FACULTAD DE MEDICINA
UNIVERSIDAD DE CANTABRIA

GRADO EN MEDICINA

TRABAJO FIN DE GRADO

El receptor de estrógenos en el cáncer de mama

Estrogen receptor in breast cancer

Autor/a: Paula López Bienert

Director/es: Carlos Manuel Martínez Campa

Santander, junio 2023

CONTENTS

ABSTRACT **1**

1. INTRODUCTION **1**

2. NUCLEAR RECEPTORS **2**

2.1. CLASSIFICATION 2
2.2. COMMON STRUCTURE 6
2.3. LIGAND INTERACTION 8
2.4. DNA INTERACTION 9

3. ESTROGEN RECEPTORS **10**

3.1. STRUCTURAL PROPERTIES OF ERS 13
3.2. ISOFORMS OF ERS 16
3.2.1. ERα isoforms *16*
3.2.2. ERβ isoforms *17*
3.2.3. ER-X *18*
3.3. ERS SIGNALING PATHWAYS AND ITS EFFECTS 18
3.3.1. Ligand-dependent pathways *18*
3.3.2. Signaling crosstalk *22*
3.3.3. Ligand-independent signaling *22*
3.3.4. GPER1 signaling pathways *23*

4. ESTROGEN RECEPTORS IN BREAST CANCER **25**

4.1. THE ROLE OF ERS IN BREAST CANCER 25
4.1.1. ERα and breast cancer *26*
4.1.2. ERβ and breast cancer *26*
4.1.3. GPER1 and breast cancer *28*
4.2. ER CLASSIC LIGANDS AND THEIR THERAPEUTIC IMPLICATIONS 29
4.2.1. Selective estrogen receptor modulators (SERMs) *29*
4.2.2. Selective estrogen receptor degraders (SERDs) *33*
4.2.3. Sulfatase and Aromatase inhibitors *35*
4.3. PROGNOSIS 36
4.3.1. Mechanisms of endocrine resistance *36*
4.3.2. Clinical management of endocrine resistance *38*

5. ESTROGEN – MELATONIN INTERACTIONS **46**

6. CONCLUSIONS **49**

7. REFERENCES **51**

ABSTRACT

Estrogens play important modulatory roles in multiple complex physiological and pathophysiological processes. They perform their roles mainly by interacting with estrogen receptors (ERs) which are ligand-dependent transcription factors that regulate gene transcription through different pathways, thereby facilitating the normal biological functions of estrogens.

However, abnormal ER signaling can result in multiple disorders, including gynecological disorders and various types of cancer, such as breast cancer, where they play a fundamental role in initiation, progression and metastasis, especially in hormone receptor-positive or luminal breast cancer. Therefore, targeting ERs with endocrine therapy, alone or in combination with other targeted agents, represents the primary approach for treating this type of breast cancer. In addition, quantification of estrogen receptors is usually used as a predictor of breast cancer prognosis.

The aim of this work is to provide an overview of different aspects of estrogen receptors ranging from their types, isoforms, structures, signaling pathways, pathological roles in breast cancer and therapeutic strategies to overcome unleashed endocrine resistance.

1. INTRODUCTION

The estrogen receptors (ERs) belong to the superfamily of nuclear receptors (NRs), a group of approximately 48 members in humans, behaving as ligand-activated transcription factors that act as a bridge between environmental and hormonal signals and genomic responses, regulating various cell fate decisions at the level of gene expression. They are activated by steroid hormones and a variety of other lipid-soluble signals, such as oxysterols, retinoic acid and thyroid hormones, since these compounds can cross the plasma membrane and bind to their respective receptors inside the cell.

Members of this superfamily share a common architecture consisting of six main functional and structural domains, which result in various quaternary configurations. These receptors are typically composed of a disordered N-terminal domain (NTD), a highly conserved DNA-binding domain (DBD), a hinge D region, a ligand-binding domain (LBD) and a C-terminal domain (CTD). However, there are some variations between different receptors.

This superfamily can be sub-divided into subfamilies based on their cellular location and their genomic response to ligands. An important group of these receptors include the high-affinity receptors for steroid hormones, which are usually located in the cytoplasm and include the estrogen receptors as well as the androgen receptors (ARs),

the mineralocorticoid receptors (MRs), the progesterone receptors (PRs) and the glucocorticoid receptors (GRs) [1].

As mentioned above, steroid receptors are typically found in the cytoplasm. However, with regards to ERs, a significant portion is present in the nucleus, while only about 5% is in the cytoplasm. It is for this reason that there are different approaches to target cytoplasmic and nuclear ERs, which will be further discussed.

ERs are normally activated by estrogens or other ligands that can easily diffuse across the cell membrane and bind the estrogen receptors inside the cell triggering intracellular events leading to regulation of various physiological processes. However, in some cases, estrogen receptors can also be activated without the presence of estrogen or other receptor agonists [2].

Despite playing a role in regulating various physiological systems, such as the central nervous, cardiovascular and reproductive systems, ERs are also involved in regulating cell proliferation through the cell cycle. This association between ER activity and cell proliferation has been linked to the development of different estrogen-mediated tumors, being breast cancer the most frequent one [3].

This review provides an overview of nuclear receptors focusing on estrogen receptors, including their structures, isoforms and signaling pathways, as well as the pathological and clinical implications of these receptors, especially in breast cancer. We will also summarize the state-of-the-art of antiestrogen ligands used in breast cancer therapy, strategies to overcome resistance to these ligands and, finally, the oncostatic roles of melatonin on hormone-dependent mammary tumors.

2. NUCLEAR RECEPTORS

The superfamily of nuclear receptors is a group of transcription factors that are widely expressed in many tissues throughout the body and have a significant role in various biological functions, such as metabolism, inflammation and reproduction. This superfamily operates through highly coordinated signaling pathways that are heavily dependent on the tissue microenvironment. Any disruption in these pathways, whether endogenous or exogenous, can lead to organ dysfunction, tissue loss or even cancer [4].

2.1. Classification

The first NR members were cloned in 1985. Since then, many other members have been discovered, and presently the human NR family has grown to comprise 48 members, that can be classified in different ways depending on the specific characteristics that are being considered (Figure 1) [5].

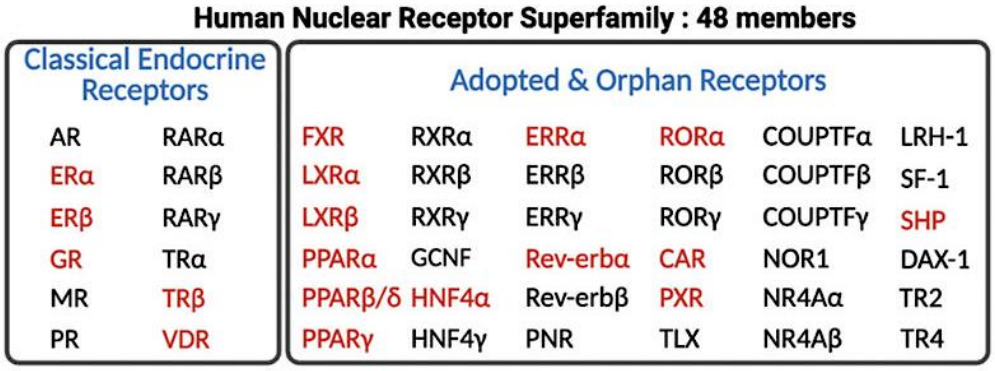

Figure 1. Human nuclear receptor superfamily. There are about 48 different types of nuclear receptors encoded by the human genome that can be classified in different ways depending on the specific characteristics that are being considered. This figure shows one of the existing classifications of NRs based on their ligands that divides them into two groups. The first group is called classical endocrine receptors and includes steroid hormone receptors and RXR heterodimeric receptors. The second group is referred to as adopted and orphan receptors and includes several receptors that bind to dietary lipids, cholesterol derivatives, phospholipids, bile acids and some receptors whose specific ligands remain unidentified. Taken from [6].

Based mainly on structure but also on some other characteristics, NRs can be divided into seven subgroups:

Subgroup 0 includes a subset of unusual nuclear receptors such as DAX and small heterodimer partner (SHP). Their main characteristic is that, unlike the rest of NRs, they only have a ligand-binding domain (LBD) on their structure. Moreover, their LBDs have typical NR co-activator motifs that interact with the LBDs of other NRs for transcriptional regulation.

Subgroup 1 comprises a set of many receptors, which are activated by different lipophilic signaling molecules. These include retinoic acid receptors (RAR) and retinoic acid related receptors (ROR), thyroid hormone receptors (TR), reverse-Erb receptors (REV-ERB), vitamin D receptors (VDR), peroxisome proliferator activated receptors (PPAR), liver X receptors (LXR) and farnesoid X receptors (FXR).

Subgroup 2 consists of orphan receptors that can bind to fatty acids, although it is not clear whether these ligands have a role in regulating the receptors' activity. Examples of these receptors are chicken ovalbumin upstream promoter transcription factors (COUP-TF), the hepatocyte nuclear Factor 4 (HNF4) and retinoid X receptors (RXR). The last group mentioned is particularly significant as they have the ability to form heterodimeric complexes with numerous NRs and are the only receptors in this subgroup with a known activating ligand, which is 9-cis retinoic acid.

Subgroup 3 contains the steroid receptors (SRs), which are activated by direct binding of cholesterol-derived hormones and play a crucial role in regulating various metabolic, reproductive and developmental processes. This family includes the androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR) and estrogen receptor (ER), the main objective of this review.

Subgroup 4 consists of three orphan nuclear receptors that play a crucial role in the development and maintenance of neurons: neuron-derived orphan Receptor-1 (NOR-1), nerve growth Factor 1B (NGF1-B) and nurr-related Factor-1 (NURR1).

Subgroup 5 is comprised of two members: steroidogenic Factor 1 (SF-1) and liver receptor Homolog-1 (LRH-1). They are implicated in the regulation of numerous metabolic processes and are critical for the development and function of several organs, including the liver, pancreas and reproductive system. While these receptors are typically considered orphan receptors, research has shown that they are indeed regulated by phospholipids.

Subgroup 6 contains a single receptor called germ cell nuclear factor (GCNF), which is an orphan receptor that plays an important role in development of germ cells. It contains a DNA-binding domain and a ligand-binding domain, but unlike many other nuclear receptors, it does not have an activation function domain, which means that it does not directly activate gene transcription which, ultimately, is the reason why it is placed in a separate category. Instead, GCNF is known to repress the expression of genes by recruiting co-repressor proteins to the DNA and blocking the binding of other transcription factors [7,8].

Based on their mechanistic properties, that is, on type of dimerization (homo, hetero or mono), DNA binding (direct repeat or inverted repeat) and ligand binding (required or not required), NRs have been classified into four classes as illustrated below (Figure 2).

Steroid Receptors make up class I of NRs. These transcription factors are initially sequestered to the cytoplasm, where they are bound to chaperone proteins. However, when the receptor is activated by its respective ligand, it undergoes a conformational change that allows the release of chaperone proteins and nuclear translocation. Once in the nucleus, SRs typically bind to DNA response elements (REs) as homodimers, which consist of two inverted palindromic repeats. The binding of the receptor to DNA results in the recruitment of co-regulators, which in turn either enhance or suppress their transcriptional activity. Thus, SRs play a critical role in regulating a variety of biological processes [9].

Class II NRs remain in the nucleus regardless of the presence of an activating ligand. After binding to the ligand, the receptor is released from a co-repressor complex and exchanged with co-activators and the transcriptional machinery. The key feature of class II NRs is that they require RXR dimerization for activation. RXR can promote activation after homodimerization, but other members of this class such as PPAR, Pregnane X Receptor and LXR need heterodimerization with RXR to get activated [10]. Moreover, it is noteworthy that RXR is able to bind DNA and perform functions like recruiting co-repressor complexes to repress gene expression, even without ligand binding, through heterodimerization with RAR [11].

Class III NRs are distinct from Class I and II receptors as they lack identified activating ligands and exhibit different sequence-specific binding to DNA. They form homodimers when binding to their REs, which are palindromic or direct repeat sequences. Due to these characteristics, they are referred to as homodimeric orphan receptors, such as VDR. Some of these receptors may play a crucial role in alternative pathway activation as they recognize similar target genes as the other nuclear hormone receptors [10]. Rev-ERbA α (NR1D1), for instance, when it binds as a dimer to DNA, functions as a transcriptional repressor for genes that are typically activated by class II RXR-RAR receptors. This shows that these receptors can have an important regulatory role in gene expression, even though their mechanism of activation remains unclear [12].

Class IV NRs are known as monomeric orphan receptors. They are structurally similar to class II NRs, but they differ in that they bind to DNA as a monomer, recognizing extended half-sites within REs. The DNA binding domain of class IV receptors has a unique fold that enables monomeric binding. Examples of class IV nuclear receptors include LRH-1 and SF-1. As mentioned earlier, unlike class III receptors, class IV receptors have been shown to bind specific ligands, including phospholipids, but their endogenous ligands remain largely unknown [10].

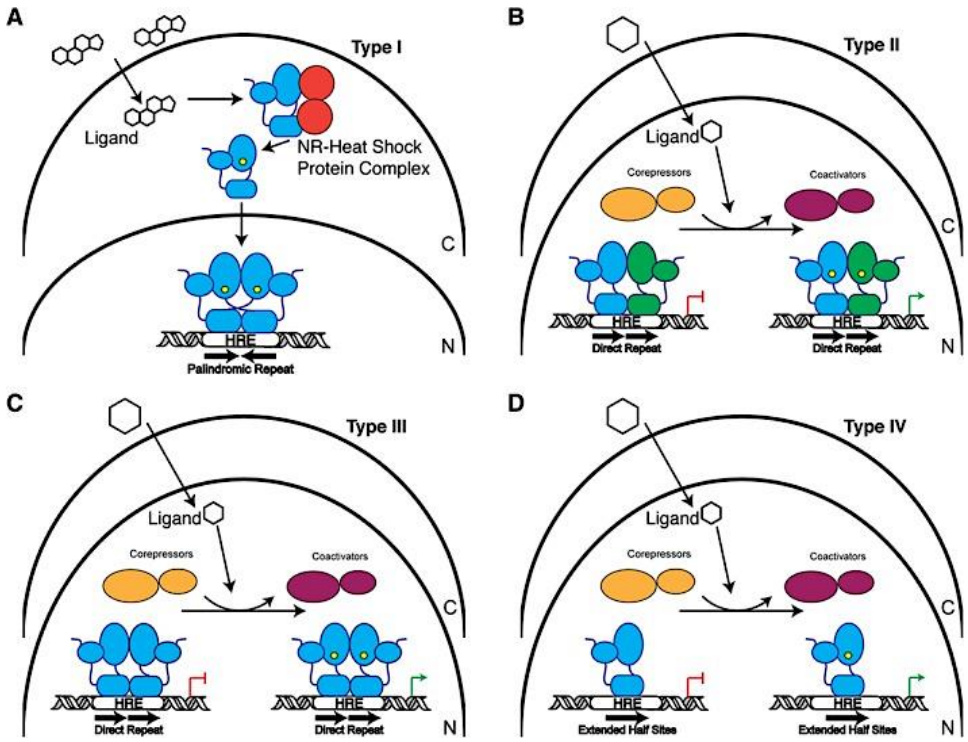

Figure 2. NR classes and signaling mechanisms. (A) The Class I receptors are found in the cytoplasm in conjunction with chaperone proteins. When a ligand binds to the receptor, it separates from the chaperone protein and moves into the nucleus. In the nucleus, the receptor typically forms a homodimer and attaches to palindromic hormone response elements (HREs) to control transcription. (B) Class II receptors are found in the nucleus. When they are not bound to a ligand, they interact with co-repressor proteins, but upon ligand binding, they switch to co-activator proteins. NRs in this group usually form heterodimeric complexes with RXR. (C) Class III receptors, like Class II, are present in the nucleus and exchange bound co-repressors and co-activators. These receptors bind to direct repeat HREs as homodimers. (D) Class IV receptors are similar to Class III receptors, but they attach to HREs that are extended half sites as monomers. Taken from [8].

2.2. Common structure

Even though nuclear receptors differ in shape, size and activating ligands, most of them have a common structure consisting of six modular domains, labeled A to E (Figure 3). These individual subdomains have distinct functions in the biological activity of the receptor.

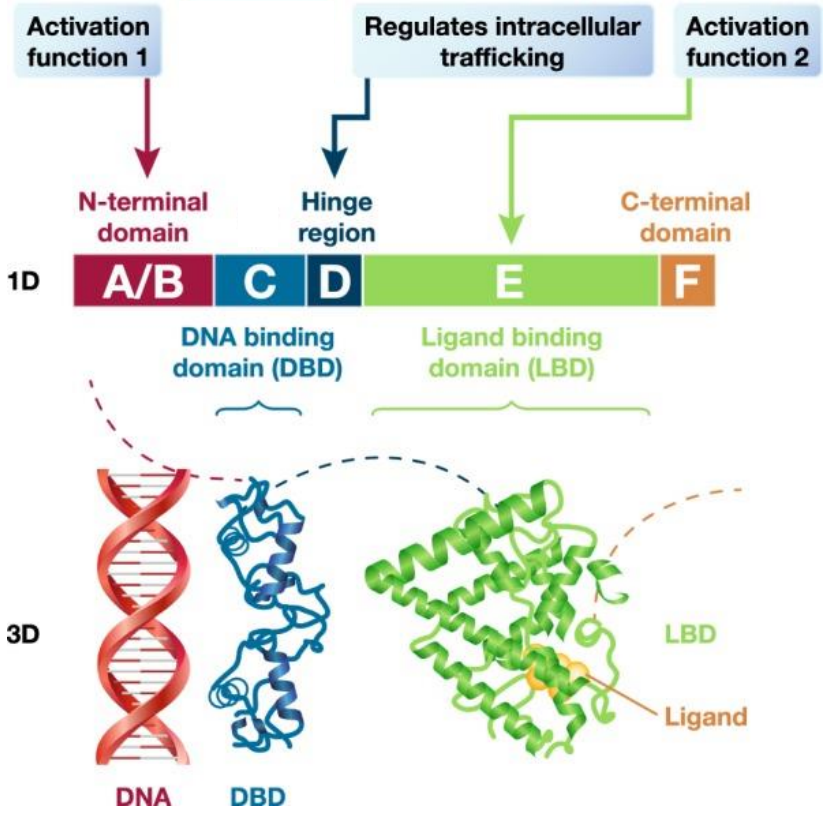

Figure 3. NRs common structure. The N-terminal domain is highly variable and contains multiple regulatory regions such as the activation function 1 (AF-1). The central region or DNA binding domain (DBD), consists of two highly conserved zinc fingers that direct the receptor to specific DNA motifs, known as response elements. The hinge region, located between the DBD and LBD, is flexible and facilitates receptor dimerization and DNA binding, and contains a nuclear localization signal. The C-terminal domain of the receptor contains a large ligand binding domain (LBD) that recognizes specific ligands, activating the receptor for transcriptional regulation. This LBD also includes an important pocket called the activating function 2 (AF-2). Taken from [16].

The first domain is the A/B or N-terminal domain (NTD) which is a highly disordered domain whose specific structure is difficult to describe since there is little sequence similarity between NTDs of different nuclear receptors and the size of this domain varies considerably. The A/B domain contains the activator function-1 region (AF-1), an intrinsically disordered region that can change its shape to fit the different co-regulators it interacts with, allowing for flexibility in the regulation of gene expression [13]. Moreover, the NTD is subjected to multiple post-translational modifications, such as phosphorylation, SUMOylation and acetylation, which affect transcription in different ways, either by enhancing or inhibiting it [14]. Finally, the NTD contains a phenylalanine-x-x-leucine-phenylalanine (FxxLF) motif, which is composed of five amino acids. This motif helps in binding and stabilizing the N-terminal domain (as well as the C-terminal

domain). This binding process promotes the stability of dimers by keeping them in an active conformational state, thus preventing dissociation caused by the binding of a ligand [15].

The next domain is called the DNA binding domain (DBD) or C domain which is the most conserved among all domains of this superfamily of proteins. It is composed of a cysteine-rich sequence that encodes two zinc (Zn) finger motifs with a conserved amino acid sequence. The first zinc finger motif is formed by C1 to C4, while the second is formed by C5 to C8. Following each zinc finger there is an amphipathic helix and a peptide loop. These zinc finger motifs are referred to as the P-box and D-box [17]. The P-box is the term for the initial zinc finger motif that contains the DNA-binding helix, which interacts with the major groove of DNA to make specific base interactions on genomic response elements. On the other hand, the D-box refers to the second zinc finger motif, which is an helix that engages in DBD dimerization and creates non-specific contacts with the DNA backbone (Figure 4) [8].

The zinc finger-binding half site sequences on DNA are conserved across different nuclear receptors, as it has been demonstrated for the estrogen and glucocorticoid receptors. Because of this similarity, a single nucleotide or amino acid change in a zinc finger domain can cause the receptor to recognize hormone response elements present on the DNA sequence target of other receptors, resulting in the activation of genes that are not appropriate for the external signal received [17]. However, the specificity of the response element is determined by how the zinc fingers interact with each other once a dimer is formed. The zinc fingers can interact either head-to-head or head-to-tail, which affects the orientation of the DNA binding and therefore the specific sequence that is recognized [18].

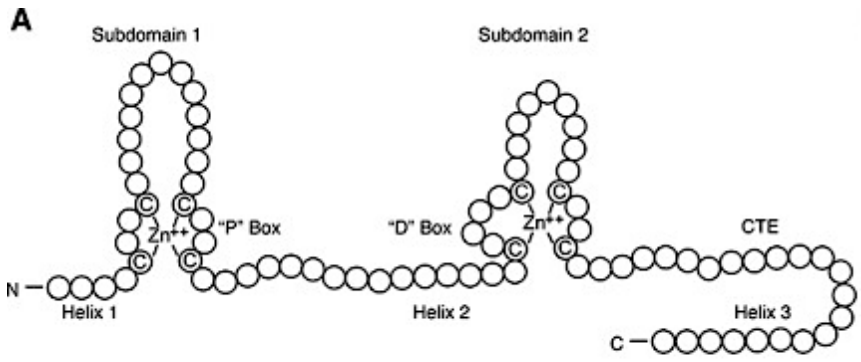

Figure 4. Representation of the two subdomains of the DBD of nuclear receptors. The first subdomain engages with the DNA's major groove, establishing specific interactions with the response elements present in the genome. The second subdomain is involved in dimerization of the DBD and interacts non-specifically with the DNA backbone. Certain NRs, have C-terminal extensions (CTEs) that interact specifically with the minor groove of DNA. Taken from [8].

Furthermore, certain nuclear receptors, such as LRH-1 and GCNF, have an additional part at the end of their DNA binding domain called the C-terminal extension

(CTE). This extension allows the receptor to make even more specific contacts with the DNA, but instead of interacting with the major groove like the rest of the DBD, it interacts with the minor groove of the DNA. This extra level of specificity can help ensure that the receptor only binds to the correct hormone response elements on the DNA and not to other non-target sequences [19].

The D domain, also known as the hinge region, is a region of low sequence conservation, which makes it the least conserved domain among nuclear receptors. It is a flexible linker region that connects DBD and LBD. It contains various sequences that allow for flexibility and mobility of the LBD, which is important for the receptor to undergo conformational changes upon ligand binding. Besides, the D domain can be modified by post-translational modifications such as phosphorylation, which can alter the function and activity of nuclear receptors [14].

The last domain is the E/F or C-terminal domain (CTD), which contains the ligand binding domain (LBD), the second most conserved region among the different nuclear receptors that has the ability to bind ligands but also directly interact with co-regulator proteins. This domain typically consists of 12 α-helices and four β-strands that arrange into three parallel layers to create an alpha-helical sandwich. As a result of this folding, a hydrophobic pocket, referred to as the ligand-binding pocket (LBP), is formed at the bottom of the receptor [20]. Comparison of different NRs LBD structures reveals that the upper part of the receptor is more alike, whereas the lower region, which encompasses the LBP, is more variable. This variability at the LBP among NRs enables them to recognize a wide range of ligands. The LBD contains another activation function surface (AF-2) that requires ligand-binding to become active but remains ordered in all states. Unfortunately, in cases where AF-2 is spliced out, the protein may acquire gain-of-function mutations that enable activation without the need for ligand binding. These mutations can lead to aberrant protein expression. AF-2 is composed of helices 3, 4 and 12. Helix 12 (H12), or the activation function helix (AF-H), contains a hot spot for steroid co-activator binding (SRC) mediated through its leucine-x-x-leucine-leucine (LxxLL) motif, which promotes transcriptional activity [21]. The importance of positional displacement of H12 in NRs, especially in ERs, after binding to either estradiol or ER antagonists will be also discussed below.

2.3. Ligand interaction

Nuclear receptors can directly attach to a range of small, fat-soluble molecules, which vary depending on the specific receptor. Out of the 48 types of nuclear receptors found in humans, 24 are known to have ligands, while the other 24 are categorized as "orphans" or "adopted orphans," which means that a potential ligand has not been identified for them yet. When there is no ligand present, nuclear receptors tend to be unstable, which is why there are relatively few structures available for the LBD of these receptors in their apo form. However, when a ligand binds to the receptor, it significantly

enhances the stability of the LBD. This increased stability, along with other factors, enables the binding of co-regulators and subsequent activation of various signaling pathways [22].

The ligands attach to the receptor through the LBP located at the bottom of the LBD. This pocket is predominantly made up of hydrophobic amino acid residues (~75%), but it also has some crucial polar residues that participate in essential hydrogen bonding interactions with the ligand. These hydrogen bonds are responsible for correctly orienting the ligand, which ensures that the natural ligands are specifically bound to their respective receptors [21]. For example, the endogenous SR ligands contain a rigid fused 4-ring scaffold that places different hydrogen bond donors and acceptors in a particular arrangement to interact with the receptor. The SRs use a conserved glutamine on H3 and arginine on H5 to lock the ligand's ring in place, so that no other ligand can bind to that specific site [23].

2.4. DNA interaction

DBDs of nuclear receptors attach to various types of DNA response elements that have different nucleotide sequences but with some common features. These sequences can be constituted in the form of a palindrome, direct repeat or extended monomeric sites. It is important to note that the similarity among most of these sequences suggests a common ancestral origin (Figure 5).

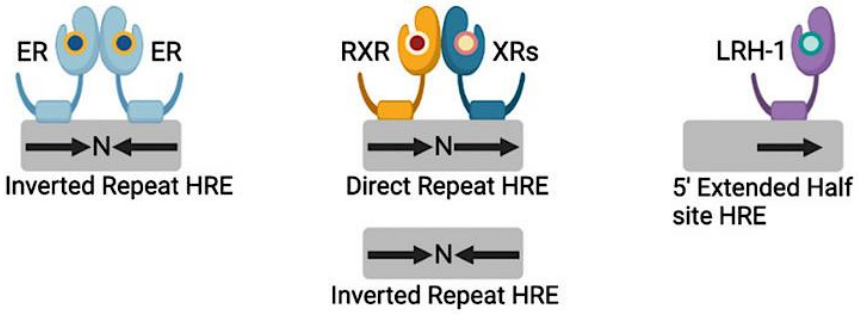

Figure 5. Hormone response elements. The core sequence AGGTCA of the hormone response element (HRE) is denoted by black arrows, and the letter "N" represents any nucleotide found between the half sites of the HRE. The first drawing represents endocrine receptors which typically attach, as homodimers, to palindromic DNA sequences that are inverted repeats separated by three nucleotides. The second picture shows how some nuclear receptors form heterodimers with RXR and attach to direct repeats, which are separated by zero to six nucleotides, or inverted repeats separated by zero or one nucleotide. The last drawing displays how only a few nuclear receptors bind to HRE as monomers with a three-nucleotide extension at the 5' end of the sequence. Taken from [6].

Palindromic repeats are composed of two AGGACA repeats that are separated by a spacer region, which can differ in length, being the most common spacer length 3 base pairs (bp). The length of this spacer has been found to modulate the receptors allosterically, leading to different levels of transcriptional output. SRs are an example of nuclear receptors that attach to these palindromic repeats [24]. The RXR-RAR heterodimer, GCNF and VDR are examples of nuclear receptors that attach to DNA response elements consisting of direct repeats. These repeats are composed of two

AGGTCA sites that are separated by a spacer sequence ranging in length from 0 to 5 bp [25]. Finally, LRH-1 and SF-1 are examples of receptors that attach to DNA response elements with extended monomeric sequences. These sequences comprise of one AGGTCA site along with an A/T rich region that is situated immediately upstream [26].

3. ESTROGEN RECEPTORS

To comprehend the function of estrogen receptors (ERs) completely, it is essential to emphasize some of the key traits of their activating molecules, estrogens. Estrogens are lipid-soluble steroid hormones and ones of the most important female sex hormones. However, they also have important regulatory functions in the development and homeostasis of various organs and systems in both men and women, including the liver, pancreas, bone, brain, cardiovascular system, immune system and even spermatogenesis and male fertility. They are primarily synthesized by the ovaries, testes and adrenal cortex. There are two main types of estrogens: endogenous and exogenous. Endogenous estrogens are produced naturally by glands or cells within an organism's body. Exogenous estrogens, on the other hand, come from external sources such as synthetic estrogens, certain foods and medications [27].

Currently, there are four known endogenous estrogens present in humans, which are estrone (E1), 17β-estradiol (E2), estriol (E3) and estetrol (E4). All of these estrogens share a similar structure and chemical properties, as they each contain 18 carbon atoms. Due to their comparable structures and functions, they are collectively known as C18 steroids.

E1 is a type of estrogen with low potence because of its low ability to bind with classical estrogen receptors. It is the main form of estrogen present in women who are experiencing menopause. When we use the term "estrogen", we typically refer to E2 because of its widespread presence and vital physiological functions across various tissues and organs. E2 is involved in the development of secondary female sex characteristics, menstrual cycle regulation and growth of the endometrial lining from menarche to menopause. Additionally, E2 exhibits a strong binding affinity for all estrogen receptors. E3 is the major estrogen hormone produced by the placenta during pregnancy. Therefore, in non-pregnant women or men, E3 levels are usually very low compared to the substantial increase during pregnancy. Much like E3, E4 is a fetal estrogen hormone that is only measurable during pregnancy. Unlike E2, E3 and E4 have a lower binding affinity for ERs [28].

The most prevalent sources of exogenous estrogens include those produced and eliminated by living organisms into the environment, certain medications and environmental contaminants generated from industrial and agricultural practices. These exogenous estrogens have a relatively lower binding affinity to native estrogen receptors and have a structure that differs from that of natural estrogens [29].

The physiological functions regulated by estrogens are mediated by ERs. In humans, there are three types of ERs involved in several biological processes, the classical alpha (ERα) and beta (ERβ) receptors and the more recently discovered G protein-coupled estrogen receptor 1 (GPER1). Various characteristics these receptors are summarized in table 1.

The first existence of ERs was established in 1958 by Elwood Jensen, who discovered the first receptor for any hormone. Jensen demonstrated that female reproductive tissues were capable of absorbing estrogen from the bloodstream by binding to proteins, thus postulating the existence of estrogen receptors. He later demonstrated that estrogen-bound receptors could migrate to the nucleus and activate gene transcription [30]. This fact led to the discovery of the different types of ERs.

In 1985, the first human estrogen receptor, known today as ERα, was cloned using RNA obtained from the human breast cancer cell line MCF-7 [31]. Subsequently the second estrogen receptor, ERβ, was described ten years later [32]. The identification of GPER1 was distinct from that of classical receptors. In contrast to ERα and ERβ, which were isolated using traditional biochemical methods, GPER1 was identified through molecular cloning techniques in 2012 [33]. However, the history of its discovery stemmed from 1997, when a novel orphan G protein-coupled receptor (GPCR), initially named GPR30, was identified in the evaluation of two human breast cancer cell lines [34]. The structural similarities between the receptors for angiotensin II and other peptides led to the belief that GPR30's ligand was either a hormone or a chemotactic peptide. Even so, despite screening numerous chemotactic peptides and factors, no molecules that bound to GPR30 were discovered, leading to the continued classification of the receptor as orphan [35]. However, in the year 2005, specific research groups were able to show that 17β-estradiol could directly bind to GPR30 in breast cancer cell lines and GPR30-transfected cells [36]. GPR30 was officially renamed GPER1 in 2007.

Genetically speaking, ERα and ERβ are encoded by genes ESR1 and ESR2, located on chromosome 6 (locus 6q25.1) and chromosome 14 (locus 14q23.2), respectively. Full-length ERα is slightly heavier with a molecular size of 66 kDa containing 595 amino acids, whereas ERβ is smaller and has a molecular size of 59 kDa that contains a total of 530 amino acids. Lastly, the gene encoding the membrane receptor GPER1 is situated on chromosome 7 (locus 7p22.3). This receptor has a molecular mass of approximately 41 kDa and contains 375 amino acids, which makes it the smallest of the estrogen receptors [37].

It is well known that ERs are present in female reproductive tissues, but they are also expressed in other body systems such as the skeletal, cardiovascular, muscle, central nervous, male reproductive and immune systems where they control different physiological functions.

ERα is primarily expressed in the reproductive tissues, both in the female (uterus and thecal cells of the ovaries) and in the male (testes, epididymis and prostate's stroma). It is also present in the mammary gland, fatty tissues, bone and liver. Similarly, ERβ is highly expressed in the immune system, bladder, prostate's epithelium, adipose tissue, colon and granulosa cells of the ovaries. Furthermore, both receptors are also expressed in the central nervous system and cardiovascular system [38]. GPER1 is more widely distributed and present in the skeletal muscle, neurons, vascular endothelium and immune cells [39]. Additionally, some studies have reported that it is expressed in different types of tumors, such as breast, ovarian and lung cancer [40].

Table 1. Various characteristics of different Estrogen receptors. Taken from [2]

	ERα	*ERβ*	*GPER1*
Category	Nuclear steroid hormone receptor superfamily	Nuclear steroid hormone receptor superfamily	G protein coupled receptor superfamily
Location	Nucleus	Nucleus	Membrane- associated
Protein G involved	x	x	Gs and Gi/o
Size	595 aa	530 aa	375 aa
Numbers of isoforms	3	5	1
Major isoforms	ERα66, ERα46, ERα36	ERβ1, ERβ2, ERβ3 ERβ4, ERβ5	Not described
Chromosome region	6q25.1	14q23.2	7p22.3
Structure	DNA binding domain, ligand binding domain, N-terminal domain	DNA binding domain, ligand binding domain, N-terminal domain	7 transmembrane α-helical regions, 4 extracellular and 4 cytosolic segments
Distribution in tissues	Hypothalamus, hippocampus, testes, ovary, endometrium, uterus, prostate, kidney, liver, breast, epididymis, muscle, adipose tissue	Testes, ovary, prostate, vascular endothelium, bladder, colon, adrenal gland, pancreas, muscle, adipose tissue	Central and peripheral nervous system, uterus, ovary, mammary glands, testes, pancreas, kidney, liver, adrenal and pituitary glands, cardiovascular system, adipose tissue
Selective agonists	17- estradiol estriol xenoestrogens phytoestrogens	17-estradiol estrone xenoestrogens phytoestrogens	17-estradiol estrone, G1, xenoestrogens phytoestrogens
Selective antagonist	SERMs	PHTPP	G15, G36
Type of expression	Constitutive	Constitutive	Constitutive
Subcellular localization	Nucleus (ERα66) Cell membrane (ERα36) Cytoplasm	Nucleus Cytoplasm	Cell membrane

3.1. Structural properties of ERs

Previously in this review, we described the common structure of the nuclear receptor superfamily. In this section, we will revisit this structure briefly, with a particular emphasis on the architecture of estrogen receptors. The structure of the classical ERα and ERβ is quite similar to the other receptors in this superfamily, as they are composed of various functional domains and have several structural regions in common. However, in terms of structure, GPER1 does not share similarities with ERα or ERβ, as we will disuse further on.

As many other members of the superfamily of nuclear receptors, both ERα and ERβ have six structural domains ranging from A to F from N to C terminus (Figure 6).

The N-terminal of the A/B domains represents the amino-terminal domain which is involved in gene transcription transactivation and shares a 16% amino acid identity between ERα and ERβ [38]. As mentioned before, the amino acid sequences within the nuclear receptors' NTD possess an intrinsically disordered (ID) conformation, which is also the case of ERs. This means that the amino acid sequence lacks a defined three-dimensional structure. In ERs the ID nature of its NTD was confirmed by a circular dichroism method [41]. This domain is considered necessary in the modulation of transcriptional activity due to the presence of AF-1 which is the least conserved region with only 30% identity between ERα and ERβ, being expressed at high levels in ERα. The A/B domains also contain some amino acids that can undergo post-transcriptional modifications, such as splicing to stimulate AF-1 activity [42].

The C region corresponds to the DNA binding domain which is the most conserved domain out of all six domains, exhibiting a 97% similarity in the amino acid sequence between ERα and ERβ [38]. This domain is composed of two zinc finger modules that form the core structure necessary for ER dimerization and binding to specific sequences in chromatin, collectively known as estrogen response elements (EREs). EREs are palindromic sequences with a structure of GGTCA-3n-TGACC, where the receptor binds in the form of homodimers [43]. Similar to ER, other steroid receptors such as GR, MR, PR and AR also recognize a palindromic sequence, but this is slightly different from ERE and is referred to as HRE, with a structure of GAACA-3n-TGTTC. The specific amino acid composition of the first zinc finger determines the distinction between the recognition of ERE and HRE sequences [43, 44].

The D domain, or hinge region, acts as a connector between the C and E domains and has a homology of only 36% between ERα and ERβ [32]. It is able to bind to chaperone proteins as well as nuclear co-repressor and co-activator proteins such as L7/SPA. This region also contains the nuclear localization signal, that stabilizes the DNA-binding function of the C domain. The signal is revealed when estrogen binds to the receptor, allowing the receptor-ligand complexes to be translocated into the nucleus [41].

It was suggested that a calmodulin (CaM) binding site existed at the interface of the hinge region and the ligand binding domain [45]. Later, it was confirmed that such a site was present, but only in the ERα subtype, not in ERβ. The existence of this site is significant as research has demonstrated that the interaction between CaM and the E2-ERα complex enhances the complex's ability to bind to an ERE, implying that calmodulin may function as a modulator of ERα transcriptional activity [46].

As a result of the interaction between ERα and this calcium-binding protein, it was observed that calmodulin antagonists function as selective inhibitors of ERα-mediated transcription, in a dose-dependent fashion, without any inhibitory effect on ERβ-mediated transcription of either ERE- or AP1-driven promoters [47]. One of these calmodulin antagonists is melatonin and its potential role in breast cancer treatment will be discussed further on.

The C-terminal of the E/F domain, also known as the ligand binding domain (LBD), is considered the second most conserved domain, with a 59% amino acid similarity between ERα and ERβ. However, the differences in structure between the two subtypes' ligand binding pockets are relatively insignificant. This is noteworthy as these subtle variations in the ligand binding pockets have facilitated the development of ligands that are selective for each specific subtype [38].

As previously mentioned, the LBD contains a dimerization surface and the AF-2 which, together with AF-1, assist in regulating ER transcriptional activity and subsequently connecting co-activators or co-repressors with ER [48]. Whereas AF-1 does not require binding to hormones or steroids to be activated, AF-2 undergoes a marked conformational change in the presence of different ligands. For example, the binding of agonists to the AF-2 in LBD ERs results in the formation of a binding surface or cleft created by helices 3, 4, 5 and 12. This surface allows the binding of co-activators containing the LxxLL motif, which activates transcription. However, when partial agonists or antagonists, such as tamoxifen, bind to the LBD, helix 12 (H12) is repositioned, causing the LxxLL motif of the co-activator to be misaligned. This relocation of H12 likely prevents the co-activator from interacting with the ER [49]. Moreover, when estrogen receptor degraders, such as fulvestrant, bind to the LBD, H12 is relocated to different positions, which is believed to initiate the proteolysis of the ER [50,51]. Thus, the movement and positioning of H12 play a crucial role in ligand-dependent regulation of transcription by the ER.

Finally, it has been studied that variations in the F domain among the ERs could potentially play a role in the receptors' ability to selectively regulate the transcription of specific target genes [52].

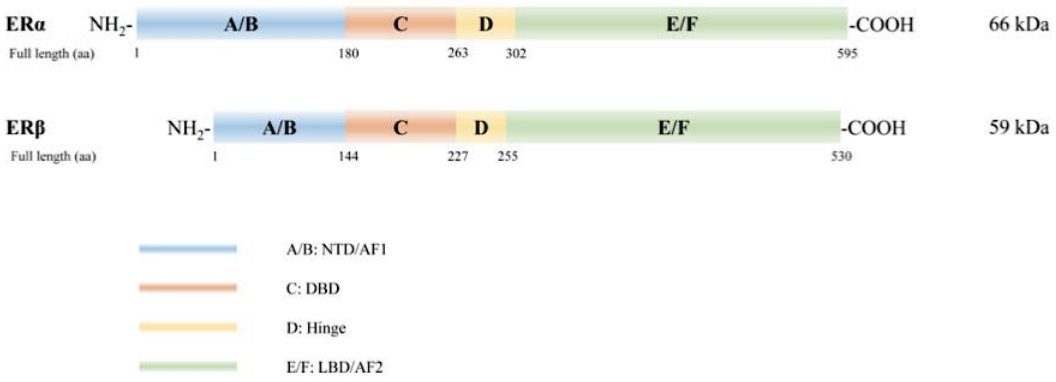

Figure 6. Structure of classical estrogen receptors (ERs). Both receptors exhibit 6 distinct structural and functional domains, which include the N- terminal (NTD, A/B domains, AF-1), DNA binding domain (DBD, C domain), the hinge region (D domain) and the C- terminal region comprising the ligand binding domain (LBD, E/F domain, AF-2). Taken from [37].

On the other hand, as GPER1 is a typical G protein coupled receptor, its structure consists of 7 transmembrane α-helical regions, 4 extracellular segments and 4 cytosolic portions.

The extracellular N-terminal domain of GPER1 is responsible for binding to ligands and initiating receptor activation. It contains two cysteine residues that form a disulfide bond, which stabilizes the structure of the receptor. The N-terminal domain is also highly glycosylated, which can affect the binding of ligands and receptor signaling. The seven transmembrane helices are stabilized by hydrophobic interactions with the cell membrane and contain several highly conserved amino acid residues that are critical for receptor function. Moreover, the intracellular domain of the third transmembrane loop has been shown to bind to the heterotrimeric G protein complex (Gαβγ) [53].

The intracellular C-terminal domain contains a PDZ domain that interacts with other plasma membrane proteins, such as protein kinase A and membrane-associated guanylate kinases, in order to activate downstream signaling pathways. The C-terminal domain of GPER1 is relatively short compared to other GPCRs and has a unique sequence that distinguishes it from other estrogen receptors [54].

The general belief is that this receptor is situated on the plasma membrane, much like most G protein-coupled receptors, because it initiates signaling through the stimulation of Gαs or Gαi [55]. However, other studies have demonstrated that over fifty percent of the total cellular GPER1 is spread out within intracellular areas, such as the endoplasmic reticulum, nuclear membrane and Golgi apparatus [56].

Compared to classical estrogen receptors, GPER1 has a poor binding affinity for 17β-estradiol and estrone, which act as agonists [57]. On the other hand, it has been reported that estriol at low concentrations can act as an antagonist [58]. Additionally, there are other compounds that have been found to act as selective ligands for GPER1: G-1, which acts as a selective agonist, and two selective antagonists, G15 and G36 [59-61].

3.2. Isoforms of ERs

In addition to the full-length ERα (66kDa) and ERβ (59kDa) isoforms, several shorter isoforms have been identified.

3.2.1. ERα isoforms

Several isoforms of ERα, also known as ERα-66, have been identified arising from alternative gene splicing, such as ERα-36 and ERα-46 (Figure 7).

ERα-36 was first identified in 2005 [62]. It has a molecular weight of approximately 35.7 kDa and it is present both inside the cytoplasm and at the plasma membrane, so it has an essential role in genomic and non-genomic estrogen signaling, as well as in GPER1-mediated responses. It contains dimerization domains, partial ligand-binding domains and DNA binding domains, but lacks the transcriptional activation domains (AF-1 and AF-2) [63]. Its gene composition is almost the same as the ERα-66 gene, but in the last two exons (exons 7 and 8) it has a unique 27 amino acid sequence that substitute the last 138 amino acids of the ERα-66 gene [62]. The activation of this isoform has been associated with clinical phenotypes and various types of cancers, specifically tamoxifen-resistant breast cancer [64].

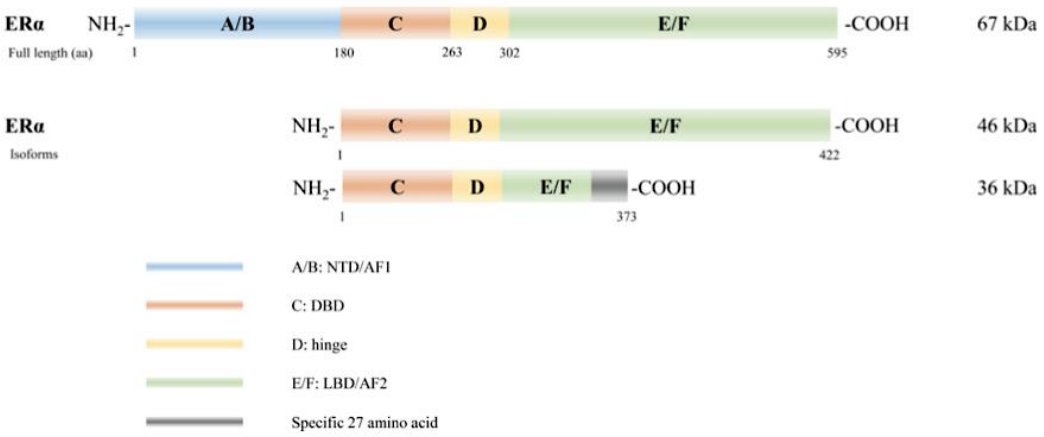

Figure 7. Structure of estrogen receptor alpha (ERα) isoforms. 6 distinct structural and functional domains: N-terminal (NTD, A/B domains, AF-1), DNA binding domain (DBD, C domain), the hinge region (D domain) and the C-terminal region comprising the ligand binding domain (LBD, E/F domain, AF-2). Taken from [37].

ERα-46 was first reported in 2000. It has a molecular weight of 46 kDa and it is found abundantly in the cell cytosol. It is called the AF-1 deficient estrogen receptor as it lacks the first 173 amino acids present at the N-terminus of ERα-66, where the AF1 domain is included. However, the rest of the amino acid sequence is the same as in ERα-66. This means that the transactivation function AF-2 and the DNA and ligand- binding activities are not abolished, thereby potentially allowing ERα-46 to act as a ligand-inducible transcription factor in some cells. Moreover, when both ERα-46 and ERα-66 are co-expressed (as seems to be the most frequent situation *in vivo*) ERα-46 acts as a

powerful competitor that can efficiently suppress the AF-1 activity of ERα-66 in a cell-specific context [65]. Finally, the ERα-46 expression has been documented in human cancer cell lines such as tamoxifen resistant breast cancer cell lines [66].

3.2.2. ERβ isoforms

There are different ERβ isoforms that exist as a result of either alternative splicing of the last coding exons, deletion of one or more coding exons, or alternative usage of untranslated exons in the 5' region [67]. Currently five full-length ERβ splice variants have been described in human, named as ERβ1–5 (Figure 8).

ERβ1, mostly referred to as ERβ, is the full- length receptor consisting of 530 amino acids coded by exons 1–8. It shares the same sequence with full-length ERβ2-5 transcripts just from exon 1 to exon 7, as ERβ2-5 isoforms have unique sequences in place of exon 8. These differences in the C-terminal part of ERβ2-5 result in a truncation of the LBD and ablation of the AF-2 domain [68]. Therefore, ERβ1 is the only isoform with ligand binding abilities capable to recruit co-activators to regulate ligand-dependent transactivation zone AF-2 functionality [69].

ERβ2 isoform, also known as ERβcx, consists of 495 amino acid residues and has a molecular weight of 55.5 kDa. Due to alternative splicing, it possesses a distinctive C-terminus wherein the amino acids corresponding to exon 8 are substituted with 26 distinct amino acids. Consequently, as mentioned above, it misses the AF-2 region and has undetectable affinity for estradiol and other tested ligands [67].

Functional studies of ERβ3 isoform have not been executed as its expression is only limited to the testis.

ERβ4 and ERβ5 isoforms were initially identified as truncated transcripts that only contained a part of exon 7 and 8. However, a later study confirmed that they exist as full-length transcripts. Additionally, it has been reported that ERβ4 and ERβ5's ability to activate transcription can be increased when they form a heterodimer with ERβ1 [70].

There were discrepancies between studies as to whether ERβ isoforms could or could not form functional homo or heterodimers. The initial study suggested that ERβ1, 2 and 3 isoforms could both homo and heterodimerize with each other [71]. However, a later study concluded that only ERβ1 was able to form a functional homodimer, the formation of which was enhanced by estrogenic ligands. Even though the study confirmed that ERβ2, 4 and 5 could not form functional homodimers, it showed that they were able to form heterodimers with both ERβ1 and ERα, the latter resulting in inhibition of ERα function [69].

Interestingly, an altered ratio of these alternative spliced variants has been found in several cancers, such as in breast and ovary tumors. However, results regarding the

correlation of ERβ isoform expression and parameters, such as tumor progression, success of treatment and relapse, have been contradictory as yet [68].

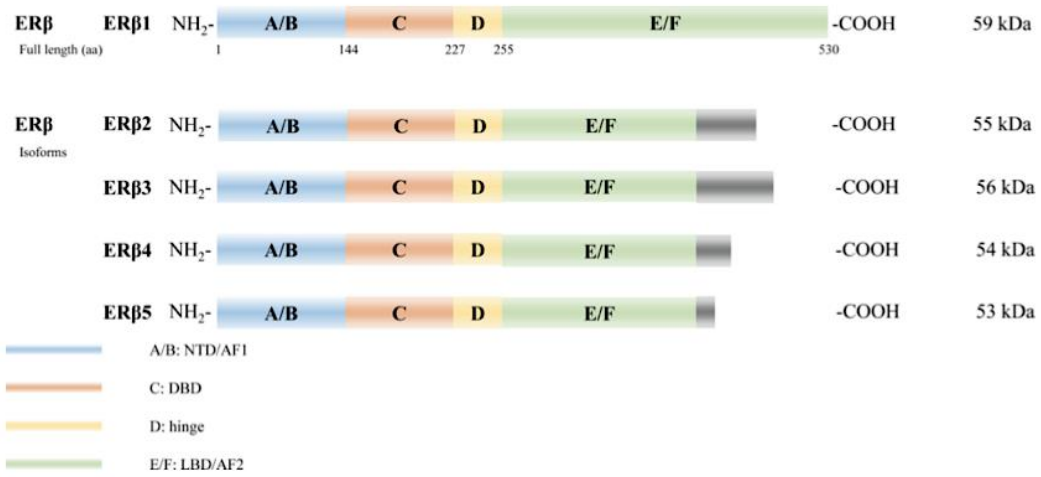

Figure 8. Structure of estrogen receptor beta (ERβ) isoforms. 6 distinct structural and functional domains: N- terminal (NTD, A/B domains, AF-1), DNA binding domain (DBD, C domain), the hinge region (D domain) and the C- terminal region comprising the ligand binding domain (LBD, E/F domain, AF-2). Taken from [37].

3.2.3. ER-X

ER-X is a plasma membrane associated estrogen receptor with a molecular mass of around 63–65 kDa. Although it shares some homology with the C-terminal region of ERα, it is not an alternative splicing variant of ERα, in fact it has a distinct pharmacological and developmental profile than ERα and ERβ. Moreover, Hsp90 proteins are required for the inactivation of ERα and ERβ receptors, whereas Hsp90 proteins working in conjunction with ER-X are considered necessary for its function [72].

3.3. ERs signaling pathways and its effects

As a steroid hormone, estrogen has the ability to cross the plasma membrane and interact with intracellular ERs, leading to direct effects by binding to DNA sequences. In addition, estrogen can also activate intracellular signaling cascades via interaction with ERs without binding directly to DNA sequences. These pathways through which estrogens trigger their effects by binding to ERs are known as ligand-dependent pathways. However, an interesting phenomenon observed in many cells is that estrogen receptors can actually be activated in the absence of estrogens or other receptor agonists, giving rise to ligand-independent pathways [73].

3.3.1. Ligand-dependent pathways

The ligand-dependent mechanism is one of the main mechanisms by which ERs are modulated. In the absence of the ligand, ERs are scattered throughout the cell and kept in an inactive state by co-repressor proteins such as NCoR1, SMRT and at least 19

more proteins that have co-repressor activity. These co-repressors recruit histone deacetylases (HDACs) which act antagonistically to inhibit chromatin and block the transcriptional machinery from approaching the DNA [74]. To activate ER-dependent transcription, co-activator proteins need to replace ER-corepressor complexes that are maintaining the ER in an inactive state. This replacement occurs when ERs undergo a conformational change as a result of ligand binding, causing ERs to enter their active state [75]. Co-activator proteins serve as platforms for the assembly of large protein complexes that contain histone modifying enzymes such as histone acetyltransferases (HATs) or histone methyltransferases (HMTs) [76]. These enzymes' modifications lead to the opening of chromatin, allowing other regulatory proteins to access it. The final outcome is the recruitment of the general transcriptional machinery and RNA Polymerase II to initiate transcription [77].

Up to date, there are three ligand dependent pathways that are used to mediate ERs known as the genomic pathway, the tethered pathway and the non-genomic pathway (Figure 9).

3.3.1.1. Genomic pathway

In the genomic pathway, also known as the classical mechanism of estrogen signaling, the nuclear estrogen receptors ERα and ERβ act as ligand-activated transcription factors.

In this way, as mentioned before, in the absence of estradiol hormone, ERs are enclosed inside the cytoplasm of the target cell by a multiprotein inhibitory complex. As estrogens or other receptor agonists diffuse through the cell membrane, they encounter ERs, leading to binding of the ligand to the receptor. This interaction results in a conformational change in the ER and dissociation of the ER-corepressor complex. The release of these inhibitory proteins activates the receptor, which triggers the formation of homodimers. These homodimers are then translocated into the nucleus to initiate transcription of ER target genes by binding to EREs [78]. Moreover, binding of ER to chromatin and subsequent activation of gene expression is additionally controlled by a distinct group of transcription factors called pioneer factors, such as forkhead box A1 (FOXA1) and GATA binding protein 3 (GATA3), even though there is an ongoing discussion about whether pioneer factors assist in the binding of ER or vice versa [79].

The progress made in computational biology has made it easier to identify EREs in various gene promoters and has enabled the prediction of genes regulated by estrogen in numerous species' genomes, in fact a study using genome-wide screening techniques recently identified more than 70,000 EREs in the genomes of humans and mice [80]. Although the EREs share a significant degree of sequence similarity, it's crucial to note that the inherent sequence composition of the EREs can affect the receptor's ability to bind DNA. For instance, while ERα exhibits high affinity for the canonical ERE

sequence present in the vitellogenin A2 gene, its affinity for the EREs located in the oxytocin gene is comparatively lower. This partly explains how variations in ERE sequences, resulting from inter-individual gene variability or mutations, can influence the activation of gene expression [81]. Furthermore, specific ERE sequences can cause changes in the receptor's structure affecting the ability of the complex to recruit co-activators and transcription factors that can contribute to the biological activity of ER [82].

3.3.1.2. Tethered or indirect pathway

Around 35% of genes that are targeted by estrogen do not have ERE-like sequences in their promoter regions, yet they can still be regulated by estrogen through a mechanism that does not involve direct binding of estrogen receptors to DNA. Instead, estrogen receptor complexes interact with other transcription factors and response elements through protein-protein interactions, leading to indirect signaling that can activate or suppress the expression of target genes [83].

A crucial mediator of this type of signaling pathway is the stimulating protein-1 (Sp-1), which is the predominant mediator of ER-DNA indirect binding. The presence of estrogen receptors can boost the binding of this transcription factor to promoter regions located at GC-rich sites. Some genes that can be induced via the Sp-1 mechanism are the progesterone receptor B, low-density lipoprotein (LDL) receptor, signal transducer and activator of transcription 5 (STAT5), GATA binding protein 1 (GATA1), endothelial nitric oxide synthase (eNOS) and the retinoic acid receptor-1α genes [84]. Interestingly, to activate transcription, 17β-estradiol binds to ERα and associates with Sp-1 in GC-rich regions. Nevertheless, the binding of E2 to ERβ does not result in the formation of a transcriptionally active complex at a promoter containing Sp-1 elements [85].

The nuclear estrogen receptors also promote the expression of genes containing the activator protein-1 (AP-1) sites though protein-protein interactions. The AP-1 complex, composed of Jun protein dimers and of Jun/Fos heterodimers, is a transcription factor that regulates crucial cellular processes such as cell differentiation, proliferation and apoptosis. Examples of genes induced by ERα through the AP-1 mechanism include insulin-like growth factor-1 (IGF1), collagenase, IGF1-receptor, ovalbumin and cyclin D1 [86]. Similar to Sp-1-mediated transcription, studies have suggested that ERα and ERβ signaling can be opposite, depending on the ligand and response elements present at the AP-1 sites. For example, 17β-estradiol induces AP-1-dependent transcription through ERα, whereas ERβ inhibits this mechanism [87].

Furthermore, studies have demonstrated that ERα has the ability to interact with the c-rel subunit of the nuclear factor-κB (NF-κB) complex, resulting in the prevention of NF-κB from binding to cytokine genes promoters and activating expression from the

interleukin-6 (IL-6) promoter. As a result, estrogen inhibits the expression of the cytokine IL-6 [88].

Finally, a few studies have indicated that ERα can interact with other transcriptional modulators, including nuclear transcription factor-Y and activating transcription factor (ATF)-2/c-jun or ATF-2/cAMP response element binding protein (CREB) [86].

In conclusion, the differences in how the two estrogen receptors regulate transcription in various cells, as well as their interactions with specific transcription factors, may explain the variations observed in the tissue-specific responses to estrogen.

3.3.1.3. Non-genomic pathway

For a considerable time, it was recognized that some of the changes brought about by estrogen in response to it were too swift to be linked with the transcription of target genes and the subsequent synthesis of proteins. This led to the development of the hypothesis that estrogen could be acting by non-genomic mechanisms.

In this pathway, the ligand activates either certain variants of ERα and ERβ or a different receptor which can be located in the membrane or in the cytoplasm, such as GPER1. The process that follows this activation is not very clear yet, but it is known that signal-transduction mechanisms are triggered. This result in the production of intracellular second messengers (such as calcium), regulation of cyclic AMP (cAMP) and activation of protein-kinase signaling cascades that ultimately leads to a rapid physiological response without involving gene regulation [89]. The protein-kinase cascades that can be activated in this pathway can be classified into four major ones: the phospholipase C (PLC)/protein kinase C (PKC) pathway [90], the Ras/Raf/mitogen-activated protein kinase (MAPK) cascade [91,92], the phosphatidyl inositol 3 kinase (PI3K)/Akt kinase cascade [93] and the cAMP/protein kinase A (PKA) signaling pathway [94,95].

The protein kinases mentioned above can modify the function of transcription factors through phosphorylation, which can impact their ability to bind to specific DNA sequences and regulate gene expression. Examples of such transcription factors include CREB, Elk-1, the NF-κB complex, CCAAT-enhancer-binding protein beta (C/EBPβ) and the STAT family [96-101]. Therefore, by triggering these non-genomic mechanisms, estrogen receptors can indirectly regulate gene transcription at alternative DNA response elements, in addition to the previously mentioned genomic effects where they directly bind to EREs.

Another important aspect to consider is that both ERα and ERβ can undergo phosphorylation by protein kinases like MAPKs. This implies that the non-genomic

actions of estrogens could potentially involve the regulation of receptor expression through self-modification [102].

3.3.2. Signaling crosstalk

In addition to the distinct genomic and non-genomic pathways outlined earlier, it has been proposed that there are additional pathways in which both genomic and non-genomic factors converge, leading to the regulation of gene transcription [83,103]. There are at least two "cross-talk" mechanisms described that involve interactions between elements of both the genomic and non-genomic pathways.

In the first mechanism, estrogen-bound nuclear estrogen receptor complexes form dimers that are translocated to the nucleus. There, they bind to phosphorylated transcription factors that have resulted from signaling mediated by GPER1. The complexes then bind to either ERE sequences via the nuclear estrogen receptors or to other DNA binding sites, including STATs, Sp1, ATF-2/c-Jun, AP-1 and/or NF-κB.

In the second mechanism, GPER1, ERα and ERβ located at the plasma membrane interact, resulting in the activation of protein kinase cascades that lead to the phosphorylation of various transcription factors, including AP-1, STATs, Elk-1, CREB and NF-κB. The estrogen receptors themselves can also be phosphorylated through this process. Once phosphorylated, these transcription factors and estrogen receptors can interact with DNA sequences to regulate transcription [103].

3.3.3. Ligand-independent signaling

Interestingly, in many cells, estrogen receptors can be activated without the presence of estrogen or other receptor agonists [83]. This activation occurs through a process called ligand-independent activation, which is mainly triggered by the activation of certain kinases by growth factor signaling (Figure 9). Once activated, these kinases can modify the estrogen receptors or associated co-regulators by adding a phosphate group, which results in their activation. Serine and tyrosine are the two most commonly phosphorylated amino acids.

This independent mechanism requires certain regulatory molecules that are necessary for the process of phosphorylation. These regulatory molecules include MAPK phosphorylation cascade components, PKC, PKA, as well as other substances such as cell cycle regulators (cyclin A and D1), cell adhesion molecules (heregulin), inflammatory cytokines (interleukin 2) and peptide growth factors like insulin, transforming growth factor β (TGFβ), epidermal growth factor (EGF) and IGF1 [104].

This pathway is thought to significantly contribute to hormone-independent growth in some tumors [105].

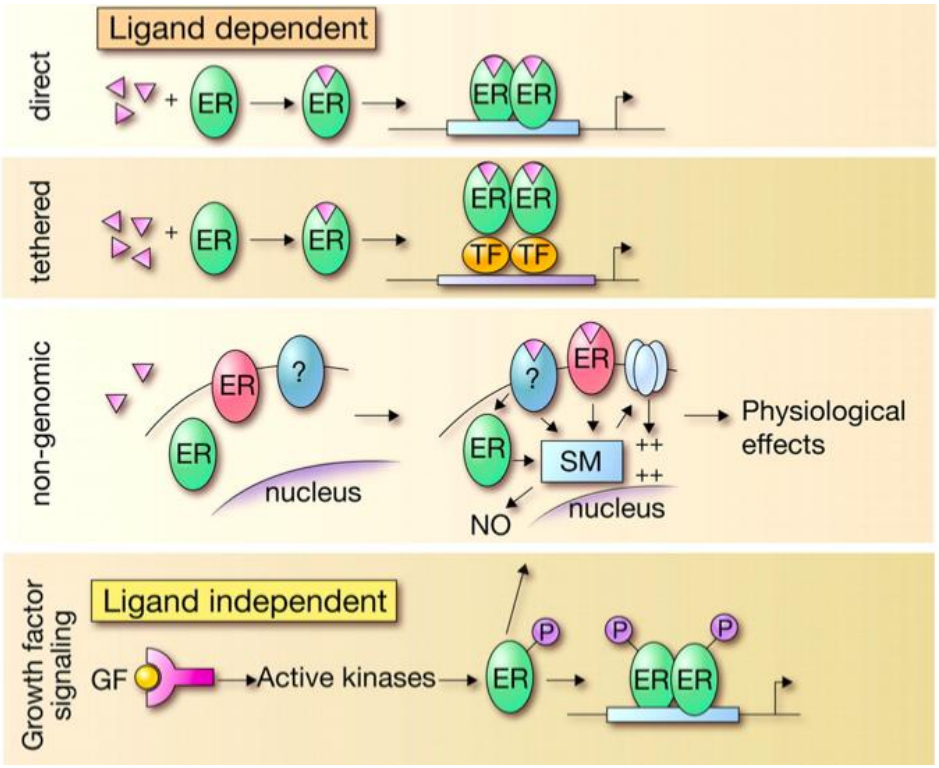

Figure 9. Schematic illustration of ER signaling mechanisms. In the classical pathway, estrogen directly binds to its receptor and the complex then binds to estrogen response elements (EREs) in the promoter regions of target genes. The tethered pathway involves indirect DNA binding, where estrogen receptor complexes interact with other transcription factors (TF) after ligand activation. In the non-genomic pathway, estrogen can bind to a receptor located on the cell membrane or in the cytoplasm, leading to signaling cascades via second messengers (SM) that result in rapid physiological responses without involving gene regulation. Finally, the ligand-independent pathway involves activation of nuclear ERs through growth factors (GF) activating protein-kinase cascades that lead to phosphorylation (P) and activation of ERs. Taken from [105].

3.3.4. GPER1 signaling pathways

GPER1 plays a role in the non-genomic activation pathways of estrogens and estrogen-like substances. As a typical G protein coupled receptor, when the ligand binds to GPER1, it activates heterotrimeric G proteins by intracellular mechanisms. This complex is made up of three subunits: α, β, and γ. In most cases, the heterotrimeric Gαβγ complex dissociates into the Gα-subunit (Gαs) and the Gβγ-complex. Both these two subunits are involved in the activation of GPER1-mediated signaling which can vary depending on the cell type and involves various molecular targets and signaling pathways [106].

After binding of 17β-estradiol or G1 to GPER1 various signaling pathways are activated in the cytosol (Figure 10).

The pathway involving the EGF-receptor begins with the activation of the kinase Src by the Gβγ-complex, which activates matrix-metalloproteases (MMPs) that release EGF from heparin-bound EGF. Binding of EGF to EGF-receptor results in auto-phosphorylation of the EGF-receptor, leading to activation of extracellular regulated kinase 1 and 2 (Erk1/2) and PI3-kinase. The activated Erk triggers transcription of c-fos,

Egr-1, ERRα and aromatase in the nucleus, followed by the induction of the connective tissue growth factor (CTGF) [107]. The phosphorylated EGF-receptor also activates PI3-kinase, which in turn phosphorylates kinase Akt. Akt phosphorylates transcription factor forkhead box 3a (FOXO3a) in the nucleus, which is then transported to the cytosol, where it is degraded [108].

The process of calcium (Ca2+) signaling begins with Gαs being released, which then activates PLC. PLC cleaves Phosphatidylinositol-4,5-bisphosphate into two molecules: diacylglycerol and inositol trisphosphate (IP3). IP3 then releases calcium ions from calcium-stores present mainly in the endoplasmic reticulum. In turn, Ca2+ activates several enzymes in the cytosol, including Erk1/2 [109]. Additionally, the binding of 17β-estradiol to GPER1 opens calcium L-channels in the plasma membrane through an unknown mechanism [110].

When Gαs is released, it also activates adenylyl-cyclase (AC) in the cytosol. Activated AC generates cAMP which in turn activates PKA that phosphorylates CREB. Phosphorylated CREB acts as a transcription factor, binding to the promoters of genes that contain a cAMP response element, such as cyclinD1, which supports the progression of the cell cycle [111].

The IκB pathway involves the activation of IκB kinase (IKKβ) by the Gβγ-complex, which results in the phosphorylation of IκB. This, in turn, leads to the degradation of IκB by the ubiquitin proteasome, ultimately preventing the proliferative and antiapoptotic effects of NFκB [112].

Estrogen also activates the Hippo-signaling pathway via GPER1. This pathway is involved in regulating organ size, cell proliferation and tumor development. When Gαs is dissociated, it stimulates the kinase mammalian sterile 20-like 1 and 2 (MST1/2), which in turn activates the large tumor suppressor (LATS) kinases. LATS then phosphorylates and inactivates the transcription factors yes-associated protein (YAP) and transcriptional co-activator with PDZ binding motif (TAZ). Phosphorylated YAP and TAZ are bound to the protein 14-3-3 and retained in the cytoplasm, where they are eventually degraded by the proteasome [113].

Finally, GPER1-dependent signaling also induce the expression of HOX transcript antisense intergenic RNA (HOTAIR). HOTAIR is one of the many long non-coding RNAs and is upregulated in different types of cancer, including breast cancer. It is involved in regulating apoptosis, metastasis and DNA repair. In this pathway, Gα-dependent signaling inhibits the expression of miR148a, which leads to an increase expression of HOTAIR, ultimately supporting metastasis. However, the exact intermediate steps leading to the transcription of this microRNA are not yet fully understood [114].

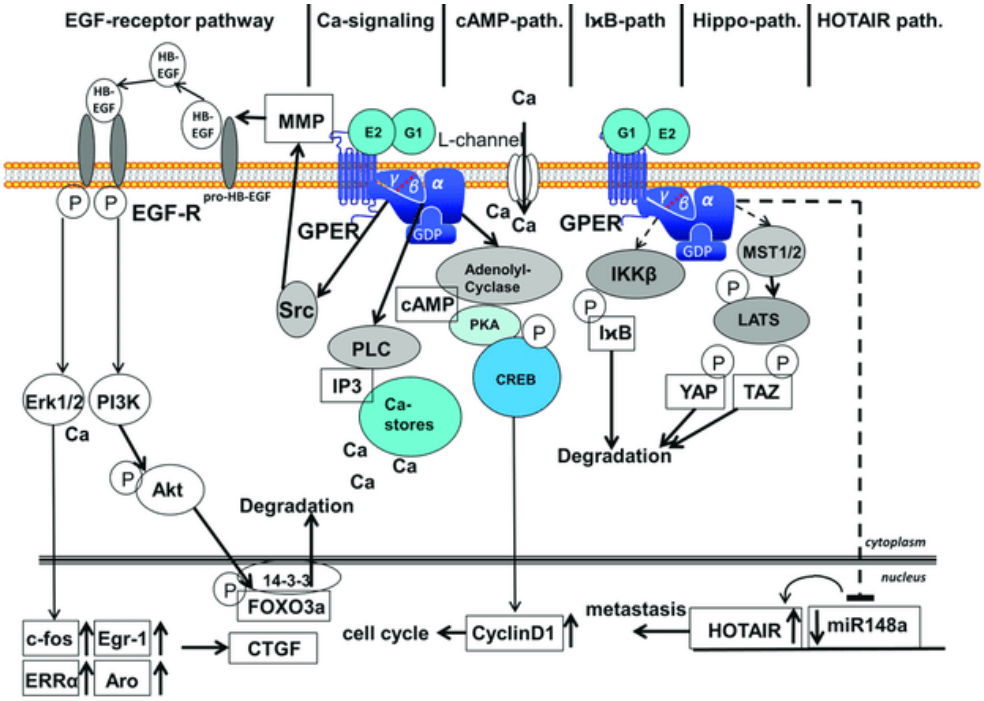

Figure 10. Signaling pathways of GPER1. Six distinct signaling pathways can be identified: the EGF receptor pathway, calcium signaling pathway, cAMP pathway, IκB pathway, Hippo pathway, and HOTAIR pathway. Taken from [115].

4. ESTROGEN RECEPTORS IN BREAST CANCER

4.1. The role of ERs in breast cancer

As previously mentioned, both the expression and activity of ERs are important for normal development and function in various tissues, including the breast, where they are also involved in tumorigenesis, leading to the development of breast cancer, which is the most prevalent cancer affecting women worldwide [1]. This relationship between estrogen and breast cancer development dates back to 1895 when Dr. George Thomas Beatson proposed a connection between estrogen and the development of breast cancer based on the idea that ovarian hormones could be responsible for the proliferation of breast cells. To test his theory, he performed bilateral oophorectomy on three women at Glasgow Cancer Hospital and observed significant improvements in their conditions, which supported his hypothesis [116]. This initial investigation was the beginning of numerous subsequent studies that validated the involvement of estrogen in the development of breast cancer.

Breast cancer subtypes have traditionally been classified based on the expression status of ER, progesterone receptor and human epidermal growth factor receptor 2 (HER2). In this way, we describe three types of breast cancer: luminal A or B (ER+PR+HER2-), HER2-positive (ER-PR-HER2+) and triple-negative (ER-PR-HER2-). The role of ERs is so important that the majority of breast cancers are luminal or ER+ (approximately 60–80%). This highlights the significant involvement of ERs in various

processes during tumor development, including cell survival, proliferation and tumor growth [37].

4.1.1. ERα and breast cancer

Approximately 50-80% of breast cancer tissues overexpress ERα, whereas only about 10% of healthy tissues exhibit this level of expression [117]. ERα's role in mammary gland development was first demonstrated through experiments on ERα knockout (ERαKO) mice. These studies revealed that ERα plays a significant role in both promoting cell growth and differentiation in the mammary gland [118]. In addition, research has demonstrated that ERα serves as a transcription factor for genes linked to the proliferation and growth of tumor cells, such as anti-apoptotic BCL-2 protein, cyclin D1, vascular endothelial growth factor (VEGF) and IGF1-receptor. When serine 118 and serine 167 of ERα are phosphorylated in breast cancer cells it triggers specific gene expression patterns that affect tumor growth, morphology and the responsiveness of patients to hormonal therapy [119]. Furthermore, multiple studies have reported a direct link between estrogen and the growth of MCF-7, in fact estrogens can stimulate the growth of MCF-7 only if ERα is present [120].

ERα splice variants are also implicated in the development of this kind of tumor. Studies have shown that ERα-46 acts against breast cancer as it has been demonstrated that it can inhibit the growth of MCF-7 breast cancer cells and prevent the expression of genes associated with cancer development, including cyclin D1 and pS2. It is believed that this effect is accomplished through the inhibition of ERα-66, the full-length form of ERα, as ERα-46 acts as a competitive inhibitor of ERα-66 when they are co-expressed [121]. Additional evidence supporting the anti-proliferative role of ERα-46 in breast cancer is provided by the observed rise in cell proliferation upon reduction of ERα-46 levels, as observed in tamoxifen-resistant breast cancer cells. Nonetheless, the reintroduction of ERα46 has demonstrated the ability to counteract this effect and impede cell proliferation [66].

On the other hand, studies have demonstrated that ERα-36 is linked to carcinogenesis, aggressiveness, metastasis and resistance to hormonal therapy in breast cancer. Despite binding to the same target DNA sequence as the wild-type (WT) ERα, ERα-36 is believed to function as a strong competitor of ERα. However, ERα-36 acts quite differently from ERα-66 as it mainly localizes in the plasma membrane and cytoplasm, where it stimulates rapid membrane-initiated non-genomic pathways (MAPK and PI3K) [122]. This isoform has been notably linked to tamoxifen-resistant breast cancer [64,123].

4.1.2. ERβ and breast cancer

ERβ expression's significance for breast cancer prognosis remains uncertain since it appears to have diverse functions depending on factors such as ERα's presence or

absence [124]. Around 80% of normal breast epithelial cells express ERβ, in fact high levels of ERβ expression in the normal mammary gland are linked with a lower risk of developing breast cancer. This is further supported by the fact that the expression of ERβ is found to decrease and even disappear during breast cancer progression, which is related to hypermethylation of its promoter [125].

As indicated before, the function of ERβ in breast cancer is still a matter of debate. Initial studies suggested that ERβ might have a negative impact on breast cancer prognosis by promoting cell proliferation [126,127]. Nevertheless, recent research has contradicted these findings. According to certain reports, ERβ suppresses cell proliferation in breast cancer by blocking the MAPK and PI3K signaling pathways [128]. Some laboratory studies have also demonstrated that reintroducing ERβ in breast cancer cells can lead to reduced cell growth, increased apoptosis and increased sensitivity to chemotherapy [129]. In addition, clinical evidence suggests that low expression of ERβ is linked to poor prognosis and resistance to endocrine therapy [130,131].

The presence of five distinct ERβ isoforms adds even more complexity to understanding the physiological function of ERβ and its potential contribution to the development of breast cancer. Studies have shown that introducing ERβ1 into ERα+ breast cancer cells has an anti-proliferative effect. In order to prove this, researchers explored T47D cells (a type of breast cancer cell line) that had been engineered to express ERβ1. The results showed that E2 or the ERα-agonist propylpyrazoletriol (PPT) stimulated proliferation, while the ERβ-selective agonist DPN inhibited proliferation in this model [132]. Similarly, it has been demonstrated that ERβ2 has anti-proliferative properties in MCF-7 cells expressing ERα through the formation of ERβ2/ERα heterodimers, which triggers the proteasomal degradation of ERα, ultimately resulting in the inhibition of ERα-regulated genes [133]. Finally, ERβ5 also shown to have a beneficial role in breast cancer patients. Clinical studies have demonstrated that higher expression of ERβ5 is associated with longer relapse-free survival (RFS) and a significant correlation between its nuclear expression and overall survival (OS) [134,135]. Furthermore, recent research has revealed that ERβ5 can enhance the sensitivity of breast cancer cell lines to apoptosis induced by chemotherapeutic agents [136].

In addition to the abovementioned evidence linking ERβ to breast cancer, it has been associated with epithelial to mesenchymal transition (EMT), which is a necessary process for normal development but also an essential early step in tumor metastasis as it is reported to promote invasion in the progression of breast carcinomas. EMT is characterized by the loss of cellular adhesion, which is mediated by the down-regulation of adhesion molecules such as E-cadherin. Recent evidence has demonstrated that ERβ1 inhibits EMT and invasion in triple-negative breast cancer (TNBC) cells both *in vitro* and *in vivo*. The inhibition of EMT is associated with ERβ1-mediated up-regulation of miR-200a/b/429 and subsequent repression of ZEB1 and SIP1, resulting in increased E-cadherin expression [137].

To summarize, the majority of the available evidence indicates that ERβ functions as a primary negative modulator of estrogen signaling, as it inhibits ERα-mediated transcription by forming heterodimers with ERα, which may explain the protective effect of ERβ. Nonetheless, additional experiments are required to validate and reinforce our current understanding of the connection between ERβ and breast cancer.

4.1.3. GPER1 and breast cancer

The role of GPER1 in breast cancer development is still uncertain, and conflicting views exist regarding its function, in fact, the proliferative effect of GPER1 has not only been studied in breast tumor tissues but also in non-tumor breast tissue explants. In culture, G1, a selective agonist of GPER1, was found to promote tissue proliferation. In contrast, G36, a GPER1 antagonist, inhibited G1-induced proliferation in non-cancerous human breast tissues [138].

Regarding the involvement of GPER1 in tumor development, there are divergent opinions.

On one hand, GPER1 has been suggested to mediate estrogen's effects in breast cancer by promoting proliferation, tumor growth, cell migration within the tumor microenvironment, higher risk of metastasis, recurrence and reduced survival rates in patients with breast cancer [139-141]. Moreover, it has been shown that GPER1 plays a direct role in EMT. A study using breast cancer cell lines treated with G15, which is another GPER1 antagonist, revealed that G15 blocks EMT in breast cancer cells by inhibiting GPER1, and a combined effect was observed when the cells were treated with doxorubicin (one chemotherapeutic agent) and G15, which resulted in increased sensitivity of breast cancer cell lines to this drug [142]. Furthermore, the involvement of GPER1 in breast cancer treatment resistance is noteworthy as some medications, like tamoxifen and fulvestrant, act as GPER1 agonists, promoting cell growth and proliferation [143]. Tamoxifen, for example, can act as a growth factor by activating EGFR through GPER1, which is the mechanism behind the development of endocrine therapy resistance in the MCF-7 cell line [144]. Based on these and other numerous studies, GPER1 has been suggested as a potential biomarker for predicting aggressive phenotypes and poor outcomes in breast cancer patients.

On the other hand, certain studies attribute a tumor-suppressing role to GPER1. Through GPER1 activation, estrogens were found to significantly inhibit breast cancer growth, resulting in cell-cycle arrest in the G1 phase under hypoxic conditions [145]. What's more, a separate investigation revealed that breast cancer cells treated with G1 exhibited reduced expression of cyclin B, resulting in cell cycle arrest at the G2/M phase and mitochondria-related apoptosis in culture. This study concluded that GPER1 activation can restrain cell proliferation by inducing apoptosis via the caspase pathway, downregulating cyclin B expression and producing reactive oxygen species (ROS) both *in*

vitro and *in vivo* [146]. Ultimately, it has been observed in cell lines and murine models of TNBC that the activation of GPER1 by G1 inhibits the expression of IL-6 and VEGF-A, ultimately suppressing the angiogenesis and progression of TNBC [147].

4.2. ER classic ligands and their therapeutic implications

As aforementioned, the most commonly diagnosed type of breast cancer is ER+ or luminal breast cancer, which accounts for around 75% of all cases. In these tumors, ER signaling, represents the main driver of tumor growth and survival [148]. Therefore, the primary approach for treating this cancer subtype is to target the ER using endocrine therapy (ET), either on its own or in combination with other targeted agents [149].

There are several inhibitors of the ER pathway that have been approved over the years. They are classified according to their mechanism of action: some compounds, such as selective estrogen receptor modulators (SERMs) and selective estrogen receptor degraders (SERDs), act directly on the ER and block its activity by functioning as antagonists, while other compounds, like sulfatase (STS) inhibitors and aromatase inhibitors (AIs), work by blocking the enzymes that control the conversion of androgenic precursors, ultimately reducing the production of endogenous estrogen. As a result, they are grouped together under the term selective estrogen enzyme modulators (SEEMs) [150]. It's important to note that the use of each antiestrogen is guided by specific clinical guidelines that consider several factors, such as the patient's reproductive age, the tumor stage and their previous treatment history.

In this section, we will discuss the historical background, tissue-specific properties, clinical research and use of conventional treatments for breast cancer, comprising classic SERMs, SERDs, STS inhibitors and AIs (Figure 11).

4.2.1. Selective estrogen receptor modulators (SERMs)

SERMs are a category of therapeutic agents developed to compete with estrogen and regulate ER activity by altering the cofactors that the receptor interacts with. As explained before, when estrogen binds to ER, H12 caps and seals the ligand within the ligand-binding pocket. This, in turn, opens up the AF-2 cleft, which allows for the binding of co-regulators through specific LxxLL motifs. However, unlike estrogen, SERMs have a side chain that causes a conformational change between helices 11 and 12. This change prevents H12 from capping over the LBD and instead causes it to block the co-activator recognition groove by mimicking the interactions of co-activator peptides with AF-2 cleft through its own LxxML motif. This interaction hinders the binding of co-activators and promotes the binding of co-repressors, which ultimately prevents the activation of the receptor [23,151].

The term "selective" in SERMs refers to their ability to selectively modulate the estrogen receptor and downstream signaling in different tissues. As a result, they can

act as estrogen agonists in some tissues like bone, liver and the cardiovascular system, while blocking estrogen activity in other sites like the breast. These agonistic or antagonistic effects are produced by distinct changes in the conformation of the ER upon ligand binding [152].

SERMs can be classified based on their chemical structure as triphenylethylenes (tamoxifen and "tamoxifen-like"), benzothiophenes (raloxifene, arzoxifene), tetrahydronaphthalenes (nalfoxidine, lasofoxifene), indoles (pipindoxifene, bazedoxifene) and benzopyrans (EM-800, acolbifene, levormeloxifene).

4.2.1.1. Triphenylethylenes

Tamoxifen and other SERMs that are similar in structure, such as droloxifene, idoxifene and toremifene, belong to a class of compounds called triphenylethylenes. These compounds are flat and have a rigid central region, exhibiting minor differences among them. For instance, droloxifene has a hydroxyl group attached to the phenyl ring of tamoxifen, idoxifene has an additional iodine group at the 4 position and toremifene has a chlorine atom replacing a hydrogen on the ethyl side chain, which is the only structural difference between it and tamoxifen [153-155].

During the 1930s, it was discovered that certain compounds that initiate tumors can also inhibit the growth of both normal and cancerous tissues [156]. This led to the realization that estrogens, which were known to promote the growth of breast cancer cells, could be used to treat advanced breast cancer. In fact, the first clinical trial with diethylstilbestrol (DES), a synthetic non-steroidal estrogen, showed positive results [157,158]. This led to a surge of research that eventually resulted in the discovery of tamoxifen, originally known as ICI 46474, which was first reported by ICI in 1967 and has been used as the first-line therapy for breast cancer since the 1970s until today [159].

At first, tamoxifen was thought to work simply as a competitive antagonist by blocking estrogen from binding to its receptor. However, subsequent research has shown that tamoxifen function as a partial or full agonist on specific DNA regulatory elements, such as certain estrogen response elements, Sp1 sites and activator protein 1 sites, with AF-1 being particularly important for tamoxifen's agonist activity [160]. The initial preclinical study that outlined the ability of tamoxifen to inhibit the growth of ER+ breast cancer cells showed a significant reduction in thymidine incorporation in cancer cells [161]. Subsequently, it was revealed that the active metabolites of tamoxifen, 4-hydroxytamoxifen and endoxifen, were responsible for tamoxifen effectiveness *in vivo* [162]. While 4-hydroxytamoxifen acts as a potent antagonist in breast cancer cells, it demonstrates partial agonist activity in tissues such as the endometrium, in fact, a potential adverse effect associated with the use of tamoxifen in clinical settings is an elevated risk of developing endometrial cancer, especially in women over the age of 50 [163]. Nevertheless, the successful outcomes of various clinical trials that have shown the

effectiveness of tamoxifen in treating and preventing the progression of breast cancer outweigh its adverse effects [164-172]. As a result, tamoxifen has become a standard treatment option for both pre- and post-menopausal women with breast cancer and continues to be widely used.

Due to the adverse effects of tamoxifen on the endometrium, researchers investigated various tamoxifen derivatives that could potentially reduce these side effects while maintaining its efficacy. However, these agents did not show any advantages over tamoxifen and demonstrated partial agonist activity, with some studies suggesting cross-resistance between them and tamoxifen. The lack of significant efficacy observed with droloxifene and idoxifene, either when compared to tamoxifen or when used in tamoxifen-resistant disease, resulted in the discontinuation of their development [173,174]. Nonetheless, a retrospective study on premenopausal women with ER+ breast cancer showed that toremifene had comparable side effects, effectiveness and safety as tamoxifen. As a result, toremifene was approved for the treatment of advanced metastatic breast cancer (mBC) in 1997 [175-179].

4.2.1.2. Benzothiophenes

Initially studied for its potential to decrease the risk of breast cancer, raloxifene (also called keoxifene) differs from tamoxifen in that its benzothiophene core binds in a planar orientation within the LBD of ER [180]. Furthermore, the interaction between raloxifene and Asp351 in helix 3 of the receptor is more potent than that of tamoxifen. This particular interaction is essential for the antiestrogenic properties of raloxifene [181]. Raloxifene functions as an antagonist and suppresses the growth of breast cancer cells. Moreover, in contrast to tamoxifen, it acts as a pure antagonist in the uterus and has been shown to completely halt tamoxifen-induced growth of endometrial carcinoma in preclinical models. In addition, it does not raise the risk of endometrial cancer in clinical settings [182]. Nevertheless, it exhibits cross-resistance to breast tumors treated with tamoxifen and only provides modest effectiveness in advanced breast carcinomas [183,184]. Despite this, it is effective in reducing the risk of ER+ breast cancer in postmenopausal women with a high risk. Therefore, it was approved in 2007 specifically for this indication [185-188].

According to reports, arzoxifene's structure is responsible for its more favorable safety and therapeutic profile when compared to raloxifene. It substitutes the carbonyl hinge with an ether oxygen and shields one of the phenolic hydroxyl groups with a methyl ether to enhance bioavailability [189]. In addition, arzoxifene has been found to have a neutral effect on the endometrium, unlike tamoxifen, with patients not showing any thickening of the endometrium after three months of arzoxifene treatment [190]. A multicenter phase III trial compared the efficacy of arzoxifene to tamoxifen as a first-line therapy for mBC. However, the trial was terminated prematurely after the initial results

indicated that arzoxifene was less effective than tamoxifen in terms of the time of progression endpoint [191].

4.2.1.3. Tetrahydronaphthalenes

Nafoxidine was proved to be active in the treatment of postmenopausal patients with advanced breast cancer. Some studies suggested that in this disease it was one of the most effective hormone-related drugs though its practical usefulness was limited by its toxicity on skin and hair [192]. However, it was never released to the market due to the availability of other drugs with more favorable safety and efficacy profiles.

Lasofoxifene binds to ERα with a potency similar to estradiol, causing a conformational change that prevents recruitment of co-activators, much like raloxifene and tamoxifen [193]. In clinical trials, lasofoxifene reduced the incidence of ER+ breast cancer [194]. Although prolonged lasofoxifene use over five years has been linked to endometrial hypertrophy, current research suggests that it can be advantageous in treating resistant breast cancer, as we will elaborate on later [195].

4.2.1.4. Indoles

ERA-923, most known as Pipindoxifene, has an indole core structure with a piperidine ring on the side chain that is similar to raloxifene. It demonstrated the ability to hinder the estrogen-mediated growth of breast cancer cells and was effective in tamoxifen-resistant tumors. Moreover, when compared to raloxifene, it did not exhibit any uterotrophic properties in immature rats [196]. Despite being well-tolerated in clinical settings and having started a phase II study for the treatment of tamoxifen-resistant mBC, it was eventually discontinued in 2005 [197,198].

An indole-derived non-steroidal compound called bazedoxifene has been studied for its estrogen agonist/antagonist activity in breast cancer, both in preclinical and clinical settings, as will be discussed in detail below.

4.2.1.5. Benzopyrans

It has been demonstrated that EM-800 is a remarkably effective inhibitor of the growth of breast and endometrial cancer cells *in vitro* and *in vivo* [199,200]. One of its derivatives, acolbifene, was considered the most potent antiestrogen in terms of inhibition of both ERα and ERβ and was studied as a breast cancer prevention agent in premenopausal women [201]. Lastly, levormeloxifene, which is commonly used as an oral contraceptive, is currently being utilized for the treatment of advanced breast cancer therapy as well as the treatment of postmenopausal osteoporosis. Additionally, it has been shown to suppress uterus growth in response to estrogen at a dose lower than 1 mg/kg [202].

4.2.1.6. Other SERMs

Finally, there are other non-typical SERMs that can also be used as treatment options for breast cancer.

On one hand, coumarin based SERMs have been found to produce effects similar to other SERMs, but they possess structural features that are different from ideal SERMs like tamoxifen. This means that they may cause a different conformational change in the ERs, leading to the recruitment of different cofactors [203]. Furthermore, unlike other SERMs, they do not activate genes through EREs. Instead, they bind with high affinity to ERα. This effectively antagonizes estrogen action in ERα-expressing breast cancer cells by inhibiting the expression of genes like IL-6 and GM-CSF. This proves that coumarin-based SERMs function as potent antiestrogens both in *in vitro* and *in vivo* models of breast cancer [204].

On the other hand, many *in vitro* studies have shown that phytoestrogens (PEs), which are dietary compounds derived from plants that have similar chemical structures and biological activities as human estrogen, have a high affinity for ERs. They trigger the transcription of genes that are dependent on ERα and ERβ, but with a greater binding affinity for ERβ. PEs may exert potent anticarcinogenic effects by influencing cell proliferation, apoptosis, and angiogenesis through various mechanisms [205,206].

4.2.2. Selective estrogen receptor degraders (SERDs)

The quest for an endocrine drug that could treat breast cancers, which were resistant to available treatments, and could act as a universal ER antagonist to minimize unwanted effects resulted in the development of SERDs. Unlike SERMs, SERDs are antiestrogens that are created to destabilize H12 of the estrogen receptor. They work by binding to ER and causing its degradation, thus preventing dimerization and halting the ER signaling pathway [207].

The initial SERDs to be discovered were ICI 164,384 and ICI 182,780. Among the two, ICI 164,384 was the first ER antagonist to be described as pure antiestrogen with lacked agonist effects in all ER+ tissues [208]. The pure antiestrogenicity property of ICI 164,384 was found to be related with the length of the carbon chain located at the 7α position, which ranged between 15 and 19 carbon atoms in order to interact with the coactivator binding groove, in fact, shorter side chains with 13-14 atoms led to agonistic or SERM activities instead of pure antiestrogenic effects [209,210].

The continued development of ICI 164,384 led to the synthesis of fulvestrant, an ER antagonist that binds to ER in its monomeric form and hinders the formation of ER dimers, thereby preventing estrogen receptor activation [211]. In addition, evidence has demonstrated that the binding of fulvestrant to ERα induces a structural change that

results in an augmented hydrophobicity on its surface. This, in turn, attracts the E3 ubiquitin ligase and proteasome, ultimately leading to the degradation of ER [212].

Fulvestrant exhibited high effectiveness in inhibiting breast and endometrial cancer in both *in vitro* and *in vivo* models [213]. Moreover, preclinical studies have shown that fulvestrant is effective in stopping the growth of tamoxifen-resistant tumors, attributed to its distinct pharmacological antagonistic mechanism [214].

Initially, the use of a 250 mg intramuscular injection of fulvestrant was authorized for treating mBC patients who were ER+ and had already failed previous endocrine therapies. Subsequently, clinical trials have demonstrated that administering a 500 mg dose results in improved bioavailability and efficacy [215]. Therefore, the 500 mg dose is the current approved treatment for postmenopausal patients with advanced ER+ breast cancer, either alone or in combination with AIs.

However, due to fulvestrant low oral bioavailability, the complaints among patients because of the pain at the injection sites and the suboptimal ER degradation caused by injection pharmacokinetics, significant efforts have been made in recent years to develop newer generations of SERDs with improved bioavailability, pharmacokinetics, and antiestrogenic activity. These efforts have led to the discovery of an increasing number of oral SERDs, which will be discussed in more detail later.

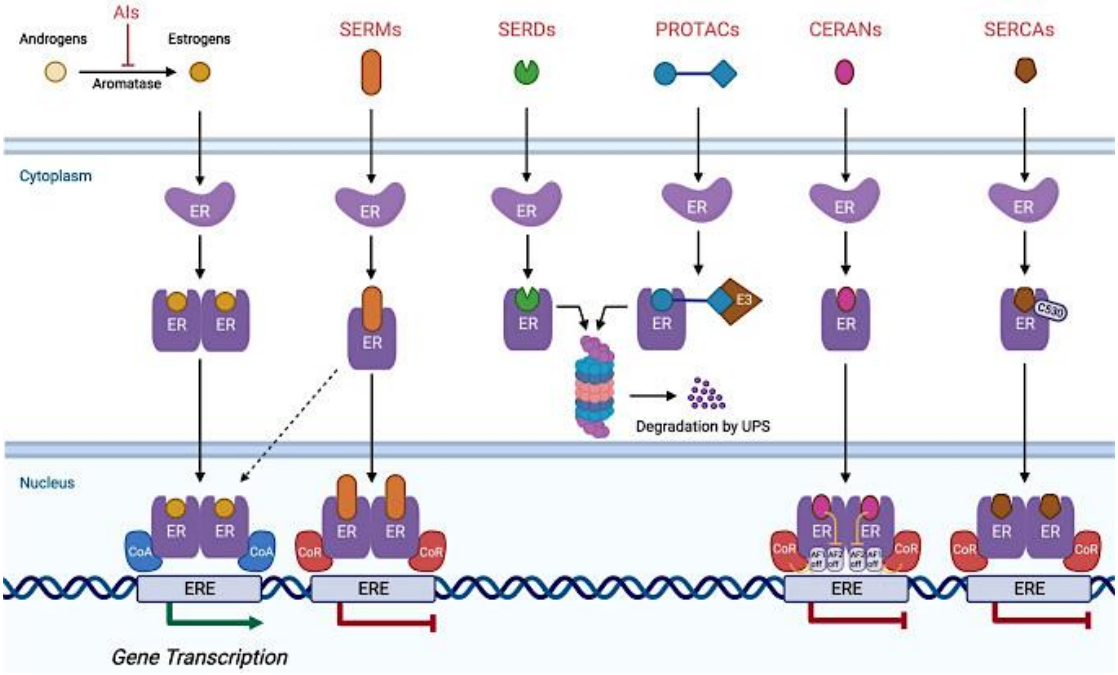

Figure 11. Mechanisms of action employed by various classes of anti-estrogen therapies. Aromatase inhibitors (AIs): They block estrogen production by inhibiting aromatase, which converts androgens to estrogens. Selective estrogen receptor modulators (SERMs): They competitively inhibit estrogen binding to ER, affecting gene transcription differently in different tissues. Selective estrogen receptor downregulators (SERDs): They are pure ER antagonists that prevent ER nuclear translocation and promote proteasomal (UPS) degradation. Proteolysis targeting chimeras (PROTACs): They use ligands to bind ER and E3 ubiquitin ligase, resulting in ubiquitination and degradation of ER. Complete estrogen receptor antagonists (CERANs): They block both activation domains of ER and recruit corepressors to inhibit gene transcription. Selective estrogen receptor covalent antagonists (SERCAs): They covalently bind to ER in cysteine 530 (C530), inactivating it and inhibiting gene transcription. Taken from [150].

4.2.3. Sulfatase and Aromatase inhibitors

The production of estrogens is mainly attributed to two pathways, namely the aromatase and sulfatase pathways. In the aromatase pathway, the aromatase (AR) enzyme complex converts the androgen precursor, androstenedione, to estrone. On the other hand, in the sulfatase (STS) pathway, the sulfotransferase enzymes convert estrone (formed via the aromatase pathway) to estrone sulfate (E1S), which is believed to act as a reservoir for the formation of active estrogens [216]. Sulfatase then converts E1S to estrone, which is subsequently reduced to the biologically active estrogen, estradiol, by 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) [217]. The aforementioned pathways are considered important sources of estrogen synthesis and upregulation in breast cancer, with sulfatase being overexpressed in nearly 90% of breast tumors and aromatase detected in 60-70% of cases [218]. Consequently, inhibiting these pathways emerged as a promising therapeutic strategy for treating breast cancer, leading to the development of inhibitors for both the sulfatase and aromatase enzymes.

STS inhibitors can be classified into three generations based on the chronology of their development. The first generation is further categorized as reversible and irreversible inhibitors. The second generation comprises D ring compounds like N-substituted piperazinedione derivatives. The third generation is known as dual aromatase-sulfatase inhibitors (DASIs), exemplified by compounds such as YM511 and STX681. They can be used alone or in combination with other compounds such as SR16157, a steroidal sulfatase inhibitor that yields a selective ERα modulator upon reaction with sulfatase [219].

AIs work by preventing the synthesis of estrogens and therefore decreasing estrogen levels which results in a reduction in cell proliferation, thus providing a preventive effect against breast cancer. Additionally, they prevent the production of estrogen metabolites such as 2-hydroxylestradiol and 4-hydroxyestradiol, which are known to cause genotoxic effects by inducing phenotypic changes that indicate neoplastic transformation [220].

Clinical studies have demonstrated that AIs are more effective antiestrogen agents than tamoxifen with more desirable toxicity profiles. However, their action varies between premenopausal and postmenopausal women. Postmenopausal women can experience a significant suppression of circulating estrogen levels by approximately 96.7–98.9% with AIs. Additionally, autocrine and paracrine estrogen production by peritumoral stromal cells found in primary and metastatic sites of the tumor can also be blocked by AIs. However, premenopausal women only experience an incomplete blockade of estrogen synthesis, which can result in a reflux rise in gonadotrophin levels that stimulate ovarian aromatase and overcome the estrogen suppression caused by AIs [221]. As a result, AIs are used as the first-line therapy only for postmenopausal women

with hormone-sensitive early breast cancer or metastatic disease and as second-line agents in cases of tamoxifen resistance [222].

AIs can be categorized into two groups based on their chemical structures: steroidal and nonsteroidal.

Steroidal AIs have a structure similar to that of androstenedione, as they are primarily composed of an androstenedione nucleus that has been modified by adding various chemical groups at different positions on the steroid. They attach irreversibly to the active site of the AR, thereby the enzyme remains inactive even after the drug is cleared from circulation. As a result, these inhibitors are marketed as either inactivators or "suicide inhibitors" [223]. Some steroidal aromatase inhibitors with prominent activity include formestane, exemestane, atamestane and MDL-18,962 [224].

Nonsteroidal AIs possess heteroatoms that are favorably located for coordination to the heme iron of aromatase, resulting in reversible binding to the enzyme [223]. They are categorized into three groups: flavonoid analogs, aminoglutethimide-like molecules and imidazole/triazole derivatives. Examples of nonsteroidal AIs that have been patented include aminoglutethimide, fadrazole, anastrozole and letrozole, among others [225].

4.3. Prognosis

Randomized clinical trials have demonstrated that endocrine therapies that modulate the level and/or activity of ER have significantly decreased the recurrence and mortality of breast cancer. This highlights the effectiveness of these agents in treating ER+ early-stage breast cancers (eBC) , which can enhance the quality of life and extend the survival of patients [226]. However, although most ER+ breast cancer patients initially respond well to the aforementioned endocrine agents, resistance can develop over time (acquired resistance), or some patients may not respond to therapy from the outset (de novo resistance). This phenomenon is known as "endocrine resistance". Changing to different endocrine therapies can sometimes be an effective approach for managing endocrine resistance. For instance, tamoxifen-resistant patients have shown increased response when treated with AIs or fulvestrant. However, the response rates to second-line hormone therapy are typically lower than those to first-line therapy, which highlights the need to identify and target novel mechanisms of endocrine resistance to enhance clinical outcomes [227].

4.3.1. Mechanisms of endocrine resistance

The development of endocrine therapy resistance is a multifactorial and complex phenomenon that involves modulation of a plethora of different processes and signaling pathways.

Endocrine resistance involves various mechanisms that result from changes in drug targets (like ER and aromatase), cellular pathways (like PI3K/AKT/mTOR and MAPK pathways) and gene expression modulators. Other factors that contribute to endocrine resistance are variations in cell cycle components (CCND1-CDK 4/6-RB), epigenetic modifications and non-genetic mechanisms, comprising of metabolic reprogramming, cancer stem cells (CSCs), EMT and alterations in ER co-activators/co-repressors. The tumor microenvironment also plays a significant role in endocrine therapy inefficacy, where several cell factors like hypoxia, inflammation and immunomodulation can lead to endocrine resistance [228].

Among all the resistance mechanisms mentioned above, changes in ER expression or activity are frequently the primary means by which the tumor evades drug inhibition, as ER is the main target of endocrine therapies. Around 20% of advanced ER+ breast cancer cases show loss of ER expression, which leads to resistance to endocrine therapy due to loss of estrogen dependence [229]. Several mechanisms contribute to this loss of ER expression, including histone modifications and promoter methylation [230,231].

Besides loss of ER expression, activating point mutations in the LBD of estrogen receptor 1 gene (ESR1), which encodes the main form of ER in the breast (ERα), represent the primary mechanism of acquired resistance to currently available ETs, although some patients may present resistance from the outset (de novo resistance) [232].While de novo ESR1 mutations are rare in newly diagnosed breast cancer patients, acquired mutations can arise in up to 40% of patients who have previously received ET, particularly tamoxifen and AIs [233].

ESR1 point mutations were first reported in breast cancer in 1997 [234]. However, it was not until the completion of genomic sequencing of mBC in 2013 that their role in maintaining resistance to endocrine therapy was firmly established [235-237]. LBD point mutations, particularly those occurring at D538G and Y537S, cause stabilization of H12 in the active conformation, mimicking the binding of estrogen to WT ER. This allows co-activators to bind and activate transcription even in the absence of ligand, which results in resistance to AIs and reduced sensitivity to tamoxifen and fulvestrant. In addition, Y537S and D538G mutants display allele-specific variations in their transcriptomes and cistromes, which promote the transcription of genes associated with metastasis [238,239]. Besides point mutations, ESR1 fusions and amplifications are also detected in clinical settings, albeit at a lower frequency [240,241].

In conclusion, the identification of key escape pathways in endocrine resistance has paved the way for the development of innovative treatment strategies aimed at addressing and delaying resistance to endocrine therapy.

4.3.2. Clinical management of endocrine resistance

There are multiple new inhibitors of ER signaling that are currently being developed and have the potential to enhance the available treatments for ER+ breast cancer.

4.3.2.1. Novel SERMs

Lasofoxifene is a tetrahydronaphthalene that has shown potential in treating breast cancer. In preclinical studies, it can inhibit ER activity, even in cases of ER mutations, which is unique compared to other available drugs [242]. It was found to be more effective than fulvestrant at inhibiting primary tumor growth and reducing metastases in mouse models of ET-resistant breast cancer [243]. In a clinical trial (ELAINE 1), lasofoxifene was found to have slightly longer median progression-free survival (mPFS) than fulvestrant in women with ER+/HER2- mBC who had ESR1 mutations and had received CDK4/6 inhibitors (CDK4/6i) as a second-line treatment after AIs therapy. The drug was also effective in patients with visceral metastasis and those with ESR1 (Y537) mutations [244]. In another trial (ELAINE 2), combining lasofoxifene with a CDK4/6i resulted in favorable outcomes in terms of mPFS and clinical benefit rate (CBR) [245].

On the other hand, we have Z-endoxifen, a metabolite of tamoxifen with antiestrogenic activity that is converted into endoxifen by the CYP2D6 enzyme. In a phase I study, endoxifen was found to be safe and well-tolerated in women with endocrine refractory mBC [246]. A randomized phase II clinical trial, comparing Z-endoxifen with tamoxifen in women with mBC, showed that Z-endoxifen was not significantly superior to tamoxifen, but patients who had not received treatment with CDK4/6i had a longer PFS when taking Z-endoxifen [247].

4.3.2.2. New-generation SERDs

New orally bioavailable SERDs that can effectively degrade both WT and mutant ERα are being developed, offering promising alternatives to fulvestrant.

Elacestrant (RAD1901) is a non-steroidal drug that acts as a SERM/SERD, with its effects varying at different doses [248,249]. It has shown potential in inhibiting ER signaling and exerting antitumor effects in ER+ breast cancer cells and patient-derived xenografts (PDX), alone or in combination with mTOR or CDK4/6 inhibitors [250]. Two phase I studies found that elacestrant had good oral bioavailability, long half-life, penetrated the blood-brain barrier and had a favorable safety profile, suggesting once-daily administration could be achievable [251]. In the EMERALD trial, elacestrant was compared to standard ET in patients with ER+/HER2- mBC who had received prior treatment with CDK4/6i. The study showed improved mPFS and reduced risk of progression or death with elacestrant in both WT and ESR1 mutant ER, making it the first oral SERD to demonstrate superior

efficacy compared to fulvestrant in second-line treatment of mBC [252]. As a result, elacestrant is expected to become the standard of care for this patient population soon, with further studies exploring its use in combination with other drugs or in earlier stages of the disease [253].

Amcenestrant (SAR439859) is an orally administered and potent ER antagonist with optimized pharmacological and pharmacokinetic properties. Its mechanism of action involves promoting the degradation and transcriptional inhibition of ER, which has been shown to induce tumor regression in both WT and mutant ESR1 ER in ER+ breast cancer models [254-256]. However, despite numerous studies (AMEERA-1, 3, 4, 5 and 6) conducted to evaluate the safety and effectiveness of amcenestrant in ER+ mBC and eBC, both alone and in combination with other treatments, the global clinical development program for amcenestrant was terminated by the sponsor due to the negative results of the trials where the drug failed to meet the primary endpoint [257-261].

Camizestrant (AZD9833) is an oral non-steroidal SERD that shows ER degradation and pure antagonism in several cancer cell lines and PDX, including those with ESR1 mutations [262]. Several clinical studies (SERENA-1, 2, 3, 4, and 6) are currently underway to assess the safety and tolerability of camizestrant, both as a monotherapy and in combination with other drugs, in women with ER+/HER2- mBC or eBC that had been already treated or not [263-267].

Giredestrant (GDC-9545) is an oral non-steroidal SERD that has the ability to fully antagonize and degrade ER. It immobilizes ER to prevent its activation and alters chromatin accessibility [268]. Additionally, it has been shown to cause tumor regression in both WT and mutant ESR1 cell lines and PDX models, either alone or in combination with CDK4/6i [269]. The coopERA study was the first randomized trial to demonstrate that an oral SERD, such as giredestrant, exhibited greater antiproliferative activity compared to an AI in ER+/HER2- eBC [270]. Currently, the persevERA and lidERA trials are ongoing in ER+/HER2- mBC and eBC respectively [271,272].

Imlunestrant (LY3484356) acts as a pure antagonist for the ER, demonstrating its ability to inhibit cell proliferation in both WT ER and ESR1 (Y537) ER mutant [273]. Several ongoing studies are currently assessing the safety and efficacy profiles of imlunestrant (EMBER), as well as its biological effects, either as a monotherapy or in combination with other drugs, in patients with ER+ mBC (EMBER-3) and eBC (EMBER-2 and 4) [274,275].

Rintodestrant (G1T48) is a newly developed orally available compound with SERD activity derived from the raloxifene structure with an added acrylic acid side chain that was selected for its favorable toxicity profile [276]. It has been shown to disrupt estrogen signaling in breast cancer models, targeting both WT ER and ESR1 (D538G) ER mutants [277]. A clinical trial currently in progress is evaluating rintodestrant, either alone or in combination with a CDK4/6i, for patients with ER+/HER2- mBC who have progressed on previous ET. Preliminary data indicates promising results, with evidence

suggesting that the combination therapy may also be effective for patients with PI3K catalytic subunit alpha gene (PIK3CA) and ESR1 mutations [278,279].

Brilanestrant (GDC-08010) is an oral non-steroidal SERD that has shown strong antitumor efficacy in xenograft models of breast cancer, including those with ESR1 activating mutations, as well as tumors that are sensitive or resistant to tamoxifen [280]. At the moment, there is an ongoing trial to evaluate the effectiveness of brilanestrant in treating postmenopausal women with ER+ mBC [281].

To conclude, there are various other SERDs currently undergoing clinical development, such as D-0502, AZD9496, borestrant (ZB716) and ZN-c5 [282-285].

4.3.2.3. SERM/SERD hybrids

Bazedoxifene is a nonsteroidal indole that has shown potential for use in breast cancer treatment due to its unique structure which includes a 2-phenyl-3-methylindole core and a larger azepane heterocyclic ring, which enables it to obstruct H12 and prevent 17β-estradiol from binding to both ERα and ERβ, with slightly higher antagonistic effect in ERα [286]. Originally developed to prevent and treat postmenopausal osteoporosis, it was found to prevent bone loss and reduce the risk of vertebral fractures without affecting the endometrium or breast tissue [287]. Researchers later found that it could reduce the stability of ER in breast cancer cells, leading to improved antagonist efficacy [288].

It has been classified as a mixed SERM/SERD hybrid due to its ability to prevent the connection between ER and its co-regulators by binding with ER, resulting in the degradation of the receptor through ubiquitination and proteasomal degradation [289]. Due to its distinctive characteristics, bazedoxifene is currently being studied in clinical trials for the treatment of mBC. Its preclinical research has shown encouraging outcomes, in both sensitive and resistant to treatment models, used alone or together with a CDK4/6i [290,291].

4.3.2.4. CDK4/6 Inhibitors

The role of the CCND1-CDK 4/6/RB pathway in endocrine resistance in breast cancer has been extensively studied. This pathway regulates cell cycle progression by controlling the decision of whether a cell arrests or advances at the G1-S phase of the cell cycle. The pathway functions by allowing cyclin-D to bind with CDK4/6 creating a complex that phosphorylates the tumor suppressor retinoblastoma protein (RB). This phosphorylation causes the dissociation of RB from the E2F transcription factors, which are responsible for promoting cell cycle progression [292].

In ERα+ breast cancer, there is often an amplification of the CCND1 gene (which encodes for cyclin-D1) and the CDK4 gene, as well as a loss of natural negative regulators

like CDKN2A (p16) and CDKN2C (p18), leading to an excessive CDK4/6 activity [293]. This indicates that CDK4/6i have the potential to be used as a therapy for ERα+ breast cancers. Various CDK4/6i are currently available or in development, such as palbociclib (PD-0332991), ribociclib and abemaciclib. These drugs are frequently used in combination with other ETs to treat recurrent ERα+ breast cancer and have been shown to improve PFS, in fact, the use of CDK4/6i in combination with fulvestrant was approved as a treatment option for mBC resistant to ET [294,295].

Some combination therapies for resistant breast cancer that have been approved or are currently in clinical trials and include CDK4/6i are summarized in table 2.

4.3.2.5. PI3K/AKT/mTOR Inhibitors

The PI3K-AKT-mTOR pathway is frequently mutated in ERα+ breast cancer and over 50% of breast cancers with ESR1 mutations or amplifications have genomic alterations in this pathway [296]. Given these findings, some clinical studies have been conducted to explore the use of PI3K-AKT-mTOR inhibitors in combination with other treatments. These studies have shown that targeting this pathway can be effective in both early and advanced stages of ERα+ breast cancer [297,298].

Everolimus is a rapamycin analog that is frequently used as a first-line therapy in many diseases. By inhibiting the mTOR complex, it interrupts the signaling pathway mediated by PI3K, which results in several downstream effects, such as altering cellular metabolism, disrupting angiogenesis and inhibiting cell growth. For this reason, it has been authorized for use in postmenopausal women with ERα+ advanced breast cancer in combination with other hormonal treatments, especially with AIs [299].

There are oral PI3K inhibitors currently being developed to treat advanced or mBC when used in combination with other antiestrogen therapies. Two of these inhibitors are taselisib (GDC-0032) and alpelisib (BYL719), which are selective for specific isoforms of PI3K. Alpelisib is particularly useful for tumors with PIK3CA mutations, as it is specific for this mutation [300,301]. In addition, two broad-spectrum PI3K inhibitors, buparlisib (BKM120) and pictilisib (GDC-0941), were being studied. However, unfavorable toxicity profiles resulted in the discontinuation of their further development [302,303].

Breast cancers carrying AKT mutation showed a positive response to the AKT-competitive inhibitor, ipatasertib (GDC-0068). However, the clinical efficacy of pan-AKT inhibitors such as AZD5363 (capivasertib), MK-2206 and GSK2141795 was found to be limited.

Approved combination therapies or therapies currently undergoing clinical trials for resistant breast cancer that involve the use of PI3K/AKT/mTOR inhibitors are showed in table 2.

Table 2. Combination therapies for anti-estrogen resistant breast cancers. Taken from [296].

Current strategies	FDA approved drugs / drugs combinations and associated clinical trials	Drugs / drugs combinations in clinical trials
Inhibition of CCND1-CDK4/6-RB pathway	1. Palbociclib and Letrozole combination (PALOMA-2 trial) 2. Palbociclib and Fulvestrant combination (PALOMA-3 trial) 3. Abemaciclib as monotherapy (MONARCH-1 trial) 4. Abemaciclib in combination with Fulvestrant (MONARCH-2 trial) 5. Abemaciclib combined with Letrozole or Amastrozole (MONARCH-3 trial)	Ribociclib (LEE011) in combination with endocrine therapy (Tamoxifen and Goserelin or a nonsteroidal AI and Goserelin) (MONALEESA-7 trial)
Inhibition of the PI3K-AKT-mTOR pathway	1. Alpelisib and Fulvestrant combination (SOLAR-1 trial) 2. Everolimus in combination with Exemestane (BOLERO-2 trial)	Ipatasertib in combination with endocrine therapy and a CDK4/6 inhibitor (TAKTIC trial)
Concurrent inhibition of ERα, CCND1-CDK4/6-RB pathway and the PI3K-AKT-mTOR pathways		Triplet therapy combining Palbociclib, Taselisib and Fulvestrant and doublet therapy combining Palbociclib and Taselisib

4.3.2.6. PROTACs

PROTACs (PROteolysis TArgeting Chimeras) are small molecules composed of two active domains and a linker that have the unique ability to target and eliminate specific proteins. These molecules contain a ligand, which is typically a small molecule inhibitor that attaches to a protein of interest (POI), and a covalently linked ligand of an E3 ubiquitin ligase (E3). By binding to POIs, PROTACs recruit E3 to ubiquitinate them, resulting in its degradation by the proteasome (Figure 12) [304].

There are more than 50 successful PROTACs that have been developed, including ARV-471, which is one of the most potent ER-targeting PROTACs. Preclinical studies have shown that ARV-471 can inhibit cell growth in ER-dependent BC cell lines (MCF7, T47D), degrade clinically relevant ESR1 variants (Y537S, D538G), inhibit the growth of cells expressing those variants in PDX and reduce the expression of ER-target genes [305]. Due to its favorable preclinical data and safety profile, the drug is currently undergoing phase I dose escalation studies, a phase Ib combination study with palbociclib and a phase II monotherapy dose-expansion study as a potential treatment for mBC [306]. There are

several other PROTACs in preclinical development that show promising *in vitro* antitumor activity. Some of these include SGK3-PROTAC1, AC682, ERD-148 and ERD-308.

4.3.2.7. MGDs

Related with PROTACs, some proteins are considered "non-targetable" because they lack the necessary binding sites for PROTAC linkers. However, a new class of drugs, called MGDs (Molecular Glue Degraders), can overcome this limitation. These degraders use small molecules, known as molecular glues, to enhance protein-protein interactions and promote ternary complex formation between proteins that do not typically interact. This leads to ubiquitination and degradation of the target protein, after which the molecular glue dissociates [307].

MGDs, similar to PROTACs, can break down early ER protein in the cytoplasm, which avoids its membrane insertion. They also degrade ESR1 and other resistance-related proteins such as CD4/6 and PI3K. According to preclinical and initial clinical results, MGDs could be more potent than SERDs and even ER PROTACs, while also less harmful [308].

4.3.2.8. LYTACs

Apart from the ubiquitin-proteasome system, the lysosome is another cellular mechanism responsible for protein degradation. Thus, in order to target specific proteins for degradation through this mechanism, researchers have developed LYTACs (LYsosome TArgeting Chimaeras).

These are compounds that have two active domains, one that binds to the extracellular domain of the target protein and another that binds to a lysosome-shuttling receptor present on the cell surface. This allows the LYTAC to transport the target protein to the lysosome for selective degradation (Figure 12) [309].

For all the above, further research is needed to explore the possibility of using LYTACs to degrade proteins such as ER.

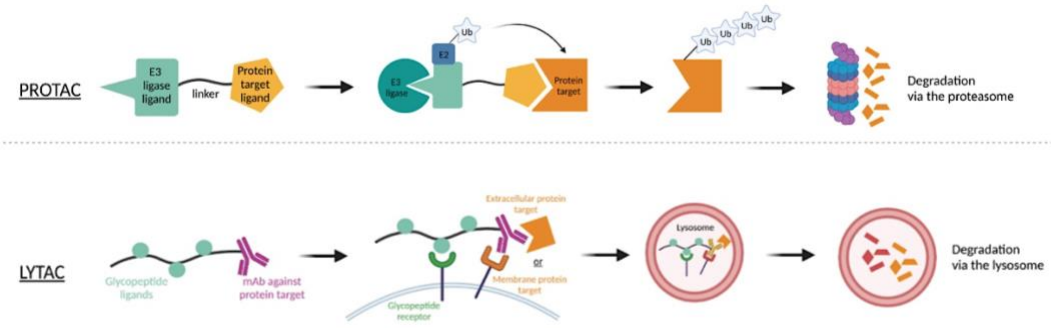

Figure 12. Two types of targeting chimera degraders. Proteolysis targeting chimeras (PROTACs) and lysosome targeting chimaeras (LYTACs) are novel technologies that utilize different protein degradation mechanisms, such as the ubiquitin-proteasome and lysosome respectively, to inhibit a target protein or biomarker. Taken from [310].

4.3.2.9. SERCAs

Given the limitations observed in certain types of therapies targeting ERα, researchers sought to discover a new approach to antagonize ERα with increased effectiveness compared to standard endocrine therapies. To achieve this goal, they proposed an intriguing hypothesis that the potency of an ER antagonist could be enhanced by forming a covalent or irreversible interaction with the receptor. This could overcome the stabilizing effects of mutations and shift the receptor's conformation towards an antagonist state [311].

Studies were conducted to test this hypothesis and the outcomes yielded promising results. They revealed that among the four cysteines present in the LBD of ERα (amino acids 381, 417, 447 and 530), C530 was a nonconserved cysteine that could be covalently modified, being the only cysteine that is directly associated with the AF-2 domain [312]. Based on this finding, scientists focused their attention on C530 as the ideal target cysteine for converting an ERα-labeling agent into a pharmacologically effective antagonist. Their objective was to determine if the formation of a covalent bond between a ligand bound in the LBD and C530 could effectively stabilize ERα in its antagonist state [313]. This exploration led to the identification of H3B-5942, an innovative orally available selective ER covalent antagonist (SERCA) which could inactivate both WT ERα and mutated (D538G, Y537S) ERα by covalent binding to C530 of helix 11 (Figure 11) [311]. Currently, H3B-6545 is undergoing evaluation in a phase 1/2 clinical trial, showing promising initial antitumor activity in women with locally advanced or metastatic ER+/HER2- breast cancer who have received prior treatment [314].

4.3.2.10. CERANs

CERANs (Complete ER ANtagonists) are a class of small molecules that possess complete ER antagonist properties. They not only have the ability to degrade ER similar to SERDs but also effectively block ER function. CERANs effectively inhibit the transcriptional activation domains (AF1 and AF2) of ER. They inhibit AF2 directly and recruit nuclear receptor co-repressors to deactivate AF1. This distinct mechanism sets them apart from SERMs, which inhibit AF2 but still allow agonist signaling through AF1 via alternative signaling pathways (Figure 11) [315].

OP-1250 is a CERAN compound that can be taken orally and has shown excellent bioavailability. In preclinical studies, OP-1250 has demonstrated the ability to inhibit cell growth, impede gene transcription and entirely degrade and deactivate ER. Furthermore, it has displayed promising anticancer effects in brain metastases and demonstrated anti-tumor activity in preclinical models involving both WT ER and mutant ESR1 ER [316]. At the moment, a phase I/II clinical trial is underway to assess the safety and tolerability of OP-1250 in premenopausal and postmenopausal women diagnosed with ER+ mBC who have experienced disease progression after previous ET [317].

4.3.2.11. ShERPAs

ShERPAs (Selective human ER Partial Agonists) are a class of benzothiophene derivatives that imitate the function of E2 but, unlike E2 and DES which act as full agonists, they function as partial agonists. In breast cancer cells and tumors that have become resistant to hormonal therapies they bind to and target the ER within the nucleus, resulting in the translocation of the ER to extranuclear sites which leads to the inhibition of growth in ER+ tumor cells. Additionally, in experimental models, ShERPAs have demonstrated the ability to prevent the growth of hormone-dependent cell lines that depend on E2 for their proliferation. They have also shown a lack of significant uterine growth induction, which is typically observed with E2 [318]. TTC-352, a specific ShERPA currently undergoing clinical development, has shown promising results in terms of safety and initial clinical evidence of its effectiveness against hormone-refractory ER+ breast cancer, especially in patients who have received extensive prior treatment [319].

4.3.2.12. SNIPERs

IAPs (inhibitor of apoptosis proteins) are a group of regulators in mammalian cells with E3 ubiquitin ligase activity that enables them to participate in the ubiquitylation of themselves and associated proteins. In addition, they play a role in inhibiting apoptosis by blocking the activity of caspase enzymes, so they are often overexpressed in cancer cells, leading to resistance to apoptosis and unfavorable prognoses [320]. As a result, they have emerged as important targets for cancer therapy.

Building upon this knowledge, SNIPERs (Specific and Non-genetic IAP-dependent Protein ERasers), which fall under the category of PROTACs, leverage the E3 ligase function of IAPs to trigger the degradation of specific target proteins. They accomplish this by efficiently forming a ternary complex that cross-links the target protein and the IAP. This complex facilitates IAP-mediated polyubiquitylation and subsequent proteasomal degradation of the target protein [321]. Numerous preclinical investigations have demonstrated the capacity of ER SNIPERs to substantially reduce the levels of ERα in human breast cancer cell lines such as MCF-7 and T47D. Moreover, they have shown efficacy in inhibiting the interaction between ER and coactivators. These encouraging findings validate the potential of SNIPERs as a viable alternative for the treatment of breast cancer [322,323].

4.3.2.13. RIBOTACs

Up until now, we have described pharmaceutical interventions primarily centered around inhibiting the activity of abnormal proteins or oncogenes through monoclonal antibodies, small molecule inhibitors or protein degraders. However, there are additional therapeutic approaches currently being developed that focus on reducing

or blocking the production of abnormally expressed gene products by targeting the messenger RNA (mRNA) or even the DNA of the gene in question. One example of these therapies are RIBOTACs.

RIBOTACs (RIBOnuclease TArgeting Chimeras) are a new class of small molecules that have the potential to target diverse types of RNAs, especially RNAs that form intricate secondary and tertiary structures. They are bivalent molecules containing an RNA-binding module and a ribonuclease (RNase L) recruitment module joined by a linker [324]. The particular component that recruits RNase L is a tetra-adenylate linked at the 2'-5' positions. This structure closely resembles the naturally produced oligoadenylates generated in cells during viral infections. These oligoadenylates have the ability to activate dormant RNase L, inducing its dimerization and transforming it into an active ribonuclease. Consequently, RIBOTACs employ this mechanism to bring an active RNase L to a target RNA of interest, leading to its degradation (Figure 13) [325].

While this approach faces numerous challenges that must be addressed before reaching the clinical development stage, one of which is determining an efficient method for delivering the treatment to tumor targets within a living organism, the current results are highly promising.

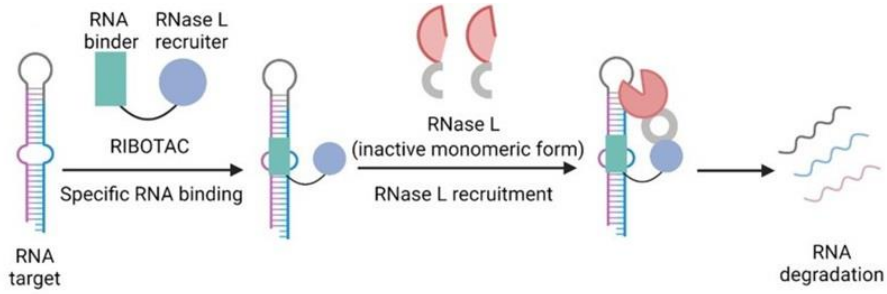

Figure 13. Mechanism of RIBOTACs. Ribonuclease targeting chimeras (RIBOTACs) are designed to selectively attract the naturally occurring ribonuclease RNase L towards a particular RNA target, resulting in effective degradation of the RNA by RNase L. Taken from [326].

5. ESTROGEN – MELATONIN INTERACTIONS

Melatonin has been the subject of extensive research in relation to various types of tumors, particularly in endocrine mammary tumors. Emerging evidence indicates that this hormone is linked to a decreased cancer risk, as demonstrated in a range of *in vitro* and *in vivo* models [327,328].

Melatonin, an indoleamine known as N-acetyl-5-methoxytryptamine, is derived from tryptophan and was initially reported by Lerner in 1958 [329]. This hormone is primarily secreted during nighttime and serves various important physiological roles. It plays a key role in synchronizing circadian rhythms as a regulator of physiological sleep, in reducing oxidative stress, in modulating the immune system by boosting both natural and acquired immunity, in regulating seasonal reproduction and in exhibiting potential

cancer-preventive effects through multiple mechanisms [330-335]. In this section, we will particularly emphasize the latter function and delve into the oncostatic effects of melatonin specifically in estrogen-dependent breast cancer.

The connection between melatonin and breast cancer development was first proposed in 1978 by Michael Cohen et al, who suggested that reduced pineal gland function could increase the risk of breast cancer [336]. This proposal marked the beginning of extensive research conducted to investigate the association between this hormone and breast cancer [337,338]. All this research yields substantial evidence supporting the inhibitory effect of melatonin on estrogen-positive cell lines in mammary cancer. This inhibition is attributed to melatonin's capacity to regulate estrogen synthesis and estrogen signaling pathways, functioning as both a SERM and SEEM (Figure 14) [339].

Unlike other clinical antiestrogenic molecules such as tamoxifen, melatonin acts as SERM without directly binding to the ER. Instead, it exerts its inhibitory effects by destabilizing the estradiol-ER complex, preventing its binding to DNA in promoters containing EREs and AP1 sites. This disruption interferes with estradiol-triggered transcriptional activation of various estradiol-responsive genes [340]. These effects of melatonin are thought to be mediated by calmodulin, as melatonin acts as an antagonist to calmodulin and this binds to ERα, as we explained earlier. By inducing structural changes in the calmodulin-ERα protein complex, melatonin disrupts its ability to bind to estrogen-responsive promoters [47,341]. Moreover, the SERM actions of melatonin can be attributed to the presence of melatonin receptor type 1 (MT-1) on the cell membrane of breast cancer cells. Upon estradiol binding to ER, adenylate cyclase is activated, leading to increased cytoplasmic levels of cAMP, which synergize with the long-term genomic effects of estradiol and enhance ER-mediated transcriptional activation. In contrast, when melatonin binds to the MT-1 receptor, it inhibits adenylate cyclase activity, resulting in decreased intracellular levels of cAMP [342,343]. Melatonin also functions as a SEEM by downregulating the transcription and activity of key enzymes involved in estrogen synthesis. It regulates the transcriptional levels of STS and 17β-HSD1, which are enzymes essential for converting inactive estrogen precursors into biologically active estradiol [344,345].

Apart from its SERM and SEEM properties, melatonin also exerts antimitotic effects, inhibits cell-cycle progression and enhances the expression of cell surface adhesion proteins, which contributes to the inhibition of cancer invasion and metastasis [346-349]. Furthermore, the impact of melatonin extends beyond tumor cells and encompasses endothelial and fibroblast cells in the tumor microenvironment. In fibroblasts, melatonin exhibits the capability to inhibit the secretion of tumor-derived cytokines, such as IL-6 [350]. This action leads to the stimulation of fibroblast differentiation and a subsequent reduction in aromatase activity and expression in both fibroblasts and adipocytes. Consequently, there is a decrease in the number of estrogen-producing cells near malignant cells [351]. On the other hand, in endothelial cells

melatonin can reduce the production of aromatase and the levels of VEGF, thereby impeding the process of angiogenesis [352].

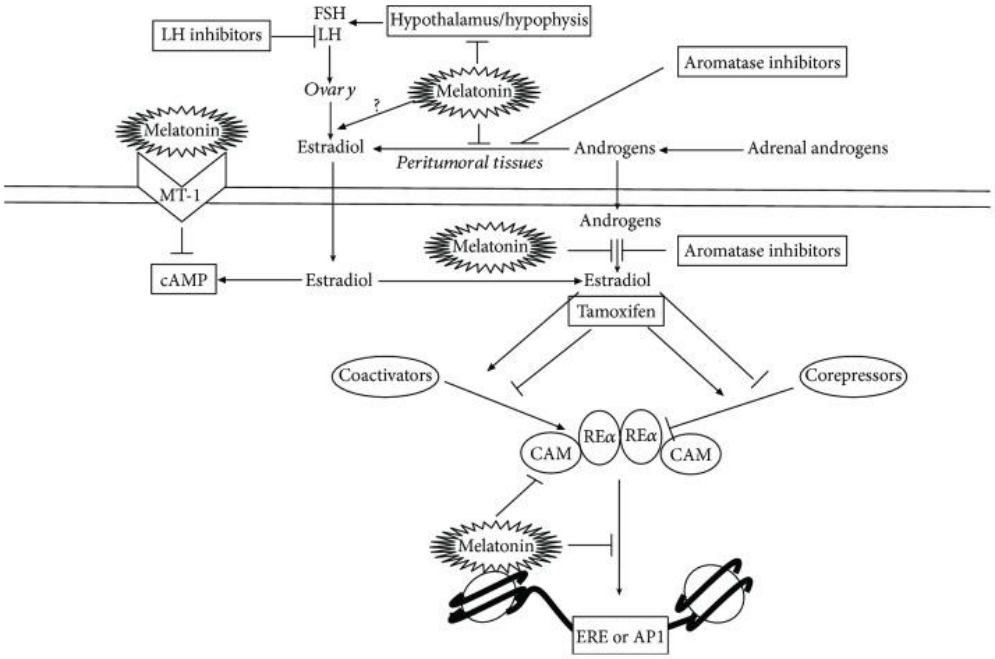

Figure 14. Melatonin antiestrogenic actions. Melatonin inhibits the enzymes involved in the conversion of adrenal androgens into estrogens in both estrogen-dependent breast cancer cells and tumor surrounding tissues. Additionally, by binding to the MT-1 membrane receptor in malignant epithelial cells, melatonin inhibits adenylate cyclase activity, thus opposing the stimulatory effect of estradiol.. Furthermore, it binds to the calmodulin-ERα complex, leading to the destabilization of the estradiol-ERα complex. This prevents its binding to DNA in promoters containing estrogen response elements (ERE) and activator protein 1 (AP1), while tamoxifen, on the other hand, directly binds to the estrogen receptor and interferes with the binding of coactivators. Taken from [353].

In addition to its previously mentioned effects, melatonin holds promise as an adjuvant to chemotherapy and radiotherapy, as well as for combination with other molecules to overcome resistance to endocrine therapy.

The impact of melatonin on chemotherapy has been thoroughly examined in various settings, including *in vitro*, *in vivo* and clinical studies involving patients. Notably, in human trials, it has been observed that breast cancer patients who received melatonin in addition to chemotherapy demonstrated fewer side effects compared to matched controls who received chemotherapy alone [354]. In *in vitro* experiments using breast cancer cell lines like MDA-MB-231 and MCF-7, the combination of melatonin with various chemotherapeutic agents such as doxorubicin and docetaxel has shown enhanced chemotherapeutic effects. This combination led to increased apoptosis, antiproliferative responses, mitochondrial oxidative stress, and caspase activity, while potentially reducing certain undesirable effects of chemotherapy [355,356]. Lastly, *in vivo* experiments conducted on rats with induced breast tumors demonstrated that melatonin augmented the therapeutic efficacy of various chemotherapeutic agents when administered in combination. This combined treatment approach resulted in a decrease in tumor occurrence, a delay in tumor onset and an improved survival rate [357-

[359]. Furthermore, certain experiments revealed that the decrease in melatonin levels caused by exposure to light-at-night (LAN) led to a complete loss of tumor responsiveness to chemotherapeutic agents, suggesting that chemotherapy resistance could be attributed to disruptions in the circadian rhythm [360].

Radiotherapy is a highly efficient approach for eliminating any residual cancer cells in the breast following surgery, as it induces cellular damage through various direct and indirect mechanisms, ultimately leading to in DNA damage and chromosomal abnormalities. However, radiotherapy can also result in adverse effects on normal tissues [361]. Consequently, there is significant interest in identifying new molecules that can protect normal tissues and organs from these radiation-induced side effects. Melatonin emerges as a promising candidate for a radioprotective agent due to its capacity to scavenge hydroxyl radicals [362]. Furthermore, melatonin has the ability to enhance the efficacy of radiation therapy by sensitizing breast cancer cells to its effects. One mechanism by which it achieves this is by reducing the expression of proteins essential for DNA repair, such as RAD51, which are commonly elevated in cancer cells [363]. Additionally, melatonin can induce cell cycle arrest, inhibit cell proliferation and promote apoptosis by increasing the levels of the tumor suppressor protein p53 [364].

Ultimately, several *in vitro* and *in vivo* studies have presented compelling evidence suggesting that melatonin can enhance the effectiveness of tamoxifen, potentially addressing its resistance issues [365,366]. As extensively discussed in this review, cells can acquire resistance to drugs through various mechanisms, including the activation of crucial signaling pathways, increased expression of specific proteins and the development of metabolic abnormalities, all of which diminish the ability of drugs to inhibit cancer growth. Melatonin, by inhibiting key kinase signaling pathways and transcription factors, modifies the phosphorylation activation of ERα and consequently alters its responsiveness to tamoxifen, ensuring that tamoxifen functions as an antagonist to ERα [367].

In conclusion, all these findings highlight the multi-faceted role of melatonin in modulating estrogen-related processes and its potential as a therapeutic agent in the management of mammary cancer.

6. CONCLUSIONS

Numerous members of the nuclear receptor superfamily have been implicated in the development of cancer, including estrogen receptors, which play a significant role in the development of breast cancer. Therefore, it is crucial to understand their characteristics to gain insights into their involvement in this tumor development. While numerous studies have investigated estrogens from various perspectives, this review aims to consolidate and provide a comprehensive examination of estrogen receptors. We have extensively covered different aspects of the estrogen receptors ERα, ERβ and

GPER1, including their types, structures and isoforms. Additionally, we have delved into the intricate signaling pathways of estrogen, shedding light on how it exerts its diverse physiological and pathological effects. Furthermore, understanding these signaling mechanisms of ERα, ERβ and GPER1, encompassing both genomic and non-genomic pathways, is essential for elucidating their aberrant functioning and exploring potential strategies to counteract it.

Given that ER+ is the most prevalent type of breast cancer, significant efforts have been devoted by various research groups over the past decades to suppress estrogen synthesis. Remarkable progress has been made in inhibiting estrogen synthesis or controlling its concentration within a therapeutic range to manage malignancy. Consequently, in this review we have extensively examined the well-known ER ligands, particularly SERMs, along with other classical ER ligands like SERDs, sulfatase inhibitors and aromatase inhibitors collectively known as SEEMs. Despite the potential side effects associated with SERMs, they have demonstrated favorable therapeutic profiles compared to other antiestrogens in terms of safety and efficacy, establishing them as the first-line treatment for ER+ breast cancer.

Nevertheless, many of the available medications used to control estrogen-dependent breast malignancies act as estrogen agonists in other tissues such as the uterus, thereby increasing the risk of uterine cancer in the same individuals. Similarly, some of these drugs face challenges in selectively binding to the target, compromising their effectiveness. Furthermore, drug resistance frequently develops shortly after initiating treatment with many currently available drugs. Therefore, it is imperative to explore and develop novel drugs with improved selectivity, high binding affinity and potency to suppress breast cancer proliferation effectively. In this review, we have discussed potential strategies to overcome drug resistance, including the role of melatonin as a SERM/SEEM molecule capable of modulating estrogen-related processes and enhancing the efficacy of chemotherapy and radiotherapy without the undesirable side effects associated with these treatments.

In summary, further investigations are warranted to unveil more promising outcomes and facilitate the development of novel synthetic approaches for the effective treatment of breast cancer.

7. REFERENCES

[1] Font-Díaz J, Jiménez-Panizo A, Caelles C, et al. Nuclear receptors: Lipid and hormone sensors with essential roles in the control of cancer development. *Semin Cancer Biol*. 2021;73:58-75.

[2] Khan MZI, Uzair M, Nazli A, Chen JZ. An overview on Estrogen receptors signaling and its ligands in breast cancer. Eur J Med Chem. 2022;241:114658.

[3] Jameera Begam A, Jubie S, Nanjan MJ. Estrogen receptor agonists/antagonists in breast cancer therapy: A critical review. Bioorg Chem. 2017;71:257-274.

[4] Gustafsson JA. Historical overview of nuclear receptors. J Steroid Biochem Mol Biol. 2016;157:3-6.

[5] Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. Cell. 1995;83(6):835-839.

[6] Kim EY, Lee JM. Transcriptional Regulation of Hepatic Autophagy by Nuclear Receptors. Cells. 2022;11(4):620. Published 2022 Feb 10.

[7] Germain P, Staels B, Dacquet C, Spedding M, Laudet V. Overview of nomenclature of nuclear receptors. Pharmacol Rev. 2006;58(4):685-704.

[8] Weikum ER, Liu X, Ortlund EA. The nuclear receptor superfamily: A structural perspective. Protein Sci. 2018;27(11):1876-1892.

[9] O'Malley BW, Tsai MJ. Molecular pathways of steroid receptor action. Biol Reprod. 1992;46(2):163-167.

[10] Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. Cell. 1995;83(6):841-850.

[11] Srivastava J, Robertson CL, Rajasekaran D, et al. AEG-1 regulates retinoid X receptor and inhibits retinoid signaling. Cancer Res. 2014;74(16):4364-4377.

[12] Harding HP, Lazar MA. The monomer-binding orphan receptor Rev-Erb represses transcription as a dimer on a novel direct repeat [published correction appears in Mol Cell Biol 1995 Nov;15(11):6479]. Mol Cell Biol. 1995;15(9):4791-4802.

[13] Kumar R, Thompson EB. Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions. Mol Endocrinol. 2003;17(1):1-10.

[14] Anbalagan M, Huderson B, Murphy L, Rowan BG. Post-translational modifications of nuclear receptors and human disease. Nucl Recept Signal. 2012;10:e001.

[15] Hill KK, Roemer SC, Churchill ME, Edwards DP. Structural and functional analysis of domains of the progesterone receptor. Mol Cell Endocrinol. 2012;348(2):418-429.

[16] Fruchart JC, Santos RD, Aguilar-Salinas C, et al. The selective peroxisome proliferator-activated receptor alpha modulator (SPPARMα) paradigm: conceptual framework and therapeutic potential: A consensus statement from the International Atherosclerosis Society (IAS) and the Residual Risk Reduction Initiative (R3i) Foundation. Cardiovasc Diabetol. 2019;18(1):71. Published 2019 Jun 4.

[17] Umesono K, Evans RM. Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell. 1989;57(7):1139-1146.

[18] Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT. Structural basis of androgen receptor binding to selective androgen response elements. Proc Natl Acad Sci U S A. 2004;101(14):4758-4763.

[19] Weikum ER, Tuntland ML, Murphy MN, Ortlund EA. A Structural Investigation into Oct4 Regulation by Orphan Nuclear Receptors, Germ Cell Nuclear Factor (GCNF), and Liver Receptor Homolog-1 (LRH-1). J Mol Biol. 2016;428(24 Pt B):4981-4992.

[20] Moras D, Gronemeyer H. The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol. 1998;10(3):384-391.

[21] Wurtz JM, Bourguet W, Renaud JP, et al. A canonical structure for the ligand-binding domain of nuclear receptors [published correction appears in Nat Struct Biol. 1996 Feb;3(2):206]. Nat Struct Biol. 1996;3(1):87-94.

[22] Keidel S, LeMotte P, Apfel C. Different agonist- and antagonist-induced conformational changes in retinoic acid receptors analyzed by protease mapping. Mol Cell Biol. 1994;14(1):287-298.

[23] Brzozowski AM, Pike AC, Dauter Z, et al. Molecular basis of agonism and antagonism in the estrogen receptor. Nature. 1997;389(6652):753-758.

[24] Schwabe JW, Chapman L, Rhodes D. The estrogen receptor recognizes an imperfectly palindromic response element through an alternative side-chain conformation. Structure. 1995;3(2):201-213.

[25] Rastinejad F, Perlmann T, Evans RM, Sigler PB. Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature. 1995;375(6528):203-211.

[26] Little TH, Zhang Y, Matulis CK, et al. Sequence-specific deoxyribonucleic acid (DNA) recognition by steroidogenic factor 1: a helix at the carboxy terminus of the DNA binding domain is necessary for complex stability. Mol Endocrinol. 2006;20(4):831-843.

[27] Flasch M, Bueschl C, Del Favero G, et al. Elucidation of xenoestrogen metabolism by non-targeted, stable isotope-assisted mass spectrometry in breast cancer cells. Environ Int. 2022;158:106940.

[28] Kumar RS, Goyal N. Estrogens as regulator of hematopoietic stem cell, immune cells and bone biology. Life Sci. 2021;269:119091.

[29] Delfosse V, Maire AL, Balaguer P, Bourguet W. A structural perspective on nuclear receptors as targets of environmental compounds. Acta Pharmacol Sin. 2015;36(1):88-101. doi:10.1038/aps.2014.133

[30] Jensen EV, Suzuki T, Numata M, Smith S, DeSombre ER. Estrogen-binding substances of target tissues. Steroids. 1969;13(4):417-427.

[31] Walter P, Green S, Greene G, et al. Cloning of the human estrogen receptor cDNA. Proc Natl Acad Sci U S A. 1985;82(23):7889-7893.

[32] Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A. 1996;93(12):5925-5930.

[33] Filardo EJ, Thomas P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. Endocrinology. 2012;153(7):2953-2962.

[34] Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ. Identification of a gene (GPR30) with homology to the G-protein-coupled receptor superfamily associated with estrogen receptor expression in breast cancer. Genomics. 1997;45(3):607-617.

[35] Feng Y, Gregor P. Cloning of a novel member of the G protein-coupled receptor family related to peptide receptors. Biochem Biophys Res Commun. 1997;231(3):651-654.

[36] Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology. 2005;146(2):624-632.

[37] Chen P, Li B, Ou-Yang L. Role of estrogen receptors in health and disease. Front Endocrinol (Lausanne). 2022;13:839005. Published 2022 Aug 18.

[38] Jia M, Dahlman-Wright K, Gustafsson JÅ. Estrogen receptor alpha and beta in health and disease. Best Pract Res Clin Endocrinol Metab. 2015;29(4):557-568.

[39] Olde B, Leeb-Lundberg LM. GPR30/GPER1: searching for a role in estrogen physiology. Trends Endocrinol Metab. 2009;20(8):409-416.

[40] Mizukami Y. In vivo functions of GPR30/GPER-1, a membrane receptor for estrogen: from discovery to functions in vivo. Endocr J. 2010;57(2):101-107.

[41] Kumar R, Zakharov MN, Khan SH, et al. The dynamic structure of the estrogen receptor. J Amino Acids. 2011;2011:812540.

[42] Metzger D, Ali S, Bornert JM, Chambon P. Characterization of the amino-terminal transcriptional activation function of the human estrogen receptor in animal and yeast cells. J Biol Chem. 1995;270(16):9535-9542. doi:10.1074/jbc.270.16.9535

[43] Helsen C, Claessens F. Looking at nuclear receptors from a new angle. Mol Cell Endocrinol. 2014;382(1):97-106.

[44] Mader S, Kumar V, de Verneuil H, Chambon P. Three amino acids of the estrogen receptor are essential to its ability to distinguish an estrogen from a glucocorticoid-responsive element. Nature. 1989;338(6212):271-274.

[45] Castoria G, Migliaccio A, Nola E, Auricchio F. In vitro interaction of estradiol receptor with Ca2+-calmodulin. Mol Endocrinol. 1988;2(2):167-174.

[46] García Pedrero JM, Del Rio B, Martínez-Campa C, Muramatsu M, Lazo PS, Ramos S. Calmodulin is a selective modulator of estrogen receptors. Mol Endocrinol. 2002;16(5):947-960.

[47] del Río B, García Pedrero JM, Martínez-Campa C, Zuazua P, Lazo PS, Ramos S. Melatonin, an endogenous-specific inhibitor of estrogen receptor alpha via calmodulin. J Biol Chem. 2004;279(37):38294-38302.

[48] Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS, Tung L. Nuclear receptor coactivators and corepressors. Mol Endocrinol. 1996;10(10):1167-1177.

[49] Bourguet W, Germain P, Gronemeyer H. Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci. 2000;21(10):381-388.

[50] Lonard DM, Nawaz Z, Smith CL, O'Malley BW. The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol Cell. 2000;5(6):939-948.

[51] Dauvois S, Danielian PS, White R, Parker MG. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. Proc Natl Acad Sci U S A. 1992;89(9):4037-4041.

[52] Montano MM, Müller V, Trobaugh A, Katzenellenbogen BS. The carboxy-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists. Mol Endocrinol. 1995;9(7):814-825.

[53] Moreira IS. Structural features of the G-protein/GPCR interactions. Biochim Biophys Acta. 2014;1840(1):16-33.

[54] Broselid S, Berg KA, Chavera TA, et al. G protein-coupled receptor 30 (GPR30) forms a plasma membrane complex with membrane-associated guanylate kinases (MAGUKs)

and protein kinase A-anchoring protein 5 (AKAP5) that constitutively inhibits cAMP production. J Biol Chem. 2014;289(32):22117-22127.

[55] Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol. 2008;70:165-190.

[56] Cheng SB, Quinn JA, Graeber CT, Filardo EJ. Down-modulation of the G-protein-coupled estrogen receptor, GPER, from the cell surface occurs via a trans-Golgi-proteasome pathway. J Biol Chem. 2011;286(25):22441-22455.

[57] Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology. 2005;146(2):624-632.

[58] Lappano R, Rosano C, De Marco P, De Francesco EM, Pezzi V, Maggiolini M. Estriol acts as a GPR30 antagonist in estrogen receptor-negative breast cancer cells. Mol Cell Endocrinol. 2010;320(1-2):162-170.

[59] Bologa CG, Revankar CM, Young SM, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat Chem Biol. 2006;2(4):207-212.

[60] Dennis MK, Burai R, Ramesh C, et al. In vivo effects of a GPR30 antagonist. Nat Chem Biol. 2009;5(6):421-427.

[61] Dennis MK, Field AS, Burai R, et al. Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. J Steroid Biochem Mol Biol. 2011;127(3-5):358-366.

[62] Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF. Identification, cloning, and expression of human estrogen receptor-alpha36, a novel variant of human estrogen receptor-alpha66. Biochem Biophys Res Commun. 2005;336(4):1023-1027.

[63] Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF. A variant of estrogen receptor-{alpha}, hER-{alpha}36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. Proc Natl Acad Sci U S A. 2006;103(24):9063-9068.

[64] Shi L, Dong B, Li Z, et al. Expression of ER-{alpha}36, a novel variant of estrogen receptor {alpha}, and resistance to tamoxifen treatment in breast cancer. J Clin Oncol. 2009;27(21):3423-3429.

[65] Flouriot G, Brand H, Denger S, et al. Identification of a new isoform of the human estrogen receptor-alpha (hER-alpha) that is encoded by distinct transcripts and that is able to repress hER-alpha activation function 1. EMBO J. 2000;19(17):4688-4700.

[66] Klinge CM, Riggs KA, Wickramasinghe NS, et al. Estrogen receptor alpha 46 is reduced in tamoxifen resistant breast cancer cells and re-expression inhibits cell proliferation and estrogen receptor alpha 66-regulated target gene transcription. Mol Cell Endocrinol. 2010;323(2):268-276.

[67] Zhao C, Dahlman-Wright K, Gustafsson JA. Estrogen receptor beta: an overview and update. Nucl Recept Signal. 2008;6:e003. Published 2008 Feb 1.

[68] Swedenborg E, Power KA, Cai W, Pongratz I, Rüegg J. Regulation of estrogen receptor beta activity and implications in health and disease. Cell Mol Life Sci. 2009;66(24):3873-3894.

[69] Green CA, Peter MB, Speirs V, Shaaban AM. The potential role of ER beta isoforms in the clinical management of breast cancer. Histopathology. 2008;53(4):374-380.

[70] Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling [published correction appears in Proc Natl Acad Sci U S A. 2006 Oct 3;103(40):14977]. Proc Natl Acad Sci U S A. 2006;103(35):13162-13167.

[71] Moore JT, McKee DD, Slentz-Kesler K, et al. Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun. 1998;247(1):75-78.

[72] Toran-Allerand CD. Estrogen and the brain: beyond ER-alpha, ER-beta, and 17beta-estradiol. Ann N Y Acad Sci. 2005;1052:136-144.

[73] Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol. 2019;116:135-170.

[74] Légaré S, Basik M. Minireview: The Link Between ERα Corepressors and Histone Deacetylases in Tamoxifen Resistance in Breast Cancer. Mol Endocrinol. 2016;30(9):965-976.

[75] Li Y, Lambert MH, Xu HE. Activation of nuclear receptors: a perspective from structural genomics. Structure. 2003;11(7):741-746.

[76] Bulynko YA, O'Malley BW. Nuclear receptor coactivators: structural and functional biochemistry. Biochemistry. 2011;50(3):313-328.

[77] Acevedo ML, Kraus WL. Transcriptional activation by nuclear receptors. Essays Biochem. 2004;40:73-88.

[78] Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. J Biol Chem. 2001;276(40):36869-36872.

[79] Jozwik KM, Carroll JS. Pioneer factors in hormone-dependent cancers. Nat Rev Cancer. 2012;12(6):381-385. Published 2012 May 4.

[80] Bourdeau V, Deschênes J, Métivier R, et al. Genome-wide identification of high-affinity estrogen response elements in human and mouse. Mol Endocrinol. 2004;18(6):1411-1427.

[81] Sausville E, Carney D, Battey J. The human vasopressin gene is linked to the oxytocin gene and is selectively expressed in a cultured lung cancer cell line. J Biol Chem. 1985;260(18):10236-10241.

[82] Yaşar P, Ayaz G, User SD, Güpür G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. Reprod Med Biol. 2016;16(1):4-20. Published 2016 Dec 5.

[83] Vrtačnik P, Ostanek B, Mencej-Bedrač S, Marc J. The many faces of estrogen signaling. Biochem Med (Zagreb). 2014;24(3):329-342. Published 2014 Oct 15.

[84] Porter W, Saville B, Hoivik D, Safe S. Functional synergy between the transcription factor Sp1 and the estrogen receptor. Mol Endocrinol. 1997;11(11):1569-1580.

[85] Marino M, Galluzzo P, Ascenzi P. Estrogen signaling multiple pathways to impact gene transcription. Curr Genomics. 2006;7(8):497-508.

[86] O'Lone R, Frith MC, Karlsson EK, Hansen U. Genomic targets of nuclear estrogen receptors. Mol Endocrinol. 2004;18(8):1859-1875.

[87] Paech K, Webb P, Kuiper GG, et al. Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. Science. 1997;277(5331):1508-1510.

[88] Galien R, Garcia T. Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the NF-kappaB site. Nucleic Acids Res. 1997;25(12):2424-2429.

[89] Lösel R, Wehling M. Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol. 2003;4(1):46-56.

[90] Marino M, Pallottini V, Trentalance A. Estrogens cause rapid activation of IP3-PKC-alpha signal transduction pathway in HEPG2 cells. Biochem Biophys Res Commun. 1998;245(1):254-258.

[91] Dos Santos EG, Dieudonne MN, Pecquery R, Le Moal V, Giudicelli Y, Lacasa D. Rapid nongenomic E2 effects on p42/p44 MAPK, activator protein-1, and cAMP response element binding protein in rat white adipocytes. Endocrinology. 2002;143(3):930-940.

[92] Watters JJ, Campbell JS, Cunningham MJ, Krebs EG, Dorsa DM. Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signalling cascade and c-fos immediate early gene transcription. Endocrinology. 1997;138(9):4030-4033.

[93] Marino M, Acconcia F, Trentalance A. Biphasic estradiol-induced AKT phosphorylation is modulated by PTEN via MAP kinase in HepG2 cells. Mol Biol Cell. 2003;14(6):2583-2591.

[94] Gu Q, Moss RL. 17 beta-Estradiol potentiates kainate-induced currents via activation of the cAMP cascade. J Neurosci. 1996;16(11):3620-3629.

[95] Picotto G, Massheimer V, Boland R. Acute stimulation of intestinal cell calcium influx induced by 17 beta-estradiol via the cAMP messenger system. Mol Cell Endocrinol. 1996;119(2):129-134.

[96] Cavalcanti FN, Lucas TF, Lazari MF, Porto CS. Estrogen receptor ESR1 mediates activation of ERK1/2, CREB, and ELK1 in the corpus of the epididymis. J Mol Endocrinol. 2015;54(3):339-349.

[97] Fox EM, Andrade J, Shupnik MA. Novel actions of estrogen to promote proliferation: integration of cytoplasmic and nuclear pathways. Steroids. 2009;74(7):622-627.

[98] Furth PA. STAT signaling in different breast cancer sub-types. Mol Cell Endocrinol. 2014;382(1):612-615.

[99] Laliotis A, Vrekoussis T, Kafousi M, et al. Immunohistochemical study of pElk-1 expression in human breast cancer: association with breast cancer biologic profile and clinicopathologic features. Breast. 2013;22(1):89-95.

[100] Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature. 1999;401(6748):82-85.

[101] Romashkova JA, Makarov SS. NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature. 1999;401(6748):86-90.

[102] Kato S, Endoh H, Masuhiro Y, et al. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science. 1995;270(5241):1491-1494.

[103] Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol. 2005;19(4):833-842.

[104] Nilsson S, Mäkelä S, Treuter E, et al. Mechanisms of estrogen action. Physiol Rev. 2001;81(4):1535-1565. doi:10.1152/physrev.2001.81.4.1535

[105] Heldring N, Pike A, Andersson S, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev. 2007;87(3):905-931.

[106] Hurowitz EH, Melnyk JM, Chen YJ, Kouros-Mehr H, Simon MI, Shizuya H. Genomic characterization of the human heterotrimeric G protein alpha, beta, and gamma subunit genes. DNA Res. 2000;7(2):111-120.

[107] Filardo EJ, Quinn JA, Bland KI, Frackelton AR Jr. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via

trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol Endocrinol. 2000;14(10):1649-1660.

[108] Zekas E, Prossnitz ER. Estrogen-mediated inactivation of FOXO3a by the G protein-coupled estrogen receptor GPER. BMC Cancer. 2015;15:702. Published 2015 Oct 15.

[109] Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science. 2005;307(5715):1625-1630.

[110] Tica AA, Dun EC, Tica OS, et al. G protein-coupled estrogen receptor 1-mediated effects in the rat myometrium. Am J Physiol Cell Physiol. 2011;301(5):C1262-C1269.

[111] Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Mol Endocrinol. 2002;16(1):70-84.

[112] Chen ZJ, Wei W, Jiang GM, et al. Activation of GPER suppresses epithelial mesenchymal transition of triple negative breast cancer cells via NF-κB signals. Mol Oncol. 2016;10(6):775-788.

[113] Zhou X, Wang S, Wang Z, et al. Estrogen regulates Hippo signaling via GPER in breast cancer. J Clin Invest. 2015;125(5):2123-2135.

[114] Tao S, He H, Chen Q. Estradiol induces HOTAIR levels via GPER-mediated miR-148a inhibition in breast cancer. J Transl Med. 2015;13:131. Published 2015 Apr 25.

[115] Girgert R, Emons G, Gründker C. Estrogen Signaling in ERα-Negative Breast Cancer: ERβ and GPER. Front Endocrinol (Lausanne). 2019;9:781. Published 2019 Jan 9.

[116] Beatson GT. On the Treatment of Inoperable Cases of Carcinoma of the Mamma: Suggestions for a New Method of Treatment, with Illustrative Cases. Trans Med Chir Soc Edinb. 1896;15:153-179.

[117] Huang B, Omoto Y, Iwase H, et al. Differential expression of estrogen receptor α, β1, and β2 in lobular and ductal breast cancer. Proc Natl Acad Sci U S A. 2014;111(5):1933-1938.

[118] Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? [published correction appears in Endocr Rev 1999 Aug;20(4):459]. Endocr Rev. 1999;20(3):358-417.

[119] Anbalagan M, Rowan BG. Estrogen receptor alpha phosphorylation and its functional impact in human breast cancer. Mol Cell Endocrinol. 2015;418 Pt 3:264-272.

[120] Dao KL, Hanson RN. Targeting the estrogen receptor using steroid-therapeutic drug conjugates (hybrids). Bioconjug Chem. 2012;23(11):2139-2158.

[121] Penot G, Le Péron C, Mérot Y, et al. The human estrogen receptor-alpha isoform hERalpha46 antagonizes the proliferative influence of hERalpha66 in MCF7 breast cancer cells. Endocrinology. 2005;146(12):5474-5484.

[122] Zhang X, Wang ZY. Estrogen receptor-α variant, ER-α36, is involved in tamoxifen resistance and estrogen hypersensitivity. Endocrinology. 2013;154(6):1990-1998.

[123] Wang Q, Jiang J, Ying G, et al. Tamoxifen enhances stemness and promotes metastasis of ERα36+breast cancer by upregulating ALDH1A1 in cancer cells. Cell Res. 2018;28(3):336-358.

[124] Haldosén LA, Zhao C, Dahlman-Wright K. Estrogen receptor beta in breast cancer. Mol Cell Endocrinol. 2014;382(1):665-672.

[125] Zhao C, Lam EW, Sunters A, et al. Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation. Oncogene. 2003;22(48):7600-7606.

[126] Markey GC, Cullen R, Diggin P, et al. Estrogen receptor-beta mRNA is associated with adverse outcome in patients with breast cancer. Tumour Biol. 2009;30(4):171-175.

[127] Speirs V, Malone C, Walton DS, Kerin MJ, Atkin SL. Increased expression of estrogen receptor beta mRNA in tamoxifen-resistant breast cancer patients. Cancer Res. 1999;59(21):5421-5424.

[128] Cotrim CZ, Fabris V, Doria ML, et al. Estrogen receptor beta growth-inhibitory effects are repressed through activation of MAPK and PI3K signalling in mammary epithelial and breast cancer cells. Oncogene. 2013;32(19):2390-2402.

[129] Treeck O, Juhasz-Boess I, Lattrich C, Horn F, Goerse R, Ortmann O. Effects of exon-deleted estrogen receptor beta transcript variants on growth, apoptosis and gene expression of human breast cancer cell lines. Breast Cancer Res Treat. 2008;110(3):507-520.

[130] Omoto Y, Inoue S, Ogawa S, et al. Clinical value of the wild-type estrogen receptor beta expression in breast cancer. Cancer Lett. 2001;163(2):207-212.

[131] Hopp TA, Weiss HL, Parra IS, Cui Y, Osborne CK, Fuqua SA. Low levels of estrogen receptor beta protein predict resistance to tamoxifen therapy in breast cancer. Clin Cancer Res. 2004;10(22):7490-7499.

[132] Sotoca AM, van den Berg H, Vervoort J, et al. Influence of cellular ERalpha/ERbeta ratio on the ERalpha-agonist induced proliferation of human T47D breast cancer cells. Toxicol Sci. 2008;105(2):303-311.

[133] Zhao C, Matthews J, Tujague M, et al. Estrogen receptor beta2 negatively regulates the transactivation of estrogen receptor alpha in human breast cancer cells. Cancer Res. 2007;67(8):3955-3962.

[134] Davies MP, O'Neill PA, Innes H, et al. Correlation of mRNA for estrogen receptor beta splice variants ERbeta1, ERbeta2/ERbetacx and ERbeta5 with outcome in endocrine-treated breast cancer. J Mol Endocrinol. 2004;33(3):773-782.

[135] Shaaban AM, Green AR, Karthik S, et al. Nuclear and cytoplasmic expression of ERbeta1, ERbeta2, and ERbeta5 identifies distinct prognostic outcome for breast cancer patients. Clin Cancer Res. 2008;14(16):5228-5235.

[136] Lee MT, Ho SM, Tarapore P, Chung I, Leung YK. Estrogen receptor β isoform 5 confers sensitivity of breast cancer cell lines to chemotherapeutic agent-induced apoptosis through interaction with Bcl2L12. Neoplasia. 2013;15(11):1262-1271.

[137] Thomas C, Rajapaksa G, Nikolos F, et al. ERbeta1 represses basal breast cancer epithelial to mesenchymal transition by destabilizing EGFR. Breast Cancer Res. 2012;14(6):R148. Published 2012 Nov 16.

[138] Scaling AL, Prossnitz ER, Hathaway HJ. GPER mediates estrogen-induced signaling and proliferation in human breast epithelial cells and normal and malignant breast. Horm Cancer. 2014;5(3):146-160.

[139] Lappano R, Pisano A, Maggiolini M. GPER Function in Breast Cancer: An Overview. Front Endocrinol (Lausanne). 2014;5:66. Published 2014 May 6.

[140] Girgert R, Emons G, Gründker C. Inactivation of GPR30 reduces growth of triple-negative breast cancer cells: possible application in targeted therapy. Breast Cancer Res Treat. 2012;134(1):199-205.

[141] Filardo EJ, Graeber CT, Quinn JA, et al. Distribution of GPR30, a seven membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. Clin Cancer Res. 2006;12(21):6359-6366.

[142] Liu Y, Du FY, Chen W, Fu PF, Yao MY, Zheng SS. G15 sensitizes epithelial breast cancer cells to doxorubicin by preventing epithelial-mesenchymal transition through inhibition of GPR30. Am J Transl Res. 2015;7(5):967-975. Published 2015 May 15.

[143] Ignatov A, Ignatov T, Weissenborn C, et al. G-protein-coupled estrogen receptor GPR30 and tamoxifen resistance in breast cancer. Breast Cancer Res Treat. 2011;128(2):457-466.

[144] Ignatov A, Ignatov T, Roessner A, Costa SD, Kalinski T. Role of GPR30 in the mechanisms of tamoxifen resistance in breast cancer MCF-7 cells. Breast Cancer Res Treat. 2010;123(1):87-96.

[145] Sathya S, Sudhagar S, Lakshmi BS. Estrogen suppresses breast cancer proliferation through GPER / p38 MAPK axis during hypoxia. Mol Cell Endocrinol. 2015;417:200-210.

[146] Wei W, Chen ZJ, Zhang KS, et al. The activation of G protein-coupled receptor 30 (GPR30) inhibits proliferation of estrogen receptor-negative breast cancer cells in vitro and in vivo. Cell Death Dis. 2014;5(10):e1428. Published 2014 Oct 2.

[147] Liang S, Chen Z, Jiang G, et al. Activation of GPER suppresses migration and angiogenesis of triple negative breast cancer via inhibition of NF-κB/IL-6 signals [published correction appears in Cancer Lett. 2017 Nov 15;:] [published correction appears in Cancer Lett. 2021 Feb 1;498:251-252]. Cancer Lett. 2017;386:12-23.

[148] Dai X, Xiang L, Li T, Bai Z. Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes. J Cancer. 2016;7(10):1281-1294. Published 2016 Jun 23.

[149] Pagliuca M, Donato M, D'Amato AL, et al. New steps on an old path: Novel estrogen receptor inhibitors in breast cancer. Crit Rev Oncol Hematol. 2022;180:103861.

[150] Patel R, Klein P, Tiersten A, Sparano JA. An emerging generation of endocrine therapies in breast cancer: a clinical perspective. NPJ Breast Cancer. 2023;9(1):20. Published 2023 Apr 5.

[151] Shiau AK, Barstad D, Loria PM, et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell. 1998;95(7):927-937.

[152] Jordan VC. Selective estrogen receptor modulation: concept and consequences in cancer. Cancer Cell. 2004;5(3):207-213.

[153] Hasmann M, Rattel B, Löser R. Preclinical data for Droloxifene. Cancer Lett. 1994;84(2):101-116.

[154] McCague R, Parr IB, Haynes BP. Metabolism of the 4-iodo derivative of tamoxifen by isolated rat hepatocytes. Demonstration that the iodine atom reduces metabolic conversion and identification of four metabolites. Biochem Pharmacol. 1990;40(10):2277-2283.

[155] Kallio S, Kangas L, Blanco G, et al. A new triphenylethylene compound, Fc-1157a. I. Hormonal effects. Cancer Chemother Pharmacol. 1986;17(2):103-108.

[156] Haddow A, Watkinson JM, Paterson E, Koller PC. Influence of Synthetic estrogens on Advanced Malignant Disease. Br Med J. 1944;2(4368):393-398.

[157] Carter AC, Sedransk N, Kelley RM, et al. Diethylstilbestrol: recommended dosages for different categories of breast cancer patients. Report of the Cooperative Breast Cancer Group. JAMA. 1977;237(19).

[158] Wittes JT, Kaufman RJ, Wittes RE. Diethylstilbestrol in breast cancer: dose-response analysis. JAMA. 1977;238(13):1362-1363.

[159] Cole MP, Jones CT, Todd ID. A new anti-estrogenic agent in late breast cancer. An early clinical appraisal of ICI46474. Br J Cancer. 1971;25(2):270-275.

[160] Kurl RN, Borthwick NM. Clomiphene and tamoxifen action in the rat uterus. J Endocrinol. 1980;85(3):519-524.

[161] Lippman ME, Bolan G. Oestrogen-responsive human breast cancer in long term tissue culture. Nature. 1975;256(5518):592-593.

[162] Johnson MD, Zuo H, Lee KH, et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. Breast Cancer Res Treat. 2004;85(2):151-159.

[163] Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. J Natl Cancer Inst. 1994;86(7):527-537.

[164] Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst. 1998;90(18):1371-1388.

[165] Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. J Natl Cancer Inst. 2005;97(22):1652-1662.

[166] Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst. 2007;99(4):272-282.

[167] Powles TJ, Ashley S, Tidy A, Smith IE, Dowsett M. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. J Natl Cancer Inst. 2007;99(4):283-290.

[168] Veronesi U, Maisonneuve P, Rotmensz N, et al. Tamoxifen for the prevention of breast cancer: late results of the Italian Randomized Tamoxifen Prevention Trial among women with hysterectomy. J Natl Cancer Inst. 2007;99(9):727-737.

[169] Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet. 2011;378(9793):771-784.

[170] Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial [published correction appears in Lancet. 2013 Mar 9;381(9869):804] [published correction appears in Lancet. 2017 May 13;389(10082):1884]. Lancet. 2013;381(9869):805-816.

[171] Staley H, McCallum I, Bruce J. Postoperative Tamoxifen for ductal carcinoma in situ: Cochrane systematic review and meta-analysis. Breast. 2014;23(5):546-551.

[172] Cuzick J, Sestak I, Cawthorn S, et al. Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial. Lancet Oncol. 2015;16(1):67-75.

[173] Buzdar A, Hayes D, El-Khoudary A, et al. Phase III randomized trial of droloxifene and tamoxifen as first-line endocrine treatment of ER/PgR-positive advanced breast cancer. Breast Cancer Res Treat. 2002;73(2):161-175.

[174] Arpino G, Nair Krishnan M, Doval Dinesh C, Bardou VJ, Clark GM, Elledge RM. Idoxifene versus tamoxifen: a randomized comparison in postmenopausal patients with metastatic breast cancer. Ann Oncol. 2003;14(2):233-241.

[175] Gershanovich M, Garin A, Baltina D, Kurvet A, Kangas L, Ellmén J. A phase III comparison of two toremifene doses to tamoxifen in postmenopausal women with advanced breast cancer. Eastern European Study Group. Breast Cancer Res Treat. 1997;45(3):251-262.

[176] Pyrhönen S, Valavaara R, Modig H, et al. Comparison of toremifene and tamoxifen in post-menopausal patients with advanced breast cancer: a randomized double-blind, the 'nordic' phase III study. Br J Cancer. 1997;76(2):270-277.

[177] Zhou WB, Ding Q, Chen L, Liu XA, Wang S. Toremifene is an effective and safe alternative to tamoxifen in adjuvant endocrine therapy for breast cancer: results of four randomized trials. Breast Cancer Res Treat. 2011;128(3):625-631.

[178] Mao C, Yang ZY, He BF, et al. Toremifene versus tamoxifen for advanced breast cancer. Cochrane Database Syst Rev. 2012;2012(7):CD008926. Published 2012 Jul 11.

[179] Ye QL, Zhai ZM. Toremifene and tamoxifen have similar efficacy in the treatment of patients with breast cancer: a meta-analysis of randomized trials. Mol Biol Rep. 2014;41(2):751-756.

[180] Gottardis MM, Jordan VC. Antitumor actions of keoxifene and tamoxifen in the N-nitrosomethylurea-induced rat mammary carcinoma model. Cancer Res. 1987;47(15):4020-4024.

[181] Dayan G, Lupien M, Auger A, et al. Tamoxifen and raloxifene differ in their functional interactions with aspartate 351 of estrogen receptor alpha. Mol Pharmacol. 2006;70(2):579-588.

[182] DeMichele A, Troxel AB, Berlin JA, et al. Impact of raloxifene or tamoxifen use on endometrial cancer risk: a population-based case-control study. J Clin Oncol. 2008;26(25):4151-4159.

[183] Buzdar AU, Marcus C, Holmes F, Hug V, Hortobagyi G. Phase II evaluation of Ly156758 in metastatic breast cancer. Oncology. 1988;45(5):344-345.

[184] Gradishar W, Glusman J, Lu Y, Vogel C, Cohen FJ, Sledge GW Jr. Effects of high dose raloxifene in selected patients with advanced breast carcinoma. Cancer. 2000;88(9):2047-2053.

[185] Martino S, Cauley JA, Barrett-Connor E, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst. 2004;96(23):1751-1761.

[186] Lippman ME, Cummings SR, Disch DP, et al. Effect of raloxifene on the incidence of invasive breast cancer in postmenopausal women with osteoporosis categorized by breast cancer risk. Clin Cancer Res. 2006;12(17):5242-5247.

[187] Grady D, Cauley JA, Geiger MJ, et al. Reduced incidence of invasive breast cancer with raloxifene among women at increased coronary risk. J Natl Cancer Inst. 2008;100(12):854-861.

[188] Vogel VG, Costantino JP, Wickerham DL, et al. Update of the National Surgical Adjuvant Breast and Bowel Project Study of Tamoxifen and Raloxifene (STAR) P-2 Trial: Preventing breast cancer. Cancer Prev Res (Phila). 2010;3(6):696-706.

[189] Palkowitz AD, Glasebrook AL, Thrasher KJ, et al. Discovery and synthesis of [6-hydroxy-3-[4-[2-(1-piperidinyl)ethoxy]phenoxy]-2-(4-hydroxyphenyl)]b
enzo[b]thiophene: a novel, highly potent, selective estrogen receptor modulator. J Med Chem. 1997;40(10):1407-1416.

[190] Suh N, Glasebrook AL, Palkowitz AD, et al. Arzoxifene, a new selective estrogen receptor modulator for chemoprevention of experimental breast cancer. Cancer Res. 2001;61(23):8412-8415.

[191] Deshmane V, Krishnamurthy S, Melemed AS, Peterson P, Buzdar AU. Phase III double-blind trial of arzoxifene compared with tamoxifen for locally advanced or metastatic breast cancer. J Clin Oncol. 2007;25(31):4967-4973.

[192] Heuson JC, Engelsman E, Blonk-Van Der Wijst J, et al. Comparative trial of nafoxidine and ethinyloestradiol in advanced breast cancer: an E.O.R.T.C. study. Br Med J. 1975;2(5973):711-713.

[193] Vajdos FF, Hoth LR, Geoghegan KF, et al. The 2.0 A crystal structure of the ERalpha ligand-binding domain complexed with lasofoxifene. Protein Sci. 2007;16(5):897-905.

[194] Cummings SR, Ensrud K, Delmas PD, et al. Lasofoxifene in postmenopausal women with osteoporosis [published correction appears in N Engl J Med. 2011 Jan 20;364(3):290. Kucu kdeveci A [corrected to Kucukdeveci, A]]. N Engl J Med. 2010;362(8):686-696.

[195] Goldstein SR, Neven P, Cummings S, et al. Postmenopausal Evaluation and Risk Reduction With Lasofoxifene (PEARL) trial: 5-year gynecological outcomes. Menopause. 2011;18(1):17-22.

[196] Greenberger LM, Annable T, Collins KI, et al. A new antiestrogen, 2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-piperidin-1-yl-ethoxy)-benzyl]-1H-indol-5-ol hydrochloride (ERA-923), inhibits the growth of tamoxifen-sensitive and -resistant tumors and is devoid of uterotropic effects in mice and rats. Clin Cancer Res. 2001;7(10):3166-3177.

[197] Cotreau MM, Stonis L, Dykstra KH, et al. Multiple-dose, safety, pharmacokinetics, and pharmacodynamics of a new selective estrogen receptor modulator, ERA-923, in healthy postmenopausal women. J Clin Pharmacol. 2002;42(2):157-165.

[198] NCT00006369. (n.d.) ERA-923 in treating postmenopausal women with metastatic breast cancer. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT00006369

[199] Simard J, Labrie C, Bélanger A, et al. Characterization of the effects of the novel non-steroidal antiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells in vitro. Int J Cancer. 1997;73(1):104-112.

[200] Labrie F, Champagne P, Labrie C, et al. Activity and safety of the antiestrogen EM-800, the orally active precursor of acolbifene, in tamoxifen-resistant breast cancer. J Clin Oncol. 2004;22(5):864-871.

[201] Fabian CJ, Kimler BF, Zalles CM, et al. Clinical Trial of Acolbifene in Premenopausal Women at High Risk for Breast Cancer. Cancer Prev Res (Phila). 2015;8(12):1146-1155.

[202] Skrumsager BK, Kiehr B, Pedersen PC, Gerrits M, Watson N, Bjarnason K. Levormeloxifene: safety, pharmacodynamics and pharmacokinetics in healthy postmenopausal women following single and multiple doses of a new selective oestrogen receptor modulator. Br J Clin Pharmacol. 2002;53(3):284-295.

[203] Osborne CK, Zhao H, Fuqua SA. Selective estrogen receptor modulators: structure, function, and clinical use. J Clin Oncol. 2000;18(17):3172-3186.

[204] Draper MW. SERMs: how do they work?. Eur J Cancer. 2002;38 Suppl 6:S35. doi:10.1016/s0959-8049(02)00278-2

[205] Jefferson WN, Padilla-Banks E, Clark G, Newbold RR. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;777(1-2):179-189.

[206] Casanova M, You L, Gaido KW, Archibeque-Engle S, Janszen DB, Heck HA. Developmental effects of dietary phytoestrogens in Sprague-Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta in vitro. Toxicol Sci. 1999;51(2):236-244.

[207] McDonnell DP, Wardell SE. The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. Curr Opin Pharmacol. 2010;10(6):620-628.

[208] Wakeling AE, Bowler J. Steroidal pure antioestrogens. J Endocrinol. 1987;112(3):R7-R10.

[209] Bowler J, Lilley TJ, Pittam JD, Wakeling AE. Novel steroidal pure antiestrogens. Steroids. 1989;54(1):71-99.

[210] Traboulsi T, El Ezzy M, Gleason JL, Mader S. Antiestrogens: structure-activity relationships and use in breast cancer treatment. J Mol Endocrinol. 2017;58(1):R15-R31.

[211] Chen D, Pace PE, Coombes RC, Ali S. Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization. Mol Cell Biol. 1999;19(2):1002-1015.

[212] Wijayaratne AL, McDonnell DP. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem. 2001;276(38):35684-35692.

[213] Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. Cancer Res. 1991;51(15):3867-3873.

[214] Hu XF, Veroni M, De Luise M, et al. Circumvention of tamoxifen resistance by the pure anti-estrogen ICI 182,780. Int J Cancer. 1993;55(5):873-876.

[215] Robertson JF, Lindemann J, Garnett S, et al. A good drug made better: the fulvestrant dose-response story. Clin Breast Cancer. 2014;14(6):381-389.

[216] Strott CA. Sulfonation and molecular action. Endocr Rev. 2002;23(5):703-732.

[217] Aka JA, Zerradi M, Houle F, Huot J, Lin SX. 17beta-hydroxysteroid dehydrogenase type 1 modulates breast cancer protein profile and impacts cell migration. Breast Cancer Res. 2012;14(3):R92. Published 2012 Jun 12.

[218] James MR, Skaar TC, Lee RY, et al. Constitutive expression of the steroid sulfatase gene supports the growth of MCF-7 human breast cancer cells in vitro and in vivo. Endocrinology. 2001;142(4):1497-1505.

[219] Howarth NM, Purohit A, Reed MJ, Potter BV. Estrone sulfamates: potent inhibitors of estrone sulfatase with therapeutic potential. J Med Chem. 1994;37(2):219-221.

[220] Fernandez SV, Russo IH, Russo J. Estradiol and its metabolites 4-hydroxyestradiol and 2-hydroxyestradiol induce mutations in human breast epithelial cells. Int J Cancer. 2006;118(8):1862-1868.

[221] Santen RJ, Yue W, Naftolin F, Mor G, Berstein L. The potential of aromatase inhibitors in breast cancer prevention. Endocr Relat Cancer. 1999;6(2):235-243.

[222] Fabian CJ. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. Int J Clin Pract. 2007;61(12):2051-2063.

[223] Recanatini M, Cavalli A, Valenti P. Nonsteroidal aromatase inhibitors: recent advances. Med Res Rev. 2002;22(3):282-304.

[224] Ahmad I, Shagufta. Recent developments in steroidal and nonsteroidal aromatase inhibitors for the chemoprevention of estrogen-dependent breast cancer. Eur J Med Chem. 2015;102:375-386.

[225] Brueggemeier RW. Aromatase, aromatase inhibitors, and breast cancer. Am J Ther. 2001;8(5):333-344.

[226] Lin NU, Winer EP. Advances in adjuvant endocrine therapy for postmenopausal women. J Clin Oncol. 2008;26(5):798-805.

[227] Augereau P, Patsouris A, Bourbouloux E, et al. Hormonoresistance in advanced breast cancer: a new revolution in endocrine therapy. Ther Adv Med Oncol. 2017;9(5):335-346.

[228] Hanker AB, Sudhan DR, Arteaga CL. Overcoming Endocrine Resistance in Breast Cancer. Cancer Cell. 2020;37(4):496-513.

[229] Gutierrez MC, Detre S, Johnston S, et al. Molecular changes in tamoxifen-resistant breast cancer: relationship between estrogen receptor, HER-2, and p38 mitogen-activated protein kinase. J Clin Oncol. 2005;23(11):2469-2476.

[230] Yang X, Ferguson AT, Nass SJ, et al. Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition. Cancer Res. 2000;60(24):6890-6894.

[231] Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. Cancer Res. 1994;54(10):2552-2555.

[232] Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol. 2015;12(10):573-583.

[233] Toy W, Weir H, Razavi P, et al. Activating ESR1Mutations Differentially Affect the Efficacy of ER Antagonists. Cancer Discov. 2017;7(3):277-287.

[234] Zhang QX, Borg A, Wolf DM, Oesterreich S, Fuqua SA. An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. Cancer Res. 1997;57(7):1244-1249.

[235] Robinson DR, Wu YM, Vats P, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. Nat Genet. 2013;45(12):1446-1451.

[236] Li S, Shen D, Shao J, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 2013;4(6):1116-1130.

[237] Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. Nat Genet. 2013;45(12):1439-1445.

[238] Katzenellenbogen JA, Mayne CG, Katzenellenbogen BS, Greene GL, Chandarlapaty S. Structural underpinnings of oestrogen receptor mutations in endocrine therapy resistance [published correction appears in Nat Rev Cancer. 2018 Oct;18(10):662]. Nat Rev Cancer. 2018;18(6):377-388.

[239] Jeselsohn R, Bergholz JS, Pun M, et al. Allele-Specific Chromatin Recruitment and Therapeutic Vulnerabilities of ESR1 Activating Mutations. Cancer Cell. 2018;33(2):173-186.e5.

[240] Szostakowska M, Trębińska-Stryjewska A, Grzybowska EA, Fabisiewicz A. Resistance to endocrine therapy in breast cancer: molecular mechanisms and future goals. Breast Cancer Res Treat. 2019;173(3):489-497.

[241] Lei JT, Gou X, Seker S, Ellis MJ. ESR1 alterations and metastasis in estrogen receptor positive breast cancer. J Cancer Metastasis Treat. 2019;5:38.

[242] Andreano KJ, Baker JG, Park S, et al. The Dysregulated Pharmacology of Clinically Relevant ESR1 Mutants is Normalized by Ligand-activated WT Receptor. Mol Cancer Ther. 2020;19(7):1395-1405.

[243] Lainé M, Fanning SW, Chang YF, et al. Lasofoxifene as a potential treatment for therapy-resistant ER-positive metastatic breast cancer. Breast Cancer Res. 2021;23(1):54. Published 2021 May 12.

[244] Goetz, M. P., Plourde, P., Stover, D. G., Bagegni, N., Vidal, G. A., Brufsky, A., ... & Gal-Yam, E. (2022). LBA20 Open-label, randomized study of lasofoxifene (LAS) vs fulvestrant (Fulv) for women with locally advanced/metastatic ER+/HER2-breast cancer (mBC), an estrogen receptor 1 (ESR1) mutation, and disease progression on aromatase (AI) and cyclin-dependent kinase 4/6 (CDK4/6i) inhibitors. Annals of Oncology, 33, S1387-S1388.

[245] Damodaran, S., et al. Open-label, phase 2, multicenter study of lasofoxifene (LAS) combined with abemaciclib (Abema) for treating pre- and postmenopausal women with locally advanced or metastatic ER+/HER2− breast cancer and an ESR1 mutation after progression on prior therapies. Journal of Clinical Oncology 2022 40:16_suppl, 1022-1022.

[246] Goetz MP, Suman VJ, Reid JM, et al. First-in-Human Phase I Study of the Tamoxifen Metabolite Z-Endoxifen in Women With Endocrine-Refractory Metastatic Breast Cancer. J Clin Oncol. 2017;35(30):3391-3400.

[247] Goetz, M.P., et al. Abstract PD7-06: A randomized phase II trial of tamoxifen versus Z-endoxifen HCL in postmenopausal women with metastatic estrogen receptor positive, HER2 negative breast cancer. PD7-PD06 Cancer Res. Am. Assoc. Cancer Res. 2020. 80 (4_Supplement).

[248] Wardell SE, Nelson ER, Chao CA, Alley HM, McDonnell DP. Evaluation of the pharmacological activities of RAD1901, a selective estrogen receptor degrader. Endocr Relat Cancer. 2015;22(5):713-724.

[249] Garner F, Shomali M, Paquin D, Lyttle CR, Hattersley G. RAD1901: a novel, orally bioavailable selective estrogen receptor degrader that demonstrates antitumor activity in breast cancer xenograft models. Anticancer Drugs. 2015;26(9):948-956.

[250] Bihani T, Patel HK, Arlt H, et al. Elacestrant (RAD1901), a Selective Estrogen Receptor Degrader (SERD), Has Antitumor Activity in Multiple ER+ Breast Cancer Patient-derived Xenograft Models. Clin Cancer Res. 2017;23(16):4793-4804.

[251] Conlan MG, de Vries EFJ, Glaudemans A, Wang Y, Troy S. Pharmacokinetic and Pharmacodynamic Studies of Elacestrant, A Novel Oral Selective Estrogen Receptor Degrader, in Healthy Post-Menopausal Women [published correction appears in Eur J Drug Metab Pharmacokinet. 2020 Aug 30;:]. Eur J Drug Metab Pharmacokinet. 2020;45(5):675-689.

[252] Bidard FC, Kaklamani VG, Neven P, et al. Elacestrant (oral selective estrogen receptor degrader) Versus Standard Endocrine Therapy for Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From the Randomized Phase III EMERALD Trial. J Clin Oncol. 2022;40(28):3246-3256.

[253] Vidal, M., et al. Abstract OT2-11-07: Solti-1905. Elacestrant in preoperative setting, a window of opportunity study (ELIPSE trial). *Cancer Res* 15 February 2022; 82 (4_Supplement): OT2–11–07.

[254] Campone, M., et a. Phase I/II study of SAR439859, an oral selective estrogen receptor degrader (SERD), in estrogen receptor-positive (ER+)/human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (mBC). Journal of Clinical Oncology 2020 38:15_suppl, 1070-1070.

[255] El-Ahmad Y, Tabart M, Halley F, et al. Discovery of 6-(2,4-Dichlorophenyl)-5-[4-[(3S)-1-(3-fluoropropyl) pyrrolidin-3-yl]oxyphenyl]-8,9-dihydro-7H-benzo[7]annulene-2-carboxylic acid (SAR439859), a Potent and Selective Estrogen Receptor Degrader (SERD) for the Treatment of Estrogen-Receptor-Positive Breast Cancer. J Med Chem. 2020;63(2):512-528.

[256] Shomali M, Cheng J, Sun F, et al. SAR439859, a Novel Selective Estrogen Receptor Degrader (SERD), Demonstrates Effective and Broad Antitumor Activity in Wild-Type and Mutant ER-Positive Breast Cancer Models. Mol Cancer Ther. 2021;20(2):250-262.

[257] Bardia A, Chandarlapaty S, Linden HM, et al. AMEERA-1 phase 1/2 study of amcenestrant, SAR439859, in postmenopausal women with ER-positive/HER2-negative advanced breast cancer. Nat Commun. 2022;13(1):4116. Published 2022 Jul 15.

[258] Tolaney, S. M., et al. 212MO AMEERA-3, a phase II study of amcenestrant (AMC) versus endocrine treatment of physician's choice (TPC) in patients (pts) with endocrine-resistant ER+/HER2− advanced breast cancer (aBC). Annals of Oncology 33 (2022): S634-S635.

[259] Campone, M., et al. AMEERA-4: A preoperative window-of-opportunity (WOO) study to assess the pharmacodynamic (PD) activity of amcenestrant or letrozole in postmenopausal patients with ER+/HER2− primary breast cancer. Journal of Clinical Oncology 2022 40:16_suppl, 528-528.

[260] Bardia A, Cortes J, Hurvitz SA, et al. AMEERA-5: a randomized, double-blind phase 3 study of amcenestrant plus palbociclib versus letrozole plus palbociclib for previously untreated ER+/HER2- advanced breast cancer. Ther Adv Med Oncol. 2022;14:17588359221083956. Published 2022 Mar 15.

[261] Meyskens, T. et al Adjuvant study of amcenestrant (SAR439859) versus tamoxifen for patients with hormone receptor-positive (HR+) early breast cancer (EBC), who have discontinued adjuvant aromatase inhibitor therapy due to treatment-related toxicity (AMEERA-6). Journal of Clinical Oncology 2022 40:16_suppl, TPS607-TPS607.

[262] Scott JS, Moss TA, Balazs A, et al. Discovery of AZD9833, a Potent and Orally Bioavailable Selective Estrogen Receptor Degrader and Antagonist. J Med Chem. 2020;63(23):14530-14559.

[263] Hamilton, E.P., et al A phase I dose escalation and expansion study of the next generation oral SERD AZD9833 in women with ER-positive, HER2-negative advanced breast cancer. Journal of Clinical Oncology 2020 38:15_suppl, 1024-1024.

[264] Oliveira, M., et al. Abstract OT-09-02: A randomized, open-label, parallel-group, multicenter phase 2 study comparing the efficacy and safety of oral AZD9833 versus fulvestrant in women with advanced ER-positive HER2-negative breast cancer (SERENA-2). Cancer Res 15 February 2021; 81 (4_Supplement): OT–09–02.

[265] Robertson, J.F., et al. Abstract OT-09-05: A randomized, pre-surgical study to investigate the biological effects of AZD9833 doses in women with ER-positive HER2-negative primary breast cancer (SERENA-3). Cancer Res 15 February 2021; 81 (4_Supplement): OT–09–05.

[266] André, F., et al. Abstract OT2-11-06: SERENA-4: A Phase III comparison of AZD9833 (camizestrant) plus palbociclib, versus anastrozole plus palbociclib, for patients with ER-positive/HER2-negative advanced breast cancer who have not previously received systemic treatment for advanced disease. Cancer Res 15 February 2022; 82 (4_Supplement): OT2–11–06.

[267] Bidard, F.-C., Kalinsky, K., et al. Abstract OT2-11-05: SERENA-6: A Phase III study to assess the efficacy and safety of AZD9833 (camizestrant) compared with aromatase inhibitors when given in combination with palbociclib or abemaciclib in patients with HR+/HER2- metastatic breast cancer with detectable ESR1m who have not experienced disease progression on first-line therapy. Cancer Res 15 February 2022; 82 (4_Supplement): OT2–11–05.

[268] Metcalfe, C., et al. Abstract 3406: GDC-9545: A pure antiestrogen clinical candidate that immobilizes the estrogen receptor and profoundly alters chromatin accessibility in vivo. Cancer Res 15 August 2020; 80 (16_Supplement): 3406.

[269] Liang J, Zbieg JR, Blake RA, et al. GDC-9545 (Giredestrant): A Potent and Orally Bioavailable Selective Estrogen Receptor Antagonist and Degrader with an Exceptional Preclinical Profile for ER+ Breast Cancer. J Med Chem. 2021;64(16):11841-11856.

[270] Fasching, P.A., et al. Neoadjuvant giredestrant (GDC-9545) plus palbociclib (P) versus anastrozole (A) plus P in postmenopausal women with estrogen receptor–positive, HER2-negative, untreated early breast cancer (ER+/HER2– eBC): Final analysis of the randomized, open-label, international phase 2 coopERA BC study. Journal of Clinical Oncology 2022 40:16_suppl, 589-589.

[271] Turner, N.C. et al. persevERA Breast Cancer (BC): Phase III study evaluating the efficacy and safety of giredestrant (GDC-9545) + palbociclib versus letrozole + palbociclib in patients (pts) with estrogen-receptor-positive, HER2-negative locally advanced or metastatic BC (ER+/HER2– LA/mBC). Journal of Clinical Oncology 2021 39:15_suppl, TPS1103-TPS1103.

[272] Bardia, A., et al. Abstract OT2-11-09: Lidera breast cancer: A phase III adjuvant study of giredestrant (GDC-9545) vs physician's choice of endocrine therapy (ET) in patients (pts) with estrogen receptor-positive, HER2-negative early breast cancer (ER+/HER2- EBC). Cancer Res 15 February 2022; 82 (4_Supplement): OT2–11–09.

[273] Bhagwat, S.V., et al. Abstract 1236: Preclinical characterization of LY3484356, a novel, potent and orally bioavailable selective estrogen receptor degrader (SERD). Cancer Res 1 July 2021; 81 (13_Supplement): 1236.

[274] Lim, E., et al. Abstract OT-09-03: EMBER: A phase 1a/b trial of LY3484356, a novel, oral selective estrogen-receptor degrader (SERD), in advanced ER+ breast cancer and endometroid endometrial cancer. Cancer Res 15 February 2021; 81 (4_Supplement): OT–09–03.

[275] Jhaveri, K., et al. Abstract OT2-11-01: EMBER-3: A randomized phase 3 study of LY3484356, a novel, oral selective estrogen receptor degrader vs investigator's choice of endocrine therapy of either fulvestrant or exemestane, in patients with estrogen receptor-positive, human epidermal growth factor receptor 2-negative, locally advanced or metastatic breast cancer previously treated with endocrine-based therapy. Cancer Res 15 February 2022; 82 (4_Supplement): OT2–11–01.

[276] Andreano KJ, Wardell SE, Baker JG, et al. G1T48, an oral selective estrogen receptor degrader, and the CDK4/6 inhibitor lerociclib inhibit tumor growth in animal models of endocrine-resistant breast cancer. Breast Cancer Res Treat. 2020;180(3):635-646.

[277] Wardell, S.E., et al. Abstract 5641: Effects of G1T48, a novel orally bioavailable selective estrogen receptor degrader (SERD), and the CDK4/6 inhibitor, G1T38, on tumor growth in animal models of endocrine resistant breast cancer. Cancer Res 1 July 2017; 77 (13_Supplement): 5641.

[278] Aftimos, P., Neven, P., et al. Abstract PS12-04: Rintodestrant (G1T48), an oral selective estrogen receptor degrader in ER+/HER2- locally advanced or metastatic breast cancer: Updated phase 1 results and dose selection. Cancer Res 15 February 2021; 81 (4_Supplement): PS12–04.

[279] Maglakelidze, M. et al. Rintodestrant (G1T48), an oral selective estrogen receptor degrader, in combination with palbociclib for ER+/HER2– advanced breast cancer: Phase 1 results. Journal of Clinical Oncology 2021 39:15_suppl, 1063-1063

[280] Joseph, J. et al. Discovery of GDC-0810 a novel, non-steroidal selective estrogen receptor degrader with robust activity in pre-clinical models of endocrine-resistant breast cancer. Cancer Research, 2015 75:15_suppl, 5053-5053.

[281] Dickler M, Villanueva R, Perez Fidalgo J, et al. Abstract PD5-10: A first-in-human phase I study to evaluate the oral selective estrogen receptor degrader (SERD), GDC-0927, in postmenopausal women with estrogen receptor positive (ER+) HER2-negative metastatic breast cancer (BC). Cancer Res 15 February 2018; 78 (4_Supplement): PD5–10.

[282] Osborne, C., et al. Abstract PS11-26: A phase 1 study of D-0502, an orally bioavailable SERD, for advanced or metastatic HR-positive and HER2-negative breast cancer. Cancer Res 15 February 2021; 81 (4_Supplement): PS11–26.

[283] Hamilton, E.P., et al. Hamilton EP, Patel MR, Armstrong AC, et al. A First-in-Human Study of the New Oral Selective Estrogen Receptor Degrader AZD9496 for ER+/HER2- Advanced Breast Cancer. Clin Cancer Res. 2018;24(15):3510-3518.

[284] Guo S, Zhang C, Bratton M, et al. ZB716, a steroidal selective estrogen receptor degrader (SERD), is orally efficacious in blocking tumor growth in mouse xenograft models. Oncotarget. 2018;9(6):6924-6937. Published 2018 Jan 8.

[285] Kalinksy, K., et al. Abstract P1-17-02: ZN-c5, an oral selective estrogen receptor degrader (SERD), in women with advanced estrogen receptor-positive (ER+)/human epidermal growth factor receptor 2 negative (HER2-) breast cancer. Cancer Res 15 February 2022; 82 (4_Supplement): P1–17–02.

[286] Komm BS, Kharode YP, Bodine PV, Harris HA, Miller CP, Lyttle CR. Bazedoxifene acetate: a selective estrogen receptor modulator with improved selectivity. Endocrinology. 2005;146(9):3999-4008.

[287] Komm BS, Chines AA. Bazedoxifene: the evolving role of third-generation selective estrogen-receptor modulators in the management of postmenopausal osteoporosis. Ther Adv Musculoskelet Dis. 2012;4(1):21-34.

[288] Haines CN, Wardell SE, McDonnell DP. Current and emerging estrogen receptor-targeted therapies for the treatment of breast cancer. Essays Biochem. 2021;65(6):985-1001.

[289] Fanning SW, Jeselsohn R, Dharmarajan V, et al. The SERM/SERD bazedoxifene disrupts ESR1 helix 12 to overcome acquired hormone resistance in breast cancer cells. Elife. 2018;7:e37161. Published 2018 Nov 29.

[290] Fanning SW, Jeselsohn R, Dharmarajan V, et al. The SERM/SERD bazedoxifene disrupts ESR1 helix 12 to overcome acquired hormone resistance in breast cancer cells. Elife. 2018;7:e37161. Published 2018 Nov 29.

[291] Wardell SE, Ellis MJ, Alley HM, et al. Efficacy of SERD/SERM Hybrid-CDK4/6 Inhibitor Combinations in Models of Endocrine Therapy-Resistant Breast Cancer. Clin Cancer Res. 2015;21(22):5121-5130.

[292] Álvarez-Fernández M, Malumbres M. Mechanisms of Sensitivity and Resistance to CDK4/6 Inhibition. Cancer Cell. 2020;37(4):514-529.

[293] Pandey K, An HJ, Kim SK, et al. Molecular mechanisms of resistance to CDK4/6 inhibitors in breast cancer: A review. Int J Cancer. 2019;145(5):1179-1188.

[294] Morrison C. Pfizer's CDK4/6 inhibitor approved for advanced breast cancer. Nat Biotechnol. 2015;33(4):323-324.

[295] Turner NC, Ro J, André F, et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2015;373(3):209-219.

[296] Khatpe AS, Adebayo AK, Herodotou CA, Kumar B, Nakshatri H. Nexus between PI3K/AKT and Estrogen Receptor Signaling in Breast Cancer. Cancers (Basel). 2021;13(3):369. Published 2021 Jan 20.

[297] Nunnery SE, Mayer IA. Targeting the PI3K/AKT/mTOR Pathway in Hormone-Positive Breast Cancer. Drugs. 2020;80(16):1685-1697.

[298] Woo SU, Sangai T, Akcakanat A, Chen H, Wei C, Meric-Bernstam F. Vertical inhibition of the PI3K/Akt/mTOR pathway is synergistic in breast cancer. Oncogenesis. 2017;6(10):e385. Published 2017 Oct 9.

[299] Lousberg L, Jerusalem G. Safety, Efficacy, and Patient Acceptability of Everolimus in the Treatment of Breast Cancer. Breast Cancer (Auckl). 2017;10:239-252. Published 2017 Jan 4.

[300] Ndubaku CO, Heffron TP, Staben ST, et al. Discovery of 2-{3-[2-(1-isopropyl-3-methyl-1H-1,2-4-triazol-5-yl)-5,6-dihydrobenzo[f]imidazo[1,2-d][1,4]oxazepin-9-yl]-1H-pyrazol-1-yl}-2-methylpropanamide (GDC-0032): a β-sparing phosphoinositide 3-kinase inhibitor with high unbound exposure and robust in vivo antitumor activity. J Med Chem. 2013;56(11):4597-4610.

[301] André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med. 2019;380(20):1929-1940.

[302] Baselga J, Im SA, Iwata H, et al. Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial [published correction appears in Lancet Oncol. 2019 Feb;20(2):e71-e72]. Lancet Oncol. 2017;18(7):904-916.

[303] Krop IE, Mayer IA, Ganju V, et al. Pictilisib for oestrogen receptor-positive, aromatase inhibitor-resistant, advanced or metastatic breast cancer (FERGI): a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol. 2016;17(6):811-821.

[304] Qi SM, Dong J, Xu ZY, Cheng XD, Zhang WD, Qin JJ. PROTAC: An Effective Targeted Protein Degradation Strategy for Cancer Therapy. Front Pharmacol. 2021 May 7;12:692574.

[305] Snyder, L.B., et al. Abstract 44: The discovery of ARV-471, an orally bioavailable estrogen receptor degrading PROTAC for the treatment of patients with breast cancer. Cancer Res 1 July 2021; 81 (13_Supplement): 44.

[306] Hamilton, E.P., et al. ARV-471, an estrogen receptor (ER) PROTAC degrader, combined with palbociclib in advanced ER+/human epidermal growth factor receptor 2–negative (HER2-) breast cancer: Phase 1b cohort (part C) of a phase 1/2 study.Journal of Clinical Oncology 2022 40:16_suppl, TPS1120-TPS1120.

[307] Alabi S. Novel Mechanisms of Molecular Glue-Induced Protein Degradation. Biochemistry. 2021;60(31):2371-2373.

[308] Nagel YA, Britschgi A, Ricci A. From degraders to molecular glues: new ways of breaking down disease associated proteins. Successful Drug Discovery. 2021 Feb 26;47–85.

[309] Banik SM, Pedram K, Wisnovsky S, Ahn G, Riley NM, Bertozzi CR. Lysosome-targeting chimaeras for degradation of extracellular proteins. Nature. 2020;584(7820):291-297.

[310] https://www.biocentury.com/article/638352

[311] Puyang X, Furman C, Zheng GZ, et al. Discovery of Selective Estrogen Receptor Covalent Antagonists for the Treatment of ERαWT and ERαMUT Breast Cancer. Cancer Discov. 2018;8(9):1176-1193.

[312] Reese JC, Katzenellenbogen BS. Mutagenesis of cysteines in the hormone binding domain of the human estrogen receptor. Alterations in binding and transcriptional activation by covalently and reversibly attaching ligands. J Biol Chem. 1991;266(17):10880-10887.

[313] Harlow KW, Smith DN, Katzenellenbogen JA, Greene GL, Katzenellenbogen BS. Identification of cysteine 530 as the covalent attachment site of an affinity-labeling estrogen (ketononestrol aziridine) and antiestrogen (tamoxifen aziridine) in the human estrogen receptor. J Biol Chem. 1989;264(29):17476-17485.

[314] Hamilton, E.P., et al. Phase I/II study of H3B-6545, a novel selective estrogen receptor covalent antagonist (SERCA), in estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-) advanced breast cancer. Journal of Clinical Oncology 2021 39:15_suppl, 1018-1018

[315] Hodges-Gallagher, L., Sun, R., Myles, D. C., Harmon, C. L. & Kushner, P. J. Abstract P5-05-02: Preclinical development of OP-1250, an oral complete estrogen receptor antagonist (CERAN) that shrinks ER-positive breast tumors in xenograft models. Cancer Res15 February 2020; 80 (4_Supplement): P5–05–02.

[316] Hodges-Gallagher, L. et al. Abstract PS18-16: The complete estrogen receptor antagonist OP-1250 shrinks tumors in xenograft models and has favorable preclinical pharmacokinetic attributes. Cancer Res 15 February 2021; 81 (4_Supplement): PS18–16.

[317] Patel, M. et al. Abstract P1-17-12: Preliminary data from a phase I/II, multicenter, dose escalation study of OP-1250, an oral CERAN/SERD, in subjects with advanced and/or metastatic estrogen receptor (ER)-positive, HER2-negative breast cancer. *Cancer Res* 15 February 2022; 82 (4_Supplement): P1–17–12.

[318] Xiong R, Patel HK, Gutgesell LM, et al. Selective Human Estrogen Receptor Partial Agonists (ShERPAs) for Tamoxifen-Resistant Breast Cancer. J Med Chem. 2016;59(1):219-237.

[319] Dudek AZ, Liu LC, Fischer JH, et al. Phase 1 study of TTC-352 in patients with metastatic breast cancer progressing on endocrine and CDK4/6 inhibitor therapy. Breast Cancer Res Treat. 2020;183(3):617-627.

[320] Yang L, Cao Z, Yan H, Wood WC. Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. Cancer Res. 2003;63(20):6815-6824.

[321] Ma Z, Ji Y, Yu Y, Liang D. Specific non-genetic IAP-based protein erasers (SNIPERs) as a potential therapeutic strategy. Eur J Med Chem. 2021;216:113247.

[322] Demizu Y, Okuhira K, Motoi H, et al. Design and synthesis of estrogen receptor degradation inducer based on a protein knockdown strategy. Bioorg Med Chem Lett. 2012;22(4):1793-1796.

[323] Ohoka N, Morita Y, Nagai K, et al. Derivatization of inhibitor of apoptosis protein (IAP) ligands yields improved inducers of estrogen receptor α degradation. J Biol Chem. 2018;293(18):6776-6790.

[324] Costales MG, Matsumoto Y, Velagapudi SP, Disney MD. Small Molecule Targeted Recruitment of a Nuclease to RNA. J Am Chem Soc. 2018;140(22):6741-6744.

[325] Chakrabarti A, Jha BK, Silverman RH. New insights into the role of RNase L in innate immunity. J Interferon Cytokine Res. 2011;31(1):49-57.

[326] https://www.biochempeg.com/article/300.html

[327] Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, Manchester L, Reiter RJ. Basic mechanisms involved in the anti-cancer effects of melatonin. Curr Med Chem. 2010;17(36):4462-4481.

[328] Martínez-Campa C, Menéndez-Menéndez J, Alonso-González C, González A, Álvarez-García V, Cos S. What is known about melatonin, chemotherapy and altered gene expression in breast cancer. Oncol Lett. 2017;13(4):2003-2014.

[329] Lerner A. B., Case J. D., Takahashi Y., Lee T. H., Mori W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. Journal of the American Chemical Society. 1958;80(10):2587–2587

[330] Zisapel N. New perspectives on the role of melatonin in human sleep, circadian rhythms and their regulation. Br J Pharmacol. 2018;175(16):3190-3199.

[331] Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. J Pineal Res. 2016;61(3):253-278.

[332] Zhang Z, Inserra PF, Liang B, et al. Melatonin, immune modulation and aging. Autoimmunity. 1997;26(1):43-53.

[333] Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev. 1991;12(2):151-180.

[334] Blask DE, Sauer LA, Dauchy RT. Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Curr Top Med Chem. 2002;2(2):113-132.

[335] Reiter RJ, Rosales-Corral SA, Tan DX, et al. Melatonin, a Full Service Anti-Cancer Agent: Inhibition of Initiation, Progression and Metastasis. Int J Mol Sci. 2017;18(4):843. Published 2017 Apr 17.

[336] Cohen M, Lippman M, Chabner B. Role of pineal gland in aetiology and treatment of breast cancer. Lancet. 1978;2(8094):814-816.

[337] Stevens RG. Electric power use and breast cancer: a hypothesis. Am J Epidemiol. 1987;125(4):556-561.

[338] Schernhammer ES, Laden F, Speizer FE, et al. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst. 2001;93(20):1563-1568.

[339] Cos S, González A, Martínez-Campa C, Mediavilla MD, Alonso-González C, Sánchez-Barceló EJ. Estrogen-signaling pathway: a link between breast cancer and melatonin oncostatic actions. Cancer Detect Prev. 2006;30(2):118-128.

[340] Rato AG, Pedrero JG, Martinez MA, del Rio B, Lazo PS, Ramos S. Melatonin blocks the activation of estrogen receptor for DNA binding. FASEB J. 1999;13(8):857-868.

[341] Bouhoute A, Leclercq G. Calmodulin decreases the estrogen binding capacity of the estrogen receptor. Biochem Biophys Res Commun. 1996;227(3):651-657.

[342] Godson C, Reppert SM. The Mel1a melatonin receptor is coupled to parallel signal transduction pathways. Endocrinology. 1997;138(1):397-404.

[343] Aronica SM, Kraus WL, Katzenellenbogen BS. Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. Proc Natl Acad Sci U S A. 1994;91(18):8517-8521.

[344] Cos S, Martínez-Campa C, Mediavilla MD, Sánchez-Barceló EJ. Melatonin modulates aromatase activity in MCF-7 human breast cancer cells. J Pineal Res. 2005;38(2):136-142.

[345] Cos S, González A, Martínez-Campa C, Mediavilla MD, Alonso-González C, Sánchez-Barceló EJ. Melatonin as a selective estrogen enzyme modulator. Curr Cancer Drug Targets. 2008;8(8):691-702.

[346] Cos S, Blask DE. Melatonin modulates growth factor activity in MCF-7 human breast cancer cells. J Pineal Res. 1994;17(1):25-32.

[347] Mediavilla MD, Cos S, Sánchez-Barceló EJ. Melatonin increases p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. Life Sci. 1999;65(4):415-420.

[348] Proietti S, Cucina A, Dobrowolny G, et al. Melatonin down-regulates MDM2 gene expression and enhances p53 acetylation in MCF-7 cells. J Pineal Res. 2014;57(1):120-129.

[349] Ortíz-López L, Morales-Mulia S, Ramírez-Rodríguez G, Benítez-King G. ROCK-regulated cytoskeletal dynamics participate in the inhibitory effect of melatonin on cancer cell migration. J Pineal Res. 2009;46(1):15-21.

[350] Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S. Melatonin interferes in the desmoplastic reaction in breast cancer by regulating cytokine production. J Pineal Res. 2012;52(3):282-290.

[351] González A, Alvarez-García V, Martínez-Campa C, Alonso-González C, Cos S. Melatonin promotes differentiation of 3T3-L1 fibroblasts. J Pineal Res. 2012;52(1):12-20.

[352] Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S. Regulation of vascular endothelial growth factor by melatonin in human breast cancer cells. J Pineal Res. 2013;54(4):373-380.

[353] Menéndez-Menéndez J, Martínez-Campa C. Melatonin: An Anti-Tumor Agent in Hormone-Dependent Cancers. Int J Endocrinol. 2018;2018:3271948. Published 2018 Oct 2.

[354] Lissoni P, Tancini G, Barni S, et al. Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin. Support Care Cancer. 1997;5(2):126-129.

[355] Koşar PA, Nazıroğlu M, Övey İS, Çiğ B. Synergic Effects of Doxorubicin and Melatonin on Apoptosis and Mitochondrial Oxidative Stress in MCF-7 Breast Cancer Cells: Involvement of TRPV1 Channels. J Membr Biol. 2016;249(1-2):129-140.

[356] Alonso-González C, Menéndez-Menéndez J, González-González A, González A, Cos S, Martínez-Campa C. Melatonin enhances the apoptotic effects and modulates the changes in gene expression induced by docetaxel in MCF-7 human breast cancer cells. Int J Oncol. 2018;52(2):560-570.

[357] Kisková T, Ekmekcioglu C, Garajová M, et al. A combination of resveratrol and melatonin exerts chemopreventive effects in N-methyl-N-nitrosourea-induced rat mammary carcinogenesis. Eur J Cancer Prev. 2012;21(2):163-170.

[358] Orendáš P, Kubatka P, Bojková B, et al. Melatonin potentiates the anti-tumour effect of pravastatin in rat mammary gland carcinoma model. Int J Exp Pathol. 2014;95(6):401-410.

[359] Ma C, Li LX, Zhang Y, et al. Protective and sensitive effects of melatonin combined with adriamycin on ER+ (estrogen receptor) breast cancer. Eur J Gynaecol Oncol. 2015;36(2):197-202.

[360] Xiang S, Dauchy RT, Hauch A, et al. Doxorubicin resistance in breast cancer is driven by light at night-induced disruption of the circadian melatonin signal. J Pineal Res. 2015;59(1):60-69.

[361] Koenig KL, Goans RE, Hatchett RJ, et al. Medical treatment of radiological casualties: current concepts. Ann Emerg Med. 2005;45(6):643-652.

[362] Vijayalaxmi, Reiter RJ, Tan DX, Herman TS, Thomas CR Jr. Melatonin as a radioprotective agent: a review. Int J Radiat Oncol Biol Phys. 2004;59(3):639-653.

[363] Alonso-González C, González A, Martínez-Campa C, Gómez-Arozamena J, Cos S. Melatonin sensitizes human breast cancer cells to ionizing radiation by downregulating proteins involved in double-strand DNA break repair. J Pineal Res. 2015;58(2):189-197.

[364] Alonso-González C, González A, Martínez-Campa C, et al. Melatonin enhancement of the radiosensitivity of human breast cancer cells is associated with the modulation of proteins involved in estrogen biosynthesis. Cancer Lett. 2016;370(1):145-152.

[365] Wilson ST, Blask DE, Lemus-Wilson AM. Melatonin augments the sensitivity of MCF-7 human breast cancer cells to tamoxifen in vitro. J Clin Endocrinol Metab. 1992;75(2):669-670.

[366] Lissoni P, Barni S, Meregalli S, et al. Modulation of cancer endocrine therapy by melatonin: a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone. Br J Cancer. 1995;71(4):854-856.

[367] Hill SM, Belancio VP, Dauchy RT, et al. Melatonin: an inhibitor of breast cancer. Endocr Relat Cancer. 2015;22(3):R183-R204.