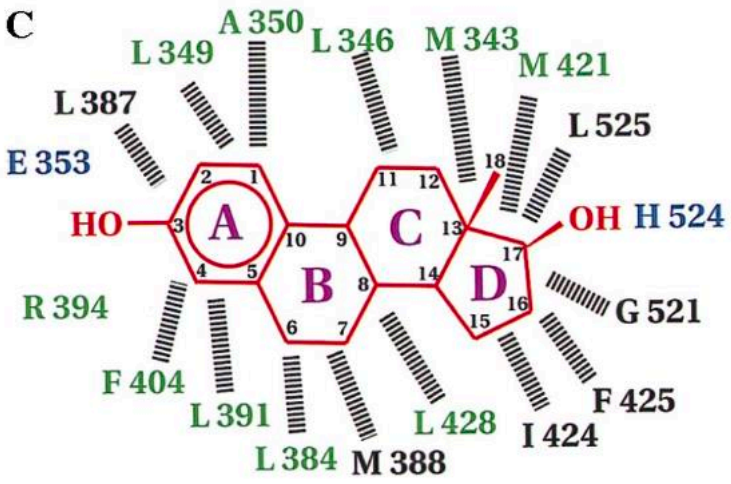

Figure 9 :Key hydrogen-bond interactions of estradiol within the ERα ligand-binding domain [50].

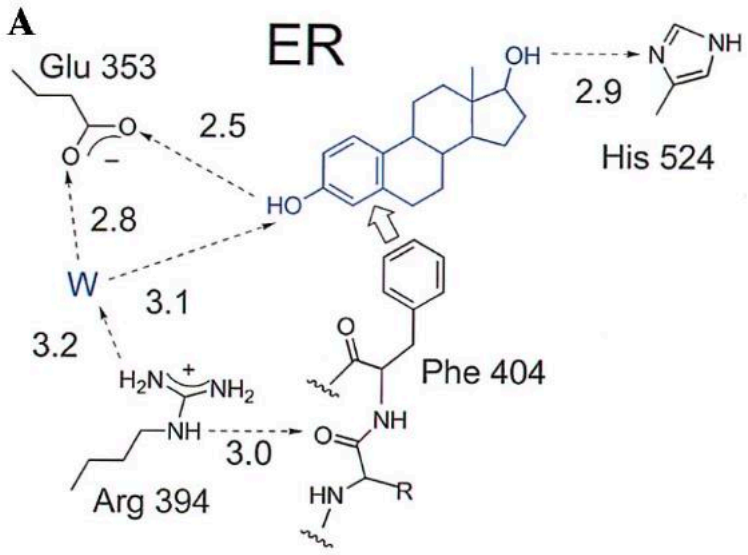

Figure 10: Estradiol in the ERα ligand-binding pocket with hydrogen bonds (blue), hydrophobic contacts (dashed), conserved (green) and variable residues (black) [50].

The LBDs of ERα and ERβ share the same overall architecture, consisting of about a dozen α-helices, a small β-sheet, and helix 12, whose position depends on the bound ligand. Their basic structure is therefore highly conserved, but the ligand-binding

cavity exhibits notable nuances. In ERβ, two amino acid substitutions within the pocket, combined with several peripheral residue variations, slightly alter the volume and geometry of the active site. This micro-adjustment creates an environment more favourable to the binding of smaller or more linear compounds than those preferred by ERα. The example of diarylpropionitrile, a selective ERβ agonist, illustrates this selectivity: its size and shape fit the spatial constraints imposed by the ERβ LBD, whereas ERα remains more permissive toward classical steroidal ligands such as estradiol. These structural particularities also explain why certain compounds—particularly phytoestrogens, SERMs, and selective agonists—display variable affinity or efficacy depending on the receptor subtype, which represents a major issue in understanding the effects of isoflavones and other non-steroidal modulators [49].

Ligand-dependent transcriptional activation then relies on the dynamics of helix 12, the twelfth α-helix of the LBD and a key structural element . When an agonist such as estradiol occupies the pocket, helix 12 folds down and stabilizes the complex. This movement creates, on the external surface of the domain, the AF-2 (Activation function 2) site, a ligand-dependent activation site within the LBD that mediates coactivator recruitment, essential for it and transcriptional complex assembly. In contrast, an antagonist such as raloxifene prevents this repositioning: helix 12 instead inserts into a hydrophobic groove, neutralizing AF-2 and blocking the activation cascade. Thus, the "lid-like" role of helix 12 directly determines the receptor's ability either to transmit the estrogenic signal or, conversely, to inhibit it depending on the ligand [48].

Beyond classical steroids, "angular" ligands—whose core is less compact and planar than that of estradiol, such as triphenylethylenes—have been studied to analyse their interaction with the LBD. It was found that they subtly alter the environment of the ERα active site. Their orientation displaces certain LBD residues, which can destabilize or slightly reposition helix 12. Depending on the extent of this movement, the AF-2 surface becomes either fully accessible to coactivators or partially disrupted, favouring the selective recruitment of coregulators or even corepressors. This mechanism highlights the plasticity of ERs: compounds whose geometry differs from estradiol, such as certain phytoestrogens or isoflavones, can induce specific LBD conformations, explaining their variable activity (agonist, antagonist, or modulator) on the receptor [51].

These differences in the ERβ pocket thus create a microenvironment able to accommodate smaller or less steroid-like compounds, such as selective agonists or phytoestrogens like isoflavones, and pave the way for the discussion of their selectivity and functional activity [49].

3.2 Isoflavones and their interactions with estrogen receptors: Mechanisms and binding affinities

Isoflavones exert most of their biological effects through the signalling pathways of ERs, owing to their chemical structure, which closely resembles that of 17β-estradiol (Figure 11 and 12) [36].

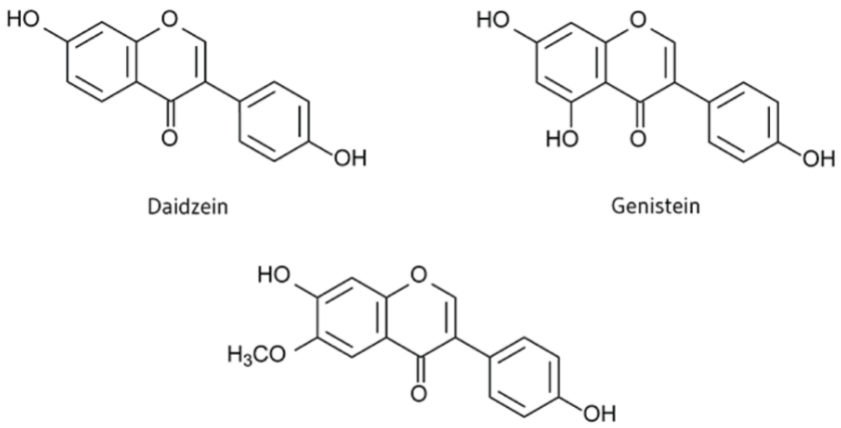

Figure 11 : Chemical structures of the main soy isoflavones: daidzein, genistein and glycitein [36].

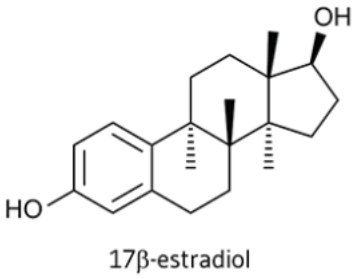

Figure 12 : Chemical structure of 17β-estradiol [36].

In contrast to estradiol and most endogenous or pharmaceutical estrogens, which display preferential affinity for ERα, isoflavones—particularly genistein, daidzein, and equol—are preferential ligands of ERβ. This selectivity is explained by subtle structural differences in the ligand-binding pockets of the two receptors: in ERα, residues Leucine384 and Methionine 421 are replaced by Methionine 336 and Isoleucine 373 in ERβ, providing ERβ with a conformation more favourable for isoflavone binding. Consequently, genistein, for example, has been shown to be between 7- and 48-fold more selective for ERβ in in vitro assays [29,52,47].

Compared with estradiol, isoflavones exhibit a much lower affinity for estrogen receptors: genistein reaches about 0.08% of the relative potency of estradiol, equol 0.06%, and daidzein only 0.01% (estradiol = 100). This apparent weakness is compensated by their circulating concentrations, which can be up to 10,000 times higher than those of estradiol, thereby conferring a significant biological potential nonetheless [53]. In general, the binding potency of isoflavones represents between 10^{-4} and 10^{-3} that of estradiol [47].

Among phytoestrogens themselves, affinity also differs. Genistein binds more strongly than daidzein, because it carries a phenolic hydroxyl group capable of stabilizing receptor binding through an intramolecular hydrogen bond. Daidzein, lacking this group, exhibits weaker binding [52].

To confirm and quantify these differences, as well as their potential effects on estrogen receptors, researchers have, over the decades, investigated their binding to receptors, the recruitment of coactivators, transcriptional activation, and the impact on cell proliferation [52]. The prevailing hypothesis is that they exert antiestrogenic activity in estrogen-rich environments and estrogenic activity under the opposite conditions. The first case can be explained by competition with estradiol for binding to the estrogen receptor; because their activity is weaker than that of estradiol, the overall effect is antiestrogenic [54]

Table 2 : Relative binding affinities (RBA, %) of 17β-estradiol (E2) and selected phytoestrogens for estrogen receptors ERα and ERβ, and β/α selectivity ratios (adapted from [52]).

Compound	RBA $_{ERα}$ (%)	RBA $_{Erβ}$ (%)	β/α
E2	100	100	1
Genistein	0.021 ± 0.009	6.80 ± 1.1	324
Daidzein	0.003 ± 0.001	0.051 ± 0.02	17
S-Equol	0.144 ± 0.02	3.50 ± 0.51	24

The affinity of isoflavones for estrogen receptors has been assessed by (name of the authors [52] using a competitive assay with E2 as the reference (set at 100%). Their results can be seen in Table 2. All isoflavones exhibit markedly lower affinities than E2, approximately 10- to 50-fold weaker, but with a pronounced preference for ERβ, which confers a distinct biological profile. Among them, genistein stands out as the most potent ligand, with 6.8% of the affinity of E2 for ERβ and a β/α ratio of 324, reflecting very high selectivity. In contrast, daidzein shows much weaker binding (ERβ = 0.051%), whereas S-equol displays an intermediate yet biologically significant affinity (ERβ = 3.5%, β/α ratio = 24). The conversion of daidzein into S-equol by the gut microbiota adds further biological relevance, underscoring the role of equol producers in modulating the effects of soy.

Overall, genistein and S-equol emerge as the most relevant candidates for preferential ERβ activation, making them particularly promising in an antiproliferative context [52, 55, 56]. Mechanistically, the physiological response to equol appears to require activation of AF-1–mediated transactivation [56].

However, binding affinity alone is insufficient to predict the actual biological activity of isoflavones, since transcriptional activation of the receptors also depends on the recruitment of coactivators such as SRC3, whose interaction directly modulates the regulation of target genes [52].

SRC3 and RIP140 play a central role in the regulation of estrogen receptor activity: SRC3 acts as a coactivator by amplifying transcription, while RIP140 functions as a corepressor. The transcriptional efficacy of a ligand therefore depends not only on its binding affinity for the receptor but also on its ability to recruit these cofactors.

Table 3 : Binding affinity (EC_{50} and RCA) of the coactivator SRC3 to ERα and ERβ complexes with 17β-estradiol (E2) and various phytoestrogens, expressed as relative coactivator activity (RCA) values (adapted from [52])

Compound	EC_{50} ERα (nM)	RCA ERα	EC_{50} ERβ (nM)	RCA ERβ	β/α
E2	0.13±0.004	[100]	0.12±0.002	[100]	[1.0]
Genistein	1.1 ± 0.1	11.30±0.7	0.20 ± 0.01	61 ± 0.9	5.4
Daidzein	14.2 ± 1.1	0.88±0.05	0.22 ± 0.02	54.4 ± 2.1	61.8
S-equol	16.7 ± 0.6	0.73±0.01	0.18 ± 0.01	78.5 ± 4.2	108

The same authors [52] also evaluated the ability of isoflavones to recruit SRC3 using a TR-FRET assay, with results expressed as Relative Coactivator Affinity (RCA), normalized to E2 set at 100 (Table 3). E2 displayed a perfectly balanced profile (RCA = 100 for both ERα and ERβ, β/α ratio = 1) and high potency (EC_{50} ~0.02 nM). Genistein showed weak efficacy through ERα (RCA ≈ 11) but much stronger activity through ERβ (RCA ≈ 61, β/α ratio = 5.4). S-equol proved to be the most selective, with virtually no recruitment via ERα (RCA ≈ 0.7) but robust activity via ERβ (RCA ≈ 79), corresponding to more than 100-fold selectivity for ERβ and approaching the efficacy of E2. Daidzein also demonstrated a clear preference for ERβ, although it remained overall the least potent. Other studies indicate that genistein, despite having a much lower binding affinity than estradiol for estrogen receptors (≈0.06% for ERα and 7.4% for ERβ), nonetheless shows a strong ability to recruit SRC3 via ERβ [57, 52]. This contrast is striking: genistein remains weakly effective through ERα, but through ERβ it induces a receptor conformation highly favorable to coactivator recruitment, with a relative recruitment potency (RRP) approximately 48 times greater for ERβ than for ERα. In summary, the biological activity of genistein depends less on its raw binding capacity than on its ability to modulate coactivator recruitment, with a clear preference for ERβ [57]. Thus, isoflavones differ from E2 by a dual selectivity: both in receptor binding and in SRC3 recruitment, thereby favoring transcriptional activation through ERβ [52]. Since ERβ activation is associated with antiproliferative effects, this functional bias may explain the protective action of isoflavones. Consequently, isoflavones are known to modify the three-dimensional structure of ERs in order to enhance binding and cofactor interactions[56].

However, their actual biological activity can only be fully understood when in vitro assays of their functional efficacy are also taken into account.

Table 4 : Potency of 17β-estradiol (E2) and selected phytoestrogens (genistein, S-equol, daidzein, liquiritigenin) through ERβ versus ERα, measured in cellular gene-stimulation assays and cell-free (ligand/coactivator binding) assays (adapted from [52]).

	Potency from cellular activities			Potency from cell-free activities		
	Gene stimulation EC_{50} (nM)		β/α potency selectivity [relative to E2)	β/α selectivity RBA x RCA [relative to E2]	β/α RCA selectivity	β/α RBA selectivity
	Erβ only, OTUB2	Erα only, PgR				
E2	1**,67**	0**,02**	0**,012[1.0]**	[**1.0]**	[**1.00]**	[**1.00]**
Genistein	4	24	6 [500]	1750	5.4	324
S-Equol	1,4	14	10 [830]	2600	108	34
Daidzein	50	200	4 [340]	1024	62	17

Therefore, the same researchers [52], used MCF-7 breast cancer cell lines selectively expressing ERα, ERβ, or both to analyze the impact of isoflavones. The results are shown in Table 4. The assays focused on gene activation (PR for ERα, OTUB2 for ERβ) and cell proliferation via Ki67. Other studies [58] employed adenoviral gene transfer to introduce ERβ, combined with ERα depletion by interfering RNA, thereby generating human MCF-7 cells expressing four different combinations of ERα and ERβ [58]. The objective was to observe the dose-dependent effects of genistein [58] or other isoflavones such as equol and daidzein [52] on genome-wide gene expression using DNA microarrays [58], and to track the recruitment of coregulators at estrogen-responsive gene regions.Transcriptomic analysis [58] demonstrated that genistein acts in a dose- and receptor-dependent manner. At low concentration (≈6 nM), it regulated gene expression much more effectively in cells co-expressing ERα and ERβ than in those expressing ERα

alone. At high concentration (≈300 nM), its transcriptomic profile became very similar to that of 17β-estradiol. The data revealed preferential activation of ERβ, with recruitment of this receptor to estrogen-responsive genomic sites, along with modulation of coregulator recruitment such as SRC3 and RIP140. These findings suggest that genistein is a potency-selective ligand, whose effects depend on dose, ligand nature, and the ability of ligand–ER complexes to recruit different coregulators.
Binding and coactivator recruitment assays confirmed the strong selectivity of isoflavones for ERβ, with genistein and particularly S-equol emerging as the most relevant [52]. In cellular conditions, however, this selectivity manifests variably depending on context. At low concentrations (<10 nM), isoflavones did not induce proliferation in cells expressing ERα alone, whereas at higher doses (>10 nM, especially 1 μM), proliferative activity via ERα was observed, particularly for S-equol. Co-expression of ERβ markedly reduced this proliferation, underscoring its protective role, while in cells expressing only ERβ, proliferative activity remained low [54].
The two sets of results converge: genistein and isoflavones display preferential affinity and activity toward ERβ, but their effects strongly depend on dose and the ERα/ERβ ratio. At low concentrations, ERβ predominates, modulating gene expression and limiting proliferation; at higher doses, the effects resemble estradiol, with ERα activation and potential induction of proliferation [52,58]. Importantly, although ERβ's antiproliferative function tends to counterbalance ERα, it is often lost in breast cancer, where tumor cells predominantly express ERα [59].
These observations demonstrate that the action of isoflavones is highly dependent on dose, ERα/ERβ ratio, and tissue context. At low doses, they favor protective ERβ activation; at high doses, their selectivity diminishes, and ERα activation prevails, leading to proliferative stimulation similar to estradiol. The ability of patients to produce equol is also critical, as this metabolite is particularly active. In sum, the transcriptional potency of genistein and other isoflavones depends on their receptor binding and their affinity for coactivators, both of which are dose-dependent. Thus, isoflavones may be protective or, conversely, stimulate proliferation depending on the conditions.
Later in vitro and in vivo studies [59,53] introduced a key concept: phytoestrogens can exert opposite effects depending on the timing of menopause. They are harmful during the first five years post-menopause, favoring proliferation, but become potentially protective after ten years, when they may exert chemopreventive effects. These divergent outcomes are explained by the evolution of the hormonal context and the sensitivity

profile of mammary cells. In the early years after menopause, tumor cells—still hypersensitive to estrogen deprivation—may use isoflavones as hormonal substitutes, thereby stimulating proliferation. In contrast, a decade after menopause, these cells become less dependent on estrogenic signals. In this context, exposure to high concentrations of phytoestrogens can trigger cellular stress and apoptosis, thereby conferring chemo preventive potential.

Finally, restricting their role to interactions with estrogen receptors would be reductive. Soy also acts through non-estrogenic mechanisms, including induction of apoptosis, inhibition of angiogenesis, and modulation of oxidative stress and signalling pathways involved in carcinogenesis. This multiplicity of effects explains the ambivalent role of isoflavones in breast cancer.

3.3. Interaction with no estrogen receptors

Beyond their role as modulators of estrogen receptors, soybean isoflavones are also thought to exert a range of ER-independent actions that may contribute to their protective effects in breast cancer, particularly genistein. These non-estrogenic mechanisms include, on the one hand, antioxidant, anti-inflammatory, and endocrine-modulating effects, and, on the other hand, a direct influence on apoptosis, angiogenesis, metastasis, and major tumor signaling pathways. Taken together, these processes illustrate the multifaceted potential of soybean isoflavones in regulating tumor growth [47,60,61]. It should nevertheless be emphasized that these findings stem almost exclusively from in vitro studies, conducted with isoflavone concentrations 50 to 100 times higher than those achievable in human plasma or tissues. While such effects—comparable to those sought in chemotherapy—could theoretically contribute to a protective action against cancer, they do not occur at the physiological concentrations observed in humans [61].

3.3.1 Antioxidant and anti-inflammatory pathways

Soy isoflavones, particularly genistein, exert direct antioxidant effects. They act as free radical scavengers, protect DNA against oxidative damage, and reduce the expression of genes involved in oxidative stress and inflammation [47]. These baseline

effects, linked to protection against oxidative stress, are subsequently integrated into the regulation of major inflammatory pathways, foremost among them NF-κB (nuclear factor-kappa B). This transcription factor promotes cell survival and is often constitutively activated in tumor cells. It inhibits apoptosis, favors proliferation and invasion, and strengthens resistance to therapy. Genistein directly inhibits NF-κB activity by reducing its DNA-binding capacity and nuclear translocation [60]. This effect occurs through inhibition of IKK (IκB kinase), which prevents the phosphorylation and degradation of IκBα (Inhibitor of kappa B alpha), thereby retaining NF-κB in the cytoplasm and blocking the induction of pro-survival genes such as Bcl-2 and Bcl-XL [59]. This blockade prevents NF-κB activation by pro-inflammatory stimuli (TNF-α (tumor necrosis factor) or H_2O_2) and leads to reduced expression of survival genes. In vitro, this inhibition is accompanied by apoptosis induction, sometimes via NF-κB–dependent pathways and sometimes independent of them. In vivo, an intervention study confirmed these results: after three weeks of isoflavone supplementation (Novasoy™, 100 mg/day), lymphocytes from healthy volunteers no longer responded to TNF-α with NF-κB activation, suggesting a direct protective effect in humans. These results highlight a role for isoflavones in sensitizing cancer cells to chemotherapy and radiotherapy through NF-κB inactivation [60]. Complementary reviews have emphasized the preventive dimension of this mechanism [47]. By inhibiting NF-κB, isoflavones reduce chronic inflammation, known to fuel the tumor microenvironment and promote the progression of hormone-dependent cancers such as breast cancer. This anti-inflammatory action, associated with the regulation of survival genes, helps explain the protective effects observed in some epidemiological studies. Thus, the downregulation of NF-κB by isoflavones constitutes a central mechanism at the crossroads of apoptosis and tumor prevention [60].

NF-κB is closely interconnected with the Akt (protein kinase B)/PI3K (phosphoinositide 3-kinase) pathway. This signaling cascade is activated by growth factors such as IGF-1 (Insulin-like Growth Factor-1) and is frequently overexpressed in breast cancers. It promotes cell survival by inhibiting apoptosis through NF-κB activation and by stimulating tumor proliferation. Akt is activated by phosphorylation; it inhibits apoptosis by neutralizing Bad (Bcl-2-associated agonist of cell death*)*, caspase-9, and FOXO3a (Forkhead box O3), key proteins and factors that block cell death, and by activating NF-κB. Isoflavones, particularly genistein, do not alter total Akt levels but reduce its phosphorylation and kinase activity, inhibit its activation by IGF-1, decrease

FOXO3a phosphorylation, and increase GSK-3β (Glycogen Synthase Kinase 3 beta), a kinase regulating apoptosis, thereby reactivating pro-apoptotic signaling [60]. They also induce cell-cycle arrest in the G0/G1 and G2/M phases [59]. Overall, genistein induces apoptosis in various cancer cell lines, including ER-negative breast cancers. There is also an indirect interaction with NF-κB, since Akt inhibition contributes to NF-κB inactivation [60]. Beyond Akt, other pro-survival cascades are modulated, notably the MAPK pathway, whose dysregulation supports angiogenesis and tumor progression.

Historically, genistein was also identified as a natural inhibitor of tyrosine kinases, enzymes involved in cell growth signaling and upstream of the PI3K/Akt and MAPK pathways. This mechanism has been widely studied but is likely secondary in vivo, since physiological concentrations achievable in humans are too low to exert significant inhibition [55].

3.3.2. Cell survival and proliferation pathways

The MAPK pathway consists of a core of kinases. It activates NF-κB, promotes cell growth and survival, and is frequently overactivated in cancers; it is associated with angiogenesis, invasion, and metastasis [60]. This signaling axis represents a major target of isoflavones, particularly genistein and daidzein. By interfering with it, they reduce cell proliferation, inhibit tumor invasion and migration, and ultimately promote apoptosis [47].

This influence on proliferative signals is accompanied by an interaction with classical tumor-suppressor mechanisms, notably the p53 pathway. p53 is a transcription factor and tumor suppressor that activates genes such as p21WAF1(Cyclin-dependent kinase inhibitor 1), a cell-cycle inhibitor and Bax (a pro-apoptotic protein), which mediate cell-cycle arrest and apoptosis. It is often mutated or inactivated in many cancers, thereby impairing the response to chemotherapy. In H460 cells with wild-type, functional p53, genistein increases p53 protein levels to trigger cell death. In H322 cells carrying mutant p53, genistein does not modify p53 levels—since the protein is defective—but it increases p21 and Bax while decreasing Bcl-2, thus inducing apoptosis through a p53-independent pathway. In MCF-7 breast cancer cells, which harbor a p53 mutation, genistein stabilizes p53 and inactivates Bcl-2, thereby promoting apoptosis [60]. These effects converge with the activation of the mitochondrial pathway: increased Bax/Bak, inhibition of Bcl-2,

release of cytochrome c, activation of Apaf-1 (Apoptotic protease activating factor 1) and caspase-9, leading to the cascade activation of caspases 3, 6, and 7, proteases that execute apoptosis [59].

Beyond tumor-suppressor genes, isoflavones also target developmental pathways hijacked by cancers, such as Wnt/β-catenin.

3.3.3 Developmental and Cellular Plasticity Pathways

Wnt/β-catenin is a key embryonic developmental pathway involved in proliferation, differentiation, and apoptosis. In cancers, it also undergoes aberrant activation, leading to the accumulation of β-catenin proteins that act as cofactors to activate genes such as c-Myc (a proto-oncogene) and cyclin D1 (a cell-cycle regulator), which promote tumor growth and metastasis. Wnt also interacts with Akt, the pro-survival kinase, thereby further reinforcing these signals. Genistein, once again, increases the expression of GSK-3β, which acts as a natural brake on the Wnt pathway by phosphorylating β-catenin, marking it for degradation. As a result, the expression of growth- and metastasis-promoting genes is reduced, allowing apoptosis induction and inhibition of tumor growth [60,47].

However, Wnt is not isolated: it functions in parallel and sometimes synergistically with the Notch pathway, which is also modulated by isoflavones. Notch is involved in embryonic development and the maintenance of stem cells. In breast cancer, Notch frequently exhibits aberrant activity, which reduces apoptosis while increasing proliferation, the induction of EMT (epithelial-mesenchymal transition)—a process by which epithelial cells (well-organized, tightly connected, and poorly mobile) transform into mesenchymal cells (more mobile, invasive, and capable of migration). Genistein acts by inhibiting Notch-1 and Notch-2 signalling receptors; this inhibition decreases NF-κB activity, reduces proliferation, and enhances apoptosis. Breaking the Notch/NF-κB connection is crucial to preventing tumor survival [60].

These effects on developmental pathways also converge with non-estrogenic endocrine regulation, as illustrated by the control of the androgen receptor (AR). This receptor is a transcription factor activated by androgens and involved in regulating growth-related genes. It plays a major role in prostate cancer but also in breast cancer. Depending on the context, it may either promote apoptosis or support tumor survival.

Genistein reduces androgen receptor transcription and expression: it blocks receptor binding, decreases PSA (prostate specific antigen, a growth-related gene) expression, and thereby promotes apoptosis [47,60]. This effect is closely linked to the Akt/β-catenin pathway, highlighting the integration of non-estrogenic hormonal regulation into tumor signaling cascades [59]. Isoflavones also interact with other nuclear receptors, such as PPARα and PPARγ (Peroxisome Proliferator-Activated Receptors), which participate in lipid metabolism regulation and, more importantly, the inflammatory response. Through this mechanism, genistein reduces monocyte adhesion to the vascular wall and contributes to its anti-inflammatory effect [55].

Finally, all these regulatory effects are accompanied by post-transcriptional remodeling through specific microRNAs, either locking in survival programs or restoring tumor-suppressor brakes.

3.3.4. Post-transcriptional regulation

MicroRNAs regulate key genes involved in survival, EMT, and therapeutic resistance. Altered microRNA profiles are often associated with aggressive or treatment-resistant cancers. Once again, isoflavones—particularly genistein—can influence them by modulating their expression; they are able to restore the expression of tumor-suppressive microRNAs and repress oncogenic microRNAs [60,47].

3.3.5. Angiogenesis and metastasis

Beyond the regulation of survival and cellular plasticity pathways, genistein exerts anti-angiogenic and anti-metastatic effects. It inhibits the activation of VEGFR (vascular Endothelial Growth Factor) COX-2 (cyclooxygenase-2), enzymes that produce pro-inflammatory and tumor-promoting prostaglandins, and AP-1 (Activator Protein-1), a transcription factor that regulates proliferation, as well as the expression of HIF-1α and VEGF, thereby limiting the formation of new tumor blood vessels (angiogenesis). It also slows invasion and migration by reducing the activation of MMPs (Matrix Metalloproteinases), enzymes that degrade the extracellular matrix and favor invasion/metastasis, and by suppressing SRC-3, a pro-tumoral coactivator [59].

Moreover, in a metastatic breast cancer model, dietary administration of genistein did not alter the size of the primary tumor but reduced the number of pulmonary

metastatic cells by 95%, confirming a potential role in limiting tumor dissemination [55]. This result could contribute to explaining the lower postmenopausal variation in breast cancer incidence observed among Asian women, who are high consumers of soy [55]. These effects converge toward reduced vascularization and metastatic dissemination, thereby reinforcing the protective potential of isoflavones in breast cancer [59].

Thus, these studies highlight the pleiotropic role of isoflavones through the regulation of multiple signaling pathways**.** Nevertheless, most of the observations originate from in vitro or animal studies, while clinical evidence remains exploratory to date, being limited to phase I/II trials that have confirmed safety and suggested modulation of specific biomarkers. This limitation justifies complementing the analysis with results from available clinical meta-analyses.

4. Clinical Evidence on Isoflavones in Breast Cancer

4.1 Observational and clinical evidence

Now that we understand how isoflavones act on the human body, we can turn to observational and clinical data to assess their impact on the prevention and management of breast cancer. In 2015, a double-blind, placebo-controlled randomized trial published in "Cancer Prevention Research" [62] was the first to evaluate, over 12 months, the effect of soy isoflavone supplementation on MD% (mammographic density) and FGV% (fibroglandular volume) in women at high risk or with a personal history of breast cancer, a high MD% is an independent risk factor for breast cancer. The denser the tissue, the higher the risk. Adherence to the protocol was excellent, with no contamination of the placebo arm, and a MD%–FGV% correlation of $R^2 = 0.62$ which means that the two measures are strongly correlated: when one varies, the other varies in the same direction in approximately 62% of cases. MRI (magnetic resonance imaging) measurements allowed a finer quantification than mammography. Results showed small declines in MD% and FGV% in both groups, with no significant difference, whether in the overall population, among breast cancer survivors, or according to menopausal status. These decreases appeared to reflect physiological aging rather than the intervention, suggesting that, at the tested doses and duration, isoflavones have no measurable impact on these biomarkers [62].

More recently, a systematic review with meta-analysis by a Chinese academic team, published in 2023 and including 24 studies (7 cohorts, 17 case–controls; >900,000 women), reported a robust inverse association between dietary isoflavone intake and breast cancer risk across populations [63]. The association remained significant after stratification by menopausal status (OR (Odds Ratio) ≈ 0.75 postmenopause; 0.76 premenopause) and by hormone receptor status (OR ≈ 0.77 for ER−), suggesting a benefit regardless of hormonal profile. Dose–response analyses indicated a 3.2% risk reduction per +10 mg/day in cohorts, and up to 19% in some case–control studies, supporting a threshold of about 10 mg/day. Data also supported a clearer preventive effect in Asia, where intake is regular (15–60 mg/day), compared with the very low exposure in Western countries (<3 mg/day). Clinical trials with doses ≤50 mg/day—and even up to 300 mg/day—showed no major adverse signals (no endometrial hyperplasia or reproductive toxicity). Finally, several studies suggested a potential benefit of early exposure (adolescence), indicating that timing may modulate protection [63].

A 2023 systematic meta-analysis [64], registered in the PROSPERO database and reported according to PRISMA guidelines pooling high-quality cohorts and prospective studies, confirmed an overall favourable effect of isoflavones after breast cancer: a significant reduction in recurrence risk (HR = 0.74; 95%CI 0.60–0.92; I^2 = 58%), more marked in postmenopausal women (HR = 0.72) and in ER+ tumours (HR = 0.82). Breast cancer–specific mortality showed a protective signal mainly for ER− disease (HR = 0.77), and all-cause mortality tended to decrease (HR = 0.88). Dose–response analysis suggested that the benefit for recurrence increased up to the highest intakes reported (~60 mg/day), then plateaued; for mortality, the advantage flattened at ~25–30 mg/day. Results were consistent in sensitivity analyses, with no harmful signal at usual dietary doses (<60 mg/day). The protective effect seemed mainly related to the actual isoflavone content, which is more stable in whole foods (tofu, tempeh) than in some depleted isolates [64].

Another meta-analysis of 40 randomized trials (3,285 postmenopausal women) [65], reported that soy isoflavone intake does not affect four key markers of estrogenicity (endometrial thickness, vaginal maturation index, FSH, estradiol). The absence of effect was robust in sensitivity and subgroup analyses, regardless of form (capsules, foods) or mean dose (66–77 mg/day). These data support previous work suggesting that, unlike pharmaceutical estrogens, isoflavones behave as SERMs, acting selectively by tissue without globally stimulating endometrial parameters. They may even slightly reduce endometrial thickness at higher doses (>54 mg/day), suggesting a potential protective effect against hyperplasia. The literature also supports their benefit in some patients on aromatase inhibitors, for recurrence prevention, while clearly distinguishing them from HRT (hormone replacement therapy), which increases ET (endometrial thickness), VMI (vaginal maturation index), and estradiol. Overall, the findings provide strong evidence for the safety of isoflavones at moderate dietary or supplemental doses [65].

Finally, two major studies published in 2025 further complete this picture. The first, a very recent critical narrative review, presented contrasting results [66]. It emphasized that isoflavone effects depend greatly on model, dose, and hormonal status. *In vitro*, at 0.1–1 µM (aglycone form), they can stimulate proliferation of ER+ breast cells, whereas below 0.1 µM the estrogenic effect disappears, and at unrealistically high concentrations (>5–10 µM) they induce apoptosis. This dose-dependent pattern is also illustrated by Jiang et al. [51,66], where S-equol and other isoflavones activated ERα/ERβ and increased proliferation of ER+ lines at high concentrations but remained neutral at levels closer to dietary exposure. In animals, neonatal exposure to low doses of

genistein promotes mammary differentiation and seems protective against later carcinogens; in "humanized" models, concentrations near 1 µM enhanced the growth of implanted ER+ cells, mainly with genistein/daidzein, and negative interactions with tamoxifen or letrozole were reported. In women, some trials suggest that 45–50 mg/day may modulate estrogenic markers (breast fluids, fibroglandular volume) in premenopausal women, with no signal in postmenopause; one study in ER+ patients noted an increase in proliferative genes, without documented clinical impact. Overall, some cohorts and case–control studies report a modest protective effect of soy, particularly in Asia and with regular low-dose exposure. However, most proliferative signals arise from *in vitro* or animal contexts at doses exceeding usual dietary intake, as Jiang's findings illustrate: mitogenic effects of equol and other isoflavones were evident only at concentrations higher than those achieved after soy ingestion [52,66].

The other study from 2025, was a narrative review by Messina and Nechuta [67], synthesizing over twenty cohorts and observational studies, represents a highly credible source. Messina, a long-standing researcher at the U.S. National Cancer Institute, and Nechuta concluded that observational and clinical data converge toward an overall reassuring profile for isoflavones after breast cancer. Studies analyzing risk markers (breast tissue density, *in vivo* cell proliferation) confirm the safety of soy foods, even in patients on tamoxifen or aromatase inhibitors. Large cohorts, notably the SBCSS (Shanghai Breast Cancer Survival Study), suggest a potential benefit on recurrence and survival, sometimes beyond 50 mg/day of isoflavones—roughly two daily servings of traditional Asian soy foods. Intakes assessed in randomized trials often exceeded this threshold without adverse signals, reinforcing the hypothesis of a modest protective effect, particularly when soy replaces less favorable items in the diet such as ….[67].

4.2. Controversies and limitations

Despite the overall reassuring evidence, several factors limit the strength of the conclusions and explain discrepancies among studies.

Even though the 2015 randomized controlled trial [62] demonstrated high methodological rigor, several aspects restrict the scope of its findings. The sample size was modest, and participants were heterogeneous (pre- and postmenopausal women, with either family or personal histories of breast cancer). Baseline MD% and FGV% values were low, particularly in postmenopausal women, reducing the ability to detect changes. Few "equol producers" were included, although this subgroup may respond differently to isoflavones. The study did not synchronize assessments with the menstrual cycle, although breast density can vary accordingly. Moreover, the supplements used differed from whole or fermented soy foods that are often associated with benefits in observational studies; the timing of exposure (late, after menopause) could also explain the absence of effect. Finally, although the statistical power was adequate to detect differences of 3–9%, the actual variations (<1%) were too small, suggesting either no effect or a potential benefit confined to earlier exposure or other soy forms [62]. Nevertheless, the absence of a measurable effect reported by the 2015 randomized trial is consistent with subsequent evidence. Indeed, the review by Messina and Nechuta published in 2025 [59] concluded that clinical studies have not demonstrated any significant impact of soyfoods or isoflavone supplements on breast cancer risk biomarkers, notably mammographic density and breast cell proliferation. This reinforces the interpretation that the null findings of the 2015 trial [62] reflect a genuine lack of effect under the tested conditions, rather than being solely attributable to methodological shortcomings. Thus, soy consumption does not appear to increase mammographic density, a major biomarker of breast cancer risk, which argues against the existence of a measurable deleterious estrogenic effect under the evaluated conditions.

In the next systematic review and meta-analysis of 24 studies [63], substantial heterogeneity again emerged, related to study design (many case–control studies prone to recall bias), the quality of food-frequency questionnaires (FFQs (Food Frequency Questionnaires), often self-administered), and imprecise intake estimates (soy "hidden" in processed foods). Results differed by source (whole foods vs isolates), chemical form (genistein, daidzein, equol), and bioavailability; equol, which is more estrogenic, was not distinguished in most studies. Protective signals from early case–control studies may have

been overestimated, whereas modern cohorts show more modest effects. A potential risk at very high intakes has been discussed, especially in postmenopausal women (endometrial hyperplasia observed in a few trials at ≥150 mg/day). Finally, causality remains uncertain due to the lack of robust prospective cohorts and confirmatory randomized trials, particularly to disentangle the effects of isolated isoflavones from those of the soy matrix and consumption patterns [63].

The second 2023 meta-analysis [64], despite rigorous methodology, also encountered marked heterogeneity (I^2 up to 82% depending on outcomes), reflecting variability in exposure (pre- vs post-diagnosis), covariate adjustments (ER/PR, stage, treatments), and dietary assessment tools (FFQs, recalls). Timing of exposure was rarely specified, although it may modulate protection. The disproportionate weight of certain large cohorts could skew pooled estimates. Recall bias and imprecise intake measurements—particularly in Western settings where soy is often "hidden" in processed foods—further complicate interpretation, as does the frequent lack of distinction between genistein, daidzein, and equol. Randomized trials remain scarce and small, so a proliferative effect in sensitive subgroups cannot be fully excluded, even if existing data are reassuring. Finally, the form of soy products (fermented vs isolates, whole foods vs refined proteins) influences bioavailability, and intakes above ~60 mg/day remain insufficiently documented [64].

Regarding the 2025 meta-analysis, several uncertainties also persist [65]. The effect on endometrial thickness shows heterogeneity: slight differences across continents were noted (opposite trend in Asia), possibly linked to metabolism (equol production). The estimate for the vaginal maturation index is less precise (wide confidence intervals). Extrapolation to other tissues (breast, bone, CNS (Central Nervous System)) or to major clinical outcomes remains limited: the study focused only on endometrial biomarkers without directly assessing tumour risk. Food quality (isolates low in isoflavones vs tofu/whole soy) may influence outcomes, as may the actual dose consumed, which is very low in Western countries (<6 mg/day). Although industrial funding did not bias the analysis, the presence of an author affiliated with the Soy Nutrition Institute Global underlines the need for vigilance about potential conflicts of interest. Overall, the evidence supports good safety but calls for additional, higher-powered trials to confirm the absence of estrogenic effects in other clinical settings [65].

In the major narrative review published in August 2025 [66], human findings remain mixed: several case–control and cohort studies report neutral or even adverse

effects in addition to protective signals. Biological measures (urine, serum) suffer from timing bias and weak correlation with actual intake. In cohorts, confounding factors (reproductive history, comorbidities) are seldom fully addressed; in Western countries, exposure is low, often concealed in processed foods, and isoflavone content varies according to processing, rarely documented. Intervention studies mainly focus on intermediate biomarkers, as ethical considerations preclude trials on recurrence. Isolated isoflavones appear less consistent than whole soy foods, and effects may depend on other bioactive compounds in soy [66].

Finally, the narrative review by Messina [67] highlights that no randomized trial has directly evaluated the impact of post-diagnosis soy intake on recurrence or mortality. Most evidence stems from observational analyses exposed to confounding (dietary habits, treatment, hormonal status). Available data are dominated by a single Chinese cohort ((Shanghai Breast Cancer Survival Study, SBCSS) (n=4856)) [59], limiting transferability to Western populations where exposure is low and often masked in processed products. Intervention trials have mainly assessed intermediate biomarkers and rarely clinical outcomes. The exact source of isoflavones (supplements vs whole foods) and the presence of other bioactive complicate attribution of effects. In this context, benefits should be regarded as possible but unproven, and moderate dietary intake should be prioritized over high or isolated doses [67].

Given these limitations, recommendations necessarily remain cautious and measured.

4.3. Official recommendations and practical guidance

The body of research now provides a relatively clear picture of the role of soy isoflavones in relation to breast cancer. On the preventive side, dietary intake of isoflavones is associated with a modest but consistent reduction in breast cancer risk, particularly when consumption is regular and exceeds roughly 10 mg/day. This effect is more evident in Asia, where intake often ranges from 15 to 60 mg/day, than in Western countries, where exposure remains below 3 mg/day. Beyond approximately 20 mg/day, the benefit tends to plateau in several syntheses.

Among women already diagnosed with breast cancer, the evidence is generally reassuring. Recent reviews and meta-analyses have found no signal of increased risk of recurrence or mortality; on the contrary, a modest benefit is sometimes reported, notably in ER-positive tumors and after menopause, for intakes corresponding to usual dietary doses (around 20–60 mg/day). Available randomized trials have shown no interference with adjuvant therapies such as tamoxifen; data remain more limited for aromatase inhibitors, but nothing of concern has been reported.

From a hormonal safety perspective, the meta-analyses reviewed show that, at usual doses (66–77 mg/day), isoflavones do not affect endometrial thickness, the vaginal maturation index, FSH, or estradiol. Some findings even suggest a slight decrease in endometrial thickness at higher doses, supporting a potential protective effect against hyperplasia. These data reinforce the idea that, unlike pharmacological estrogens, isoflavones behave more like selective estrogen-receptor modulators (SERMs), acting in a tissue-specific manner without globally stimulating the endometrium. [63,64,65,66,67]

Regarding breast biomarkers, the 12-month double-blind randomized trial found no effect of isoflavones on MD% or FGV% among women at high risk or with a history of breast cancer. The minor changes observed (<1%) seemed to reflect physiological aging rather than the intervention, suggesting that, if any benefit exists, it is unlikely to occur through short-term reductions in breast density in predominantly postmenopausal women [62].

Experimental studies also highlight a key point: the form in which soy is consumed strongly influences actual exposure. In dry soybeans, more than 99% of isoflavones occur as glucosides, which are poorly absorbed; fermentation (e.g., tempeh or miso) converts them into aglycones, which are far more bioavailable, with their content increasing up to twenty-fold after two days. Thus, 25 mg of isoflavones from tempeh does

not have the same biological impact as 25 mg from a highly diluted soy beverage. Fermented products (tempeh, natto, miso) concentrate more aglycones than tofu or drinks, which retain mainly glycosidic forms [68].

Nevertheless, several uncertainties remain. Studies display substantial heterogeneity: varied designs, different tools for estimating intake (FFQs, recalls), sources of isoflavones (whole foods vs isolates), and low exposure levels in Western populations. [63,64,65,66,67] In vitro analyses show that certain isoflavones can stimulate ER-positive cell lines at around 0.1–1 μM, whereas at higher concentrations (>5–10 μM) they induce apoptosis; these levels generally exceed those achieved through diet but illustrate how hormonal context and dose shape the response. The lack of large, randomized trials on recurrence or mortality limits confidence in post-diagnosis effects, and individual variability (equol production, hormonal status, therapies, food matrix) may influence outcomes. Very high doses (>100–150 mg/day), particularly after menopause, remain poorly documented; a few reports of endometrial hyperplasia in older studies warrant caution beyond dietary intakes [66].

In practice, current evidence supports moderate consumption of two daily servings of traditional soy foods, providing roughly 50 mg of isoflavones, including among women with or without a history of breast cancer. Whole foods such as tofu, tempeh, miso, or natto are preferable to depleted isolates, and fermentation should be favored to optimize bioavailability. There is no solid rationale for discouraging soy foods in patients taking tamoxifen or aromatase inhibitors. In low-exposure settings, the main challenge is to achieve a steady intake of about 10–20 mg/day rather than sporadically drinking soy milk.

Placed in the broader context of diet, these findings support nuanced guidance: favour moderate, regular intakes of whole soy foods rather than isolated doses like supplements—this appears to be the safest and most coherent strategy given current data. They also provide a foundation for future trials aimed at clarifying the impact of different isoflavone profiles—depending on form, dose, and timing—on breast cancer risk and progression [62, 63, 64, 65, 66, 67, 68].

Alongside scientific data, several organizations have issued guidance on soy intake for women with or without a history of breast cancer:

In 2015 EFSA concluded that, in peri- or postmenopausal women without a history of hormone-dependent cancer, isoflavones from supplements—up to 150 mg/day for approximately 30 months—showed no adverse effects on breast tissue; however, data

were insufficient to judge their safety beyond this duration or in women with (or who had) estrogen-dependent breast cancer [69].

In 2024 WCRF (World Cancer Research Fund) reported no solid evidence that soy foods increase the risk of breast cancer or recurrence; on the contrary, some data suggest a possible survival benefit after diagnosis. They, therefore, do not recommend encouraging higher intake systematically but note that those who already consume soy have no reason to stop [70].

Likewise, the American Institute for Cancer Research (AICR) states that soy foods can be safely included in a balanced diet, even after breast cancer. Early concerns about carcinogenicity have been dispelled by numerous studies. They particularly recommend favoring whole soy foods (tofu, edamame, tempeh) rather than isolated powders, which are safe but less nutrient-dense and contain fewer protective plant compounds [71].

Cancer Council Australia indicates in 2025 that soy foods form part of official dietary recommendations and are unlikely to increase cancer risk, possibly even reducing it for certain types, although evidence remains limited. They advise people, including breast-cancer survivors, to continue eating the soy foods already in their diet. However, they recommend avoiding isoflavone supplements or soy protein isolates—especially for women with a breast cancer history—and limiting very salty fermented products (miso, soy sauce, natto) due to a possible link with stomach cancer [72].

Finally, according to the American Cancer Society, consumption of whole soy foods (tofu, tempeh, edamame, soy milk, or miso) is safe, even after breast cancer, and may offer protective benefits, especially when introduced early and consumed regularly as in Asia. By contrast, concentrated isoflavone supplements are still discouraged for women with a history of breast cancer, given insufficient evidence on their safety [73].

Overall, isoflavones consumed as whole foods appear safe and likely modestly protective, provided intakes remain within dietary levels (≥10 mg/day). The absence of large randomized trials on major clinical outcomes warrants cautious, context-specific recommendations (Table 5).

Table 5 : Official recommendations from international organizations on soy isoflavone consumption in relation to breast cancer.

Organization	Year	Official Recommendations
EFSA [69]	2015	Isoflavones (≤150 mg/day for up to 30 months) show no harmful effects on the

		mammary gland in peri/postmenopausal women without a history of hormone-dependent cancer; evidence is insufficient beyond this duration or after breast cancer.
WCRF/AICR [70,71]	2024	Soy foods do not increase breast cancer risk or recurrence; a slight survival benefit is possible. No need to increase intake, but no reason to stop if already part of the diet. Soy foods are safe after breast cancer; prioritize whole forms (tofu, tempeh, edamame) over isolated powders.
Cancer Council Australia [72]	2025	Soy foods are safe, even after breast cancer; avoid isoflavone supplements and soy protein isolates, and limit very salty fermented products.
American Cancer Society [73]	2025	Whole soy foods are safe, even after breast cancer, and may be protective; avoid concentrated isoflavone supplements in women with a history of breast cancer.

Conclusion

Since the 1960s, understanding of the role of isoflavones in the prevention and therapeutic support of breast cancer has evolved considerably. Early observations from animal models and in vitro experiments suggested a potential estrogenic effect stimulating hormone-dependent tumors, but these data were based on very high doses and experimental conditions far removed from real human exposure. Epidemiological studies, particularly in Asia where soy intake is structurally higher, later highlighted an inverse association between isoflavone consumption and breast cancer risk, while Western cohorts, with lower exposure, reported more modest signals but overall converging toward the absence of harmful effects. Recent meta-analyses confirm a moderate protective association, especially above approximately 10 mg/day of isoflavones, whereas below this threshold the effect appears neutral.

The mechanisms of action are now better characterized: isoflavones act as selective estrogen receptor modulators (SERMs), showing preferential affinity for ER-β. Depending on hormonal and tissue context, they exert antiproliferative effects and also modulate oxidative stress, inflammation, and other pathways. Among postmenopausal women, randomized trials and meta-analyses have found no increase in estrogenicity biomarkers at dietary doses, supporting the absence of worrisome biological signals.

After breast cancer diagnosis, several large cohorts have shown that moderate soy consumption is not associated with increased risk and may even reduce recurrences and, in some cases, improve survival, including among patients treated with tamoxifen or aromatase inhibitors. Reviews published between 2023 and 2025 confirm both the safety and potential benefit of isoflavones in this setting. Current recommendations support the inclusion of soy foods in the diet of women with or without a history of breast cancer, whereas the use of high-dose supplements should remain cautious due to the lack of robust survival data.

The status of "equol producer" may influence the response to isoflavones. A correlation has been observed between breast cancer prevalence in Asia and the markedly higher proportion of "equol producers" in this population, equol being one of soy's most bioactive metabolites.

Nevertheless, results remain heterogeneous and do not allow for definitive conclusions. More broadly, the observed effects depend on variables such as age, menopausal status, tumor receptor profile, intake level, and other hormonal factors.

In light of these data, official recommendations converge: soy consumed as whole foods is considered safe, including for patients receiving tamoxifen or aromatase inhibitors. Highly concentrated supplements should be approached with caution until more evidence is available. Thus, the moderate inclusion of traditional soy-based foods within a balanced diet appears to be the most consistent strategy in light of current knowledge.

Finally, this work highlights that soy isoflavones exemplify contemporary challenges in nutrition and cancer research: linking biological mechanisms, clinical evidence, and public health recommendations. Future perspectives should include large, randomized trials on recurrence and mortality, investigation of the microbiota and "equol-producer" status, as well as detailed comparisons between whole foods, fermented products, and supplements. Only an approach combining scientific rigor, methodological prudence, and interdisciplinary openness will clarify the place of soy in a comprehensive strategy for breast cancer prevention and therapeutic support. Ultimately, the role of soy bioactive compounds in the prevention and management of breast cancer has fascinated researchers for more than 50 years and it has yet to reveal all its secrets.

Within this landscape, the community pharmacist occupies a strategic position. As frontline health professionals, they can identify breast cancer risk factors—smoking, alcohol use, age, overweight, chronic diseases, nulliparity—and refer patients for medical evaluation when appropriate. Beyond early detection, they contribute to patient education by advising on safe and effective intakes of soy, generally between 10 and 50 mg of dietary isoflavones per day, a range where benefits most often appear. Their role, grounded in a critical appraisal of available evidence, supports the thoughtful integration of these compounds into overall patient management, complementing validated therapies.

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