



HAL
open science

Estrogen receptor signaling as a target for novel breast cancer therapeutics

Jack-Michel Renoir, Véronique Marsaud, Gwendal Lazennec

► **To cite this version:**

Jack-Michel Renoir, Véronique Marsaud, Gwendal Lazennec. Estrogen receptor signaling as a target for novel breast cancer therapeutics. *Biochemical Pharmacology*, 2013, 85 (4), pp.449-465. 10.1016/j.bcp.2012.10.018 . hal-03002141

HAL Id: hal-03002141

<https://hal.science/hal-03002141>

Submitted on 12 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Research update

Estrogen receptor signaling as a target for novel breast cancer therapeutics

Jack-Michel RENOIR^{1,2,*}, Véronique MARSAUD³ and Gwendal LAZENNEC⁴.

¹U749 INSERM, Institut Gustave Roussy, 94805 Villejuif cedex, France; ²University Paris-Sud ;
³U1021 INSERM, CNRS UMR 3347, Institut Curie, 91405, Orsay cedex, France; ⁴INSERMU844,
Hôpital Saint-Eloi, 34295 Montpellier cedex 5, France.

Running title: new targets in estradiol receptor-positive breast cancers

*Corresponding author; phone: 33 (0) 1 4211 4259; Fax : 33 (0) 1 4211 5308

e-mail : jack-michel.renoir@igr.fr

key words: breast cancer; estrogen receptors; growth factors; hormone resistance; therapeutic targets

The authors declare that they have not any conflict of interest.

Abbreviations:

AE: antiestrogen; **AI:** aromatase inhibitors; **AIB1/ACTR/p/CIP/RAC3/TRAM-1/SRC-3:** Amplified in breast cancer 1/Activator of thyroid hormone receptor/p300/CBP-interacting protein/Receptor-associated coactivator 3/Thyroid hormone receptor activator molecule 1/**SRC-3:** steroid receptor coactivator-3; **AKT/PKB:** protein kinase B; **AP1:** Activator protein-1; **BC:** breast cancer; **Bcl2:** B cell lymphoma 2; **CARM1:** coactivator-associated arginine methyltransferase 1; **DRIP/TRAP:** Vitamin D Receptor Interacting Protein/ Thyroid Hormone Receptor-associated Proteins; **EGF,** Epidermal growth factor; **Erk-1 and Erk-2:** extracellular signal-regulated kinases 1 and 2; **17 β -estradiol: ER;** estradiol receptor; **FAK:** focal adhesion protein kinase; **Gossypol:** 2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3methylnaphthalene); **HER/Erb-B:** human epidermal growth factor receptor/avian erythroblastosis oncogene B; **IGF:** Insulin growth factor; **IRS1:** Insulin receptor substrate 1; **MAPK,** mitogen activated protein kinase; **MIBE:** ethyl 3-[5-(2-ethoxycarbonyl-1-methylvinyl)-1-methyl-1H-indol-3-yl]but-2-enoate; **mTOR:** Mammalian target Of Rapamycin; **NR:** nuclear receptor; **PAX-2:** Paired Box-2 gene product; **PDK1:** 3-phospho-inositide-dependent protein kinase; **PI3K :** phosphoinositide-3 kinase; **PR:** progesterone receptor; **PRMT1:** protein arginine methyltransferase; **PTEN:** phosphatase and TENsin homolog; **PTM:** post-translational modification; **Src:** sarcoma virus tyrosine kinase; **SERM:** selective estradiol recetor modulator; **SERD:** selective estradiol receptor downregulator; **SDF-1:** Stromal cell-Derived Factor-1; **SHARP:** SMRT/HDAC1 associated repressor protein; **SMRT:** silencing mediator for retinoid and thyroid hormone receptor; **SP1:** Specificity protein 1; **SWI/SNF:** SWItch/Sucrose Nonfermentable chromatin remodeler; **TRAP220:** Thyroid receptor-associated proteincomplex 220 kDa component.

Abstract

In breast cancer (BC) epithelial cells the mitogenic action of estradiol is transduced through binding to two receptors ER α and ER β which act as transcription factors. Antiestrogens (AEs) and aromatase inhibitors (AIs) are used clinically to arrest estrogen-dependent growth of BC. In case of resistance to AEs and AIs, therapeutic benefit is gained by inhibiting growth factor receptors with Herceptin and lapatinib. **Estrogen** effects are mediated not only through nuclear ERs but also through **cytoplasmic**/membrane ER and G-protein-coupled ER. These estrogen-binding systems associate with various proteins affecting cell cycle signaling, proliferation and survival. The partners of nuclear ER include SRC1-3, HDACs and ER β itself, but also **newly** identified proteins, such as E6-AP, LKB1, PAX-2 and FOXA1. The partners of extranuclear ER α include PI3K and the tyrosine kinase Src. These various factors are all potential targets for therapeutic intervention. In addition, BC proliferation is enhanced by insulin and EGF which stimulate signaling through the MAPK and PI3K/AKT pathways by activation of IGF-1R and EGFR axes, respectively. These pathways are tightly interconnected with ER-activated signaling and indeed, membrane ER α complexes with Src and PI3K. Chemokine-mediated signaling also modulates the estrogen response. Inhibiting these pathways with specific inhibitors or activating some of them by gene manipulation, may be therapeutically valuable for arresting BC cell cycle progression and inducing apoptosis in order to antagonize hormone-resistance. We review here some newly identified putatively targetable ER partners, and highlight the need for developing tumor-targeting drug carrier systems affecting both the tumor cells and the tumor environment.

I. Introduction

Estrogen receptors (ERs) belong to the subfamily of ligand-regulated transcription factors which **transduce hormone** signaling into a large variety of physiological responses in various organs [1]. The two structurally related ERs namely ER α and ER β are the products of two separate genes differentially distributed in tissues. ER α is responsible for estrogen-induced mitogenic signaling in epithelial cells in breast, uterine and ovarian tissues [2]. In the normal mammary gland, estradiol (E₂) binds to ER α and ER β , **which control** cell proliferation and differentiation [3]. Both ER isoforms are produced in similar, small amounts in the normal breast, whereas more ER α than ER β is expressed in breast cancer (BC) cells. Importantly, **ER α is the only ER that is detected ~~immune-histochemically~~ by immune-histochemistry** in BC biopsies. Only tumors with nuclear-free ER cells are classified **as** “ER-negative“. At least 70% of BCs are ER-positive (ER⁺) and express mainly ER α or progesterone receptor (PR) or the erythroblastosis oncogene-B2 (ErbB-2, HER2/NEU) or all three. ErbB-2 is a member of the HER family of trans-membrane receptor tyrosine kinases (RTK), which also includes the epidermal growth factor receptor (EGFR/HER 1).

Patients with ER- and PR-positive BC are currently treated with hormone therapy (HT) to inhibit ER signaling. HT uses two approaches: antagonizing agonist ligands binding to ER with antiestrogens (AE) or blocking E₂ synthesis with aromatase inhibitors (AIs). Despite the high level of success of HT, many BCs acquire resistance. Some tumors only express ErbB-2 and do not respond to HT; in such cases the use of trastuzumab (Herceptin), a humanized monoclonal antibody targeting ErbB-2, **has offered a considerable benefit**, but a significant number of breast tumors fail to respond [4]. ER and ErbB-2 have been the targets of choice for BC treatment over recent years. However, some tumors, **classified as triple negative** [5], **do not** express any ER, PR and ErbB-2, and consequently are resistant to HT and trastuzumab.

Triple negative BCs are actually considered as **cancers completely different from** hormone-dependent BCs. Their prognosis is poor and they are currently treated with chemotherapy (i. e. Paclitaxel). **Understanding of** the molecular mechanisms implicated in the development of these different malignancies, **was improved** by both clinical and fundamental research over the past decades. However, despite the progress made in our understanding of these diseases, as well as in the discovery of new treatments, the number of patients dying from BC has not **decreased substantially**. There is no doubt that new effective therapies are required. One difficulty is the lack of specific markers that can be used to distinguish malignant cells from normal cells. Indeed, current treatments simply target over-expressed factors such as ER and ErbB-2. Deciphering the mechanism of action of estrogens through the transcription activity that they trigger following binding to their cognate receptors has led to the identification of many new actors. This prompted the pharmaceutical industry to search for **new inhibitors that** can be used in BC treatment, and there are consequently numerous clinical trials underway combining several molecules. Most of them affect the regulators of post-translational modifications of ER, including phosphorylation, acetylation, prenylation and ubiquitination.

Moreover, a small pool of ER localizes in the cytoplasm and at the membrane tightly bound to adaptor proteins, forming multiprotein complexes that trigger the activation of the MAPK and AKT pathways. This also has led to envisage the use of new inhibitors. **In this review we will analyze some of the factors modulating the effects of estrogens on ER, which could serve as new targets** for the treatment of both estrogen-sensitive and insensitive breast tumors.

II. Estradiol receptors **function and endocrine therapy in breast cancers**

Like all other members of nuclear receptors (NR) family, ERs are activated through either agonist ligand binding or phosphorylation at various sites or both (see [6] for a review). The ER proteins are generally believed to shuttle between the cytoplasm and nucleus and *in vitro* experiments have demonstrated that ligand-free ER α , like other steroid NRs, is maintained in a non-DNA binding form in a multi-chaperone complex organized around Hsp90 (review in [7]). Little information is available with regard to ER β but both ERs are believed to similarly activate gene transcription upon classical estrogen binding. ER-mediated transcription is a highly complex process involving multiple coregulatory factors and “cross-talks” between different signaling pathways (figures 1 and 2). These mechanisms have been described in detail in other reviews and therefore are only briefly summarized here (for more details see [8]).

II. A. Canonical genomic ER-mediated transcription mechanism

In response to ligand binding, ER α undergoes conformational changes, which control its interaction with heat shock proteins (although the interaction between ER β and Hsp90 is poorly documented) and coregulators; these interactions determine ER binding to the 13bp estrogen response element sequence (ERE) within the promoter (figure 1). ER-dimers dynamically and sequentially recruit various regulatory protein complexes contributing to chromatin remodeling, thus strongly enhancing transcription activity [9]. The NR coactivators identified with ER include the general transcription factor p300/CBP. P300/CBP is ubiquitously expressed and serves as coadaptor between NRs and DNA. It has a critical role in cell cycle regulation, cell differentiation and apoptosis and has histone acetyltransferase (HAT) activity [10] [11]. Importantly, HATs are required for full ER-mediated transcriptional activation. P300/CBP also interacts with other HATs such as PCAF [12], and acetylates components of the

basal transcription machinery. Methyl-transferases including CARM1 and PRMT1 are also ER α -associated coactivators. Members of the p160 proteins family, namely steroid receptor coactivator 1 (SRC-1), SRC2 and SRC3 (ACTR, RAC-3, pCIP, TRAM-1, AIB1 encoded by the *AIB1* gene), play various roles in the recruitment of the pre-initiation complexes DRIP/TRAP [13].

E₂-ER α complexes affect the transcription of genes involved in proliferation, differentiation, survival, and, particularly relevant for cancer, in the stimulation of invasion, metastasis and angiogenesis. Among these genes, some are activated like those involved in cell cycle progression (such as *c-myc*, *cyclins D, A and E*) and the expression of others, such as the gene for the cyclin-dependent kinase (CDK) inhibitor p21^{Waf1/Cip1}, is decreased [14]. Consequently, the growth of ER α -expressing (ER⁺) cells from breast tumors is E₂-dependent and the removal of E₂ leads to regression. Therefore, ER α is a well-established predictive marker of hormone sensitivity and a positive prognostic marker in BC, identifying tumors for which endocrine treatment is likely to be effective. The presence of ER β inhibits both ER α -mediated transcription and E₂-induced proliferation in various cancer cells [15-17] (Bossard C., Busson M., Vindrieux D., Gaudin F., Machelon V., Brigitte M., Jacquard C., Pillon A., Balaguer P., Balabanian K. and G. Lazennec (2012) "Potential role of Estrogen Receptor beta as a tumor suppressor of epithelial ovarian cancer" PlosOne (in press)). Therefore, ER β in BC lesions is thought to be associated with more benign tumors. Both ER α and ER β may also be found in endothelial cells and vascular muscles, concomitantly with ER variants (see below). Furthermore, ER α and ER β differentially regulate both the proliferation and apoptosis of normal mammary epithelial cells [18]. It is currently believed that the ER α /ER β ratio is a key element in the regulation of E₂ activity in BC cells [19].

Ligand-activation of ER may also stimulate indirect binding of ER to DNA by protein-protein interactions with transcription factors such as AP-1 or Sp-1, which anchor the pre-initiation complex to ERE. For both direct and indirect association of ER with DNA, recruitment of co-activators modulates gene activation and subsequent protein production [8].

ERs are phosphorylated at multiple sites by a variety of kinases. Such phosphorylation may result from both estrogen binding to ER inducing activation of various growth factor receptors or other kinases [6]. Phosphorylated ER α binds directly or indirectly to DNA, recruits co-activators and triggers transcription (**figure 1**). Importantly, ER-mediated transactivation can reach its maximal level only if ER is phosphorylated, even in the absence of E₂ binding.

Various ER variants may alter the estrogenic response. This is the case of ER46, an abundant N-terminal (A/B)-deleted ER α splice variant and highly effective transducer of membrane initiated responses in endothelial cells. ER46 participates in the rapid stimulation of the vascular endothelial nitric oxide synthase (eNOS) and leads to E₂-ER-mediated vasodilatation. These effects on tumor vasculature through ER in endothelial and stroma cells may explain the AE-mediated anti-tumor activity in ER-negative BC xenografts [20, 21]. ER α -36, an ER α variant lacking the A/B N-terminal domain and a truncated ligand-binding C-terminal domain, has been implicated as a mediator of extra nuclear (non-genomic) actions.

II.B. The non-genomic pathways

II.B.1 Membrane ER

It has long been established that E₂ induces rapid effects emanating from the membrane. Various E₂-induced signaling cascades have been identified in the extra nuclear compartment (non-genomic mechanism), and involve direct interactions of a small pool of ER

(principally ER α) localized at the membrane (mbER) with other proteins. Indeed, ER α is found in multi-protein complexes that include growth factor-dependent kinases and adaptor proteins [22] (figure 2). In addition, mbER α binds in a ligand-dependent manner to the p85 α regulatory subunit of PI3K [23]. Palmitoylation enables mbER α to interact with caveolin-1. Caveolin-1 gene inactivation promotes increased ER α expression and up-regulation of cyclin D1 [24]. Binding of E₂ to mbER complexes leads to de-palmitoylation and dissociation of ER α from caveolin-1, then to the activation of many downstream signaling events such as the tyrosine kinase Src, the p85 PI3K subunit, MAPK, AKT, p21ras and protein kinase C, helping ER α to move to other membrane micro-domains [25]. Non-genomic functions resulting from E₂-binding to mbERs affect cell proliferation and survival (ER α) and apoptosis (ER β) [26].

II.B.2 GPER

Estrogen also signals through a seven trans-membrane G-protein-coupled receptor (GPCR-30) and E₂-GPCR-30 complexes (figure 2) activate Erk-1 and Erk-2. Despite alternative suggestions to attribute the non-nuclear effects of E₂ to ER α 36 and not to GPCR-30 [27], a number of evidences have established the function of GPCR-30 as membrane ER with specific binding characteristics (see [28] for a review). Indeed, E₂ remains agonist towards GPCR-30, but ER antagonists (both mix and pure) act as agonists, similarly to a variety of phyto- and xenoestrogens which stimulate cAMP production (figure 2). This receptor now named GPER-1 (G-protein-coupled ER-1), stimulates adenylyclase and the cAMP-mediated regulation of EGF-MAPK axis [29]. Conversely, GPER is up-regulated by EGF in ER-positive BC cells; moreover, GPER was suggested to act as an inducer of ER α -36 expression in various BC cells including the “ER-negative” ones (MDA-MB-231). These and diverse other findings demonstrate the tight interplay between ER- and EGFR-signaling, and illustrate the complexity

of estrogen action in BC cells. **This complexity** is exemplified by the differential activity of ER ligands **towards** GPER; GPER antagonists with ER have been identified, **such as** G15 and G36 [30] and MIBE [31] (**supplemental figure S1**). They are all promising molecules capable of inhibiting both the effects of estrogens acting as inducers of ER-mediated transcription and also those emanating from the membrane of BC cells.

II.C. Hormone therapy

Numerous reviews have already thoroughly described the various advantages and disadvantages of the use of antiestrogens and aromatase inhibitors. We will only present a brief summary here.

II. C. 1. Anti-estrogens

Two distinct classes of synthetic AE have been developed to treat ER⁺, PR⁺ but Erb-B2-negative tumors (**supplemental figure S1**). Selective Estrogen Receptor Modulators (SERMs) are a class of ER ligands, exemplified by tamoxifen (Tam) and raloxifene which act either as AE or agonist depending on the tissue and the cellular promoter context. The Selective Estrogen Receptor Downregulators (SERDs) are a class of steroidal, pure AEs, which are devoid of any agonistic activity in any tissue [32]. Faslodex[®] (fulvestrant, ICI,182780) is currently the only SERD in clinical use, employed in case of Tam resistance. Similar to the other SERD RU58668, it has a dual mode of action: first it binds to ER and thereby induces the formation of an inactive complex, **blocking** ER dimerization and nuclear localization; second it targets ER α to ubiquitination prior to its degradation by the proteasome. These effects are accompanied by the inhibition of ER-mediated transcriptional effects [33]. However, after arrest of AE

treatment, the inhibitory effects of AEs, including SERDs, are reversed by estrogens such that the efficacy of the drugs is limited, resulting in a limited efficacy of the drugs [34].

II. C. 2. Aromatase inhibitors

Approximately 50% of patients with advanced disease do not respond to first-line treatment with Tam and almost all patients with metastases relapse and die from the disease [35]. A second endocrine therapy strategy has emerged consisting of using AIs to reduce the production of estrogen in peripheral tissues and within the tumor. Aromatase converts androstenedione into androgen, then to estrone and E₂. It is expressed in many endocrine tissues including BC cells. Therefore, selective AIs have been designed to reduce circulating estrogen levels. Blocking E₂ production is considered as an option for premenopausal women with ER-positive tumors. In postmenopausal women, the ovary is no longer the primary source of E₂ which is produced in fat tissues, and AIs have been widely successfully used in the treatment of postmenopausal women with advanced BC. Indeed, anastrozole and letrozole but not exemestane (**Supplemental figure S1**) have stronger anti-proliferative activity than Tam in patients with ER-positive tumors, and this treatment can also be used to reduce the side effects of Tam. Additionally, AIs are also useful for treating AE-resistant BC [36].

III. Growth factor signaling

III. A. EGF pathways

Most BC cells express receptors for peptide growth factors, such as EGF. These tyrosine kinase receptors are activated following binding of these peptides in their extracellular part. In the case of EGF receptors ErbB-3 and ErbB-4, the EGF binding induces the formation of receptor homo- and hetero-dimers with ErbB-2, leading to enhancement of the receptors'

kinase activity (except in the case of dimers with Erb-B3 which, contrary to the other members of the EGFR family is devoid of TK activity); this ensures auto-phosphorylation of the receptors and initiates a cascade of downstream signaling. The consequences are multiple and include cell proliferation (*via* activation of the Ras/Raf/Mek/MAPK pathway), cell cycle progression and survival (*via* activation of the PI3K/AKT pathway) and expression of various genes encoding proteins such as VEGF. No ErbB-2 ligand has been identified but the ErbB-2/ErbB-3 dimers can be activated by the peptides derived from heregulin (HRG) which are ligands for ErbB-3 only. The binding of HRG to the ErbB-2/ErbB-3 hetero-dimer activates the ErbB-2 TK activity leading to mitotic response and induction of anti-apoptotic Bcl-2 family members including Mcl-1 [37]. In human BCs, an increase of Erb-B2 expression is associated with an increase of SRC-1 and SRC-3 [38].

III.B. Insulin-like growth factor signaling

Binding of IGFI and IGFI to insulin-like growth factor receptor 1 (IGF-1R) activates intracellular pathways that regulate cell growth and survival control (**figure 2**). IGF-1R is a trans-membrane receptor with TK activity. It functions as homo- or hetero-dimers with the insulin receptor I. IGF binding to IGF-1R triggers two different (although inter-connected) pathways: the recruitment of a docking specific intracellular receptor substrate (IRS-1) at the phosphorylation sites transduces the signal to the shc/Ras/raf/MAPK pathway, leading to accelerated cell proliferation. IGF I and II are secreted in practically all epithelial cells, and the binding of IGFI to IGF-1R increases tumor growth and development. Furthermore IGF-1R and the insulin receptors ($IR\alpha$ or $IR\beta$) can hetero-dimerize and transduce the signals triggered by insulin, a mechanism associated with a poor prognosis [39]. Although there are no direct interactions between ER proteins and IGF-1R, there are evidences that IGF-1R maintains cell-

induced proliferation and anti-apoptotic activity even in the presence of AE. In response, IGF-1R is phosphorylated by PI3K, leading to AKT recruitment at the membrane and consequently to its activation through phosphorylation either on ser473 by the Ric/mTOR complex or on thr308 by PDK1. Downstream from AKT, mTOR is also involved in the regulation of cell cycle proteins. The activation of this second branch of IGF-signaling is critical for cell cycle progression and survival; indeed, it has been clearly demonstrated that inhibition by phosphorylation of pro-apoptotic molecules such as the Bcl-2 family member BAD and the cleavage (activation) of caspase-9, led to suppression of apoptosis (reviewed in [40]).

IGF-1R is over-expressed in the majority of BCs (90 to 95%) and is often co-expressed with ER β . Moreover, estrogens induce the expression of IGF-1R and IRS-1, thereby reinforcing the IGF-induced responsiveness of BC and Tam resistance. IGF- and ER α -regulated pathways are thus intricately interconnected, as far as mammary development and BC are concerned. High circulating plasma concentration of IGF-1 is a marker for an increased risk of relapse under treatment with adjuvant Tam. A number of small chemical inhibitors and antibodies targeting IGF-1R inhibitors have been developed, the most advanced in clinical trials being OSI-906 (Linsitinib) and BMS 754807 (Supplemental figure S2).

IV. Resistance

Whatever the endocrine treatment used, resistance may occur, especially with Tam, which is never given for as long as a five-year period. Moreover, patients with over expression of ErbB-2 (15-20% of all BCs) are resistant to endocrine treatment. The molecular causes of endocrine resistance are incompletely understood. ER- and PR-negative menopausal BCs over-expressing Erb-B2 are currently cured with two FDA approved treatments: Trastuzumab (Herceptin) and the small chemical molecule tyrosine kinase inhibitor lapatinib. Trastuzumab

binds to an epitope in the juxtamembrane region of the ErbB-2 receptor. This binding induces uncoupling of ligand-independent HER2-HER3 heterodimers and inhibition of downstream signaling. It also causes antibody-dependent, cell-mediated cytotoxicity. Although many BCs with *HER2* gene amplification respond to trastuzumab, a significant fraction of these subsequently progress. Several mechanisms of resistance to the antibody have been reported; they include enhanced signaling by RTKs, amplification of PI3K signaling as a result of mutations in this pathway, and the presence of truncated forms of ErbB-2 devoid of the antibody-binding epitope in the receptor's ectodomain. A recent study demonstrated that exposure of ER-positive BC cells to fulvestrant increased the expression of ErbB-3 and/or ErbB-4 and sensitivity to their potent ligand heregulin, although these effects are dependent on the cell line tested [41]. This observation severely compromises the use of fulvestrant in first line hormone therapy because BC cells may be able to compensate for the growth-inhibitory effects of fulvestrant by growth stimulation via ErbB-3-4 [42]. **It remains to be determined whether** this type of fulvestrant-associated increase of ErbB-3-4 activity can occur with other AE, particularly RU 58668, another pure AE which counteracts fulvestrant-acquired resistance in xenograft models [43]. The ErbB-2 TK inhibitors (TKI) lapatinib (a dual inhibitor of ErbB-1 and ErbB-2 TK function) and neratinib have clinical activity as single agents or in combination with chemotherapy in patients who relapsed under trastuzumab [44]. These findings suggest that trastuzumab-resistant tumors continue to depend on the TK activity of ErbB-2, requiring the combination with TK activity or targeting of another pathway. Unfortunately, in case of triple negative breast cancers, there is no current treatment available to provide a good outcome.

All BCs express EGFR (**figure 2**), which regulates cell cycle and anti-apoptotic signaling. Many mechanisms other than ErbB-2 may explain Tam-acquired resistance; for example the

deregulation of **receptor** expression or maturation. The deregulation of post translational modifications of both ERs and their cofactors has been **implicated highlighted**. Also, increased and deregulated cell cycle and apoptosis signaling are certainly among the major causes of resistance [45]. In BC over-expressing Erb-B2, the concomitant over-expression of SRC-3 contributes to trastuzumab resistance by activating IGF signaling and to Tam resistance by increasing the agonistic activity of this SERM [38].

Cetuximab (Erbix) is a humanized monoclonal antibody against EGFR used in the treatments of colorectal cancers. It has been assessed in combination with TK inhibitors (erlotinib) (**supplemental figure S3**) for treating patients with ER⁺ BC, but the responses were not encouraging. However, new molecules inhibiting the HER members by competing with their ligands may be of therapeutic value, particularly in combination with drugs targeting the Erb-B2 receptor network. A combination of this type is undoubtedly required for better inhibition of this pathway and, hence, improved clinical activity. In support of this view, lapatinib is a dual inhibitor of EGFR and Erb-B2 and in combination with paclitaxel has shown good efficacy in the treatment of women with Erb-B2-positive BC [46].

V. Potential new targets

V.A. Co-activators and corepressors

V.A.1. SRC1-3

Among coactivators, which have been identified as robust enhancers of ER-regulated transcription, SRC-1 and SRC-3 are frequently over-expressed in BC tumors in association with enhancement of ErbB-2, a status leading to poor survival. SRC-1 serves as a general transcription enhancer for many transcription factors and SRC-3 over-expression participates in **positive cross-talk** with both IGF-1 pathway and AE resistance (see [38] and ref herein).

SRC-3 has also been identified as a mammary tumor-initiating factor and SRC-3^{-/-} mice are defective for oncogene- and carcinogen-induced BC initiation, and for metastasis [47]. In BC cells over-expressing ErbB-2, SRC-3 participates in the action of trastuzumab treatment through activation of the IGF signaling [48]. These various observations indicate that the ability to abolish SRC-1-3 activities would be valuable, in addition to the established arsenal of targeted therapies for BC, particularly to ~~combat~~ overcome resistance. O'Malley's laboratory has been searching for inhibitors of these coactivators and recently found that gossypol (**Supplemental figure S4**), a natural product from cottonseed, disrupts interaction between NR and SRC-3 and down-regulates SRC-3 not only in BC cells but also in lung, prostate and liver cancer cells [38]. Gossypol was described a long time ago as a male infertility molecule and was considered for use in male contraception. Gossypol binds to Bcl-2 and Bcl-XL and antagonizes their anti-apoptotic activities. Thus, gossypol represents the prototype of a new class of potent anticancer molecule, which could be used in combination with other chemotherapeutics to ~~combat~~ fight resistance in cancers. Consequently, phase II-III clinical trials to assess the value of gossypol in several types of cancer are currently underway (<http://www.clinicaltrials.gov>).

V.A.2. HDACs

Five lysines on ER α have been reported to be acetylated by p300: Lys266, Lys268, Lys299, Lys302 and Lys303, all localized in the hinge region. Other PTMs of ER α may affect the same lysine residues, but with different consequences on BC cell behavior. This is the case of Lys302, which in addition to acetylation, can be ubiquitylated, sumoylated or methylated [6]. The effects of ER α acetylation result from a two-step mechanism: **short exposure of cells** to HDAC inhibitor (HDACi) leads to acetylation and stabilization of the receptor (as well as of that

of p300/CBP); after longer time exposures, the receptor is delocalized and subsequently degraded by the proteasome [49]. By contrast, exposure of ER β -containing BC cells and ER β -rich ovarian cancer cells to HDACis stabilizes ER β isotype [50]. HDACis block cell cycle and induce apoptosis in various cancer cells. ~~They are thus anticancer agents and several phase I and II clinical trials are currently underway~~ Thus, several phase I and II clinical trials are currently underway with these anticancer agents. In breast tumor models, several HDACis show anti-proliferative effects *in vivo*. Importantly, restoration of ER α expression was observed in ER-negative BC cells following exposure of cells to pan HDACis, a process potentiated by the DNA methyl transferase inhibitor 5-aza-deoxycytidine [51]. When HDACs are inhibited, a decrease of EGFR mRNA in ER-negative MDA-MB-231 cells is observed as well as *in vivo*; concomitantly, a re-sensitization of these cells to Tam is observed, strengthening the potential usefulness of HDACis combined with AE for BC treatment [52].

HDACis are promising anticancer drugs because they have multiple targets in cancer cells [53]. They activate the acetylation process and inhibit tumor growth by repression of oncogenes including *c-myc* but they also activate tumor suppressors such as *CDKN1A* encoding the CDK inhibitor p21^{WAF1/CIP1} [54]. HDACis inhibit cell cycle, activate programmed cell death, differentiation and angiogenesis in many cancer cells and in animal models [53]. Some HDACis have already been approved by the FDA (SAHA or “Vorinostat”; CG1511 or “Belinostat”, LBH589 or “Panobinostat”) and many (Supplemental figure S5) are in clinical trials for BCs (NCI clinical protocol NCT007777049; see <http://www.cancer.gov>). Importantly, inhibitors of the class II HDACs like Etinostat (MC1575) contrary to TSA do not decrease ER α expression but enhance expression of ER β without induction of apoptosis, but via up-regulation of p21^{waf1/CIP1} gene and anti-proliferative effects [55]. This type of HDAC inhibitor could be of therapeutic

value mainly in association with other drugs, for example ER β agonist ligands, TKIs or HSP90 inhibitors (see below).

Another potentially exploitable target in BC is the microtubule-associated HDAC-6 which can deacetylate Hsp90. Specific inactivation of HDAC6 by HDAC inhibitors results in acetylation of Hsp90 leading to dissociation and proteasome-mediated degradation of client proteins, then to cell death. The G protein-coupled receptor kinase 2 (GRK2) is a key modulator of HDAC6. GRK2 phosphorylates HDAC6 leading to α -tubulin deacetylase activity that regulates key cellular processes dependent on cytoskeletal rearrangements such as migration, polarity and cell spreading [56]. Therefore, it is plausible that inhibiting HDAC6 deacetylase activity could be therapeutically beneficial against BC metastasis. However, specific inhibitors of this type of HDAC are yet to be developed.

V.A.3. PAX-2

High levels of SRC-3 (AIB-1) and ErbB-2 have been described in aggressive BC and more recently, the laboratory of J.S. Carroll showed that the Paired Box-2 gene product (PAX-2) is a critical Tam-recruited transcriptional repressor of the *ErbB2* gene [57]. Elevated AIB-1 expression can lead to competition with PAX-2 binding of Tam-ER complex to DNA, directly resulting in increased ErbB2 protein expression. PAX-2 is generally described to be a transcriptional activator with a tissue-specific activity, acting as a repressor in BC and, a determinant of SERM action in female reproductive tissues [57].

V.A.4. FOXA1

The Forkhead protein (FOXA1/HNF3 α) plays a determinant role in the transcriptional activity of E₂-ER α complex, modulating ER α -chromatin interactions and thus, the endocrine response of BC cells [58]. It is negatively regulated by the CCCTC-binding factor (CTCF), an upstream regulator of FOXA1-chromatin interactions. FOXA1 is required for E₂ and Tam action

in E₂-responsive BC cells. Moreover, FOXA1 helps in reprogramming ER α binding to gene promoters in tumors from patients with drug resistant BCs at different sites than those at which ER α binds in tumors from Tam-sensitive patients. It has also been demonstrated that FOXA1 is absolutely required for ER α -binding to promoters, even in the absence of ER-ligand binding [59]. As a consequence silencing of FOXA1 may be of therapeutic value.

V.A.5. E6-AP

E6-associated protein (E6-AP) is an E3-ubiquitin ligase, which functions as a coactivator of steroid hormone receptors including ER α [10]. Its abundance in BC tumors is inversely correlated to that of ER α . In transgenic mice that over-express the ubiquitin-ligase E6-AP, initiation of the mammary tumor development by E₂ failed, whereas such tumors develop rapidly in mice that over-express an inactive E6-AP mutant. Together with the strong inverse correlation between survival and expression of E6-AP, these findings suggest that E6-AP may act as a tumor suppressor [60]. Besides its utility in diagnosis, its gene amplification could be of potent usefulness.

V.A.6. Methyl transferases

Transient methylation of ER α on arg260 by PRMT1, a coactivator of numerous NRs, has been shown to participate in the exclusive cytoplasmic localization of the receptor and to mediate its extranuclear function by triggering its interaction with the p85 subunit of PI3K and Src [61]. As a result of this process, AKT is phosphorylated, activating the downstream cascade inducing rapid events leading to non-genomic effects of E₂. Thus, PRMT1 contributes to the regulation of E₂-induced non-genomic downstream effects. The FAK adhesion protein, a substrate of Src also interacts with arg260-methylated ER α [6]. It is possible that BC cells with methylated ER α may be involved in migration and metastasis. Consequently, targeting PRMT1 through specific inhibitor (such as the water soluble AMI-1, **Supplemental figure 4**) or siRNAs

could decrease this property for a better therapeutic success. However, no data have been obtained in *in vivo* experiments with this type of PRMT1 inhibitors.

V.A.7. LKB1/AMPK

The gene *LKB1* (liver kinase B-1) encodes a calcium-calmodulin regulated Ser/Thr kinase, which mainly phosphorylates the members of the AMPK family and being considered to be a tumor suppressor. Phosphorylation of LKB1 activates AMPK, which itself participates in the downstream inactivation of mTOR, leading to cell proliferation arrest and apoptosis control. The LKB1/AMPK complex regulates positively cell energy metabolism, and negatively cell cycle progression in various cells. In BC cells, weak expression of LKB1 is associated with high grade tumor. Over-expression of LKB1 blocks BC cell proliferation in G1 in a p21- and p53-dependent manner [62] and arrests migration and invasion through inhibition of metalloproteinases MMP-2 and MMP-9. Expression of LKB1 also negatively regulates angiogenesis through decreasing VEGF and bFGF expression and thus weak vascularization [63]. LKB1 interacts with PTEN and with the Brg1 protein encoded by the Brahma-Related-Gene1 *Brg1*, a component of the SWI/SNF chromatin remodeling complex (for a review see [64]). LKB1 also interacts directly with ER α in MCF-7 cells and plays a role of co-activator [65]. This may appear paradoxical for a tumor suppressor. However, the LKB1 promoter contains several EREs and E₂ enhances LKB1 expression. In turn, E₂ increases ER α expression, which represses LKB1 transcription, thus leading to decreased ER α expression [66]. LKB1 may be considered as a potential therapeutic target for BCs, because AMPK activating drug, metformin, used in the treatment of diabetes of type II (insulin-independent), decreases aromatase expression in BC cells [67]. In general, stimulation of LKB1 leads to inhibition of cell adhesion, invasion and migration following AMPK activation and suppression of mTOR [68].

Although no specific small molecule activators of LKB1 are available, approaches involving manipulation of *LKB1* gene expression deserve attention.

V.A.8. HOXB7 proteins

Homeobox genes express nuclear proteins, which act as transcription factors during normal development and differentiation. One of them *HOXB7* was shown to be an ER α -responsive gene significantly over-expressed in Tam-resistant MCF-7 cells and in patients with distant metastasis [69]. This elevation of *HOXB7* protein has been directly linked to the acquisition and maintenance of SERM resistance [70]. Thus, antagonists of *HOXB7* may be important tools to circumvent Tam resistance; these antagonists are not yet available but the incorporation in nanocarriers of siRNA targeting *HOXB7* warrants to be evaluated in appropriate xenograft models.

V.A.9. TLE1

The transducin-like enhancer protein 1 (TLE1) is another modulator of the transcriptional activity of ER. In particular, combining the chromatin-immunoprecipitation (ChIP) technique with high throughput sequencing Carroll et al. found a significant overlap of TLE1 binding sites in MCF-7 cells with ER targets [71]. Among these genes, some are directly involved in cell division and can be down-regulated by the transfection of TLE1 siRNAs. These data support the therapeutic use of siRNA for modulating TLE1-ER interaction.

V.A.10. The intriguing role of ER β

ERs are widely distributed in the body. ER α is mainly expressed in uterus, prostate (stroma), breast (luminal cells), ovary (theca cells), bone, epididymis, and various regions of the brain, liver and white adipose tissue. By contrast, ER β is expressed in prostate (epithelium), colon, ovary (granulosa cells), bone marrow, vascular endothelium, salivary gland and certain regions of the brain. In some tissues, both ERs are expressed, albeit in different cell types. For

example, in human testis, ER α is present in spermatogonia and Sertoli cells, and both ERs are present in other cells, such as Leydig cells and spermatocytes [8]. The two ER isotypes have different ligand binding and transcriptional activities but their affinity for E₂ and classical AE are similar. ER β , ~~but not~~ unlike ER α , binds phytoestrogens with high affinity. Although the properties of ER α and ER β overlap, studies with knockout mice revealed that these two ERs have distinct and unique roles *in vivo* [72]. ER β inhibits human ER α -positive BC cell proliferation by repressing transcription of the *c-myc*, *cyclin D₁* and *cyclin A* genes and increasing the expression of p21^{Waf1/Cip1} and p27^{kip1}, leading to cell cycle arrest in the G₂ phase [15]. ER β is also able to inhibit the proliferation of ER α -negative BC cells and it decreases their invasiveness capacity [73]. The reported inhibition of tumor growth by ER β in various mouse models in which ER β opposes the proliferative effects of ER α [16, 74] (Bossard C., Busson M., Vindrieux D., Gaudin F., Machelon V., Brigitte M., Jacquard C., Pillon A., Balaguer P., Balabanian K. and G. Lazennec (2012) "Potential role of Estrogen Receptor beta as a tumor suppressor of epithelial ovarian cancer" PlosOne (in press)), has led to the suggestion that ER β acts as a tumor suppressor [75]. Consistent with this view, ER β inhibits angiogenesis and tumor growth in a T47-D xenograft model [74], and the siRNA-mediated knockdown of ER β increases the expression of genes relevant to tumor cell proliferation such as the pro-apoptotic Bik [76]. ER β expression is linked to less aggressive tumors in BC suggesting that its re-expression in ER-positive tumors could be beneficial. Indeed, ER β seems to potentiate the anti-proliferative activity and apoptotic effects of Tam in BC cells [76]. Thus, ER β re-expression in ER-positive or negative tumors may be therapeutically useful by decreasing the survival of p53-defective cancer cells after DNA damage. There are therefore good reasons to conduct trials combining the re-expression of ER β following chemotherapy.

ER β itself may be involved in Tam-induced resistance because ER β expression increases the sensitivity of BC cells, by down-regulating the ErbB-2/ErbB-3/AKT signaling. Indeed, re-expression of ER β in MCF-7 and T47-D BC cells (both ER α^+ but ER β^-) decreases the formation of the ErbB-2/ErbB-3 receptor dimers and down-regulates their active regulator AKT, resulting in an increase sensitivity to Tam [77].

Several specific ER β ligands have been synthesized (**Supplemental figure S1**) and their anticancer activity is currently under investigations. It is not yet clear if stimulation of the transcription activity of ER β is of therapeutic relevance or if the capacity of ER β to heterodimerize with ER α is sufficient in itself to enhance the beneficial effects observed against BC proliferation and survival.

V.B. Membrane receptors and adaptor proteins

V.B. 1. Src kinase

Deregulation of the non-receptor c-Src cytoplasmic TK has been associated with many tumors including BC tumors, particularly in case of acquired resistance to treatments with either HT or anti-growth factors. Src and ER α together with PI3K are associated in several types of epithelial BC cells, where they form a complex, **involved in non-genomic pathway of E₂ induced cell proliferation** [78]. In some cases, resistance is accompanied by an invasive phenotype concomitant with an increase of Src kinase activity [79]. Src regulates the chemokine CXCL12/SDF-1, helping indolent BC cells to survive in the bone marrow. CXCL12/SDF-1 also up-regulates AKT expression, thus increasing survival and resistance to TRAIL death signals [80]. The use of the Src/Abl kinase inhibitor AZD0530 (**Supplemental figure S6**) was shown to synergize with Tam [81] or gefitinib (“Iressa”, an EGFR inhibitor) for suppressing the invasive phenotype at least *in vitro* [82]. The development of BEZ2235 (a dual nanomolar inhibitor of both PI3K and mTOR), is very promising for a new therapeutic approach

[83]. Altogether, these findings suggest that inhibiting Src activity is a potentially useful therapeutic strategy, which most likely operates via preventing dormant cells from becoming a source of future metastasis in the bone marrow. Due to the crosstalk between Src and methylated ER α [6], it is likely that combining Src kinase inhibitors with PRMT1 inhibitors may improve reduction of BC cells invasion and metastasis. Src is constitutively activated in Trastuzumab-resistant BC cells. Its targeting with specific inhibitors such as Saracitinib, re-sensitizes resistant BC tumors in xenografts to Trastuzumab [84]. This observation strongly supports the notion that combination of Src inhibitors with Erb-B2 targeted therapy would be beneficial.

V.B.2. The PI3 kinase/AKT pathway

The PI3K/ protein kinase B (AKT) pathway is a key regulator of cell proliferation and survival since PI3K-produced phospholipids favor the membrane recruitment of AKT, which is itself further phosphorylated (activated) by either the 3-phospho-inositide-dependent protein kinase 1 (PDK1) or by the Ric/TOR complex. Such a cascade of events is critical for cell cycle progression and suppression of apoptosis [40]. Importantly, ER α binds in an estrogen-dependent manner to the p85 α regulatory subunit of PI3K, leading to the activation of AKT and endothelial nitric oxide synthase (eNOS) [23]. This provides an explanation for the cardiovascular protective effects of estrogens. BC resistance to endocrine therapy can be associated with an invasive phenotype concomitantly with an increase of Src kinase activation and that of the mTOR intracellular signaling pathway [79]. Thus, targeting the Pi3K/AKT signaling must be considered as a prime strategy in cancer treatment, particularly in BC where there are evident connections with membrane ER α . Many signals emanating from the membrane, including E₂ binding to either GPER or membrane incorporated ER α leads to

phosphorylation of AKT after PI3K activation. As a consequence, cells are driven to progress in cell cycle and to survive (**figure 2**). In early studies, the mTOR inhibitor everolimus (**Supplemental figure S6**) added to endocrine therapy showed antitumor activity. Everolimus combined with an AI improved progression-free survival in patients with hormone-receptor-positive advanced BC previously treated with non steroidal AIs. Furthermore, expression of ER β in ER α -positive BC cells, such as MCF-7 and T47-D, results in a decrease of AKT signaling and down-regulation of HER2/HER3 dimers, concomitantly with a decrease of the natural inhibitor of AKT, PTEN [77]. These findings provide a strong support for a major relationship between several partners involved in resistance to AEs. They argue for initiatives to develop re-expression of ER β in BC cells in order to improve BC cells sensitivity to AE and/or AIs.

V.B.3 Chemokine receptors

Many solid tumors including BC express high levels of various chemokine receptors (review in [85]). In addition, many chemokines are produced in larger amounts by epithelial cancer cells than normal epithelial cells and also by the tumor microenvironment, resulting in enhanced tumor cell proliferation, migration, angiogenesis and then bone metastasis.

The production of a number of chemokines or their receptors in BC can be linked to ER pathway. CXCL8 is secreted by BC cells and its titer inversely correlates to ER levels [85]. Similar findings have been reported for several other chemokines including CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CCL2 and CCL4 in BC patients [86, 87]. One must note that, the weak expression of chemokines such as CXCL8 in ER-positive BC could be the result of histone deacetylase inhibition in such cells [88].

The activation of the CXCR4/CXCL12-SDF-1 (Stromal cell-Derived Factor-1) pathway (**figure 2**) has been also implicated in acquired Tam resistance. In ER-positive BC cells, the chemokine CXCL12 and one of its receptor CXCR4 are induced by estrogens [89]. This could

explain the positive correlation between CXCL12 and ER status in BC patients [90]. However, the regulation of CXCR4 by E₂ seems to be more controversial because another study did not find induction of CXCR4 by E₂ in wild-type MCF-7 cells, but observed E₂ induction in MCF-7 over-expressing Erb-B2 [91]. Significantly, CXCL12 and CXCR4 favor the hormone-independent growth of BC cells both *in vitro* and *in vivo* [89, 92]. Studies *in vivo* showed that CXCL12 can at least partially alleviate the anti-proliferative action of Faslodex implicating CXCL12 in hormone resistance [92]. E₂-induced transcriptional activation of the *SDF1* gene (and possibly other ER-regulated genes) occurs through both ERs isoforms. In turn, SDF1 interaction with its CXCR4 receptor may induce a “feed forward” loop, leading to phosphorylation of both ERs through Erk activation, a mechanism which could explain BC cell growth and Tam resistance [93]. Therefore, targeting CXCR4 (through the inhibitor AMD3100, see structure in **Supplemental figure S4**) and/or SDF1 could have a potential therapeutic usefulness.

V.B.4. The IGF axis

As described above, ligand activation of the IGF-1R and its downstream pathways (PI3K/AKT/mTOR and Ras/Raf/MEK/ERK) stimulates tumor proliferation, survival, transformation, metastasis and angiogenesis [94] (**figure 2**). In ER-positive BC cells, activation of IGF-1R can negatively affect the efficacy of both AEs and chemotherapy. Estrogens reinforce the responsiveness of BC cells to IGF by inducing the expression of IGF-1R and IRS-1; in turn, IGF/IGF-1R signaling can activate the Erk1/2 kinases, which can specifically phosphorylate ER α on ser418 and then activate ER-mediated transcription [95]. This constitutes a therapeutic potential in targeting the IGF-axis in BC. Indeed, inhibition of IGF-1R signaling is synergistic with endocrine therapy in preclinical models of ER-positive breast cancer. There have been many trials recently investigating IGF-1R as a possible cancer target. Major efforts have focused on the use of monoclonal antibodies against IGF-1R such as AMG-479 which blocks

IGF-1 ligand-mediated activation and small TK inhibitors directed against the IGF-1R TK domain [96, 97]. Several chemical molecules are currently under intense investigation and in different experimental phases [98]. Available data suggest that this class of compounds is well tolerated with mild to moderate side effects, when used alone or in combination with other therapeutic agents. In a recent work [99], it has been demonstrated that E₂ and IGF-1 down-regulate critical repressors of BC growth (like the key suppressor of tumorigenesis B-cell linker or *BLNK*) by independent mechanisms. This is of clinical significance because the restoration of BLNK expression may limit progression of the disease; this could be achieved by combining AE with anti-IGF-1 molecules. *In vivo*, the activity of IGF is regulated by its binding to IGF-binding proteins (IGFBP 1-6) which complex almost 99% of circulating IGF, thus serving as a reservoir for IGF. The development of a strategy consisting of maintaining this reservoir capacity to prevent the release of IGF and its subsequent activation of the IGF-1R is a novel possible approach to circumvent the detrimental effects of the IGF pathway on BC progression.

V.C HSP90

Following their synthesis in the ribosomes, all steroid receptors are associated in a multiprotein chaperone complex organized around Hsp90 [7] which helps to fold client proteins. This multistep folding process requires ATP binding to Hsp90 and other co-chaperones [100, 101]. HSP90 is necessary for ER and other NRs to display high affinity ligand binding and more generally for the full expression of the biological capacities of client proteins. HSP90 is a major player in the degradation through the ubiquitin-proteasome pathway of not only NRs, but also many oncogenic signaling proteins including ErbB2, c-Myc, AKT, Raf-1 and also mutated p53 (review in [102]). Many HSP90 inhibitors blocking the protein

in an ADP-binding form or the binding of ATP have been developed. These inhibitors disrupt client protein function and/or their degradation process and lead to apoptosis. Some of these inhibitors, notably geldanamycin (**Supplemental figure S7**) and several coumarin derivatives [103-105], are potential anticancer therapeutic agents due to their capacity to induce apoptosis in a large variety of cancer cells. However, **the multitude** of targets in all cells renders these molecules extremely toxic and their clinical use has not to date been authorized. However, their incorporation in nanodevices targeting BC **cells appears promising** in preclinical models (our unpublished work).

VI. Conclusions and future directions

Hormonal treatment of BC is **the first real** example of successful targeted therapy. The development of AE and of new AIs has considerably enhanced the efficacy of the treatments but long term post-treatment resistance often **develops**. Deciphering the mechanisms underlying this resistance has identified new ways to reduce re-start of cell proliferation and survival. This is particularly **true in case** of targets like HSP90 and HDACs for which a number of new inhibitors has been synthesized. The use of new humanized antibodies, other than herceptin, and targeting growth factor **receptors are also** promising. Several targets identified are of prime importance but are currently not accessible *in vivo*, **because appropriate chemical inhibitors are not available (Table 1)**. Possibly, the **targets** involved in enhancement of tumor progression could be manipulated through the use of silencing RNAs or dominant negative constructs, but **delivering** such agents to cancerous cells remains a major challenge. This is particularly **true in** the case of miRNAs. MiRNAs are a class of naturally occurring, small (19-25 nucleotides) non-coding RNA molecules. They interact with mRNAs in their 3'-untranslated region and block mRNA translation or target the transcripts to degradation. Several miRNAs

have been found in BC cells and some have been shown to be down-regulated by E₂, concomitantly with the enhanced expression of Bcl-2, cyclin D1 and survivin ([106] and references herein). Such miRNAs may also be ~~envisaged~~ considered as potential targets, although their way of administration is also challenging. Similar issues remain for targets, whose expression needs to be increased, **as for example, tumor suppressor genes**. The types of biological molecules, required for this goal (plasmids, oligonucleotides), are fragile and need to be protected against degradation, when injected in the body. They must also travel to reach a **concentration sufficient for obtaining a biological effect**. Current progress **justifies the development** of appropriate methodology for the delivery of such molecules and this has indeed been achieved with nanocarriers [110]. More than 150 molecules are currently the subject of work on encapsulation in stable and non toxic formulations. Immunotargeting of such nanocarriers based on recognizing an over-expressed marker of BC cells and carrying strong inhibitors of the cell cycle or inducers of apoptosis are amongst the most promising strategies. For example, Erb-B2 is over-expressed in a number of BC tumors, **particularly in those not responding to** classical HT, **accordingly** Trastuzumab has been used in the fabrication of Docinostat-containing devices. **These** immunoliposomes **substantially** enhance programmed-cell death in BT474 BC xenografts [107]. Trastuzumab has also been conjugated (Trastuzumab-emtansine) to DM1, an inhibitor of tubulin polymerisation and clinical trials showed that this agent is effective in patients with metastatic triple negative BC [108]. Reaching metastasis remains a major obstacle in cancer therapy and immune-nanocarriers and/or antibody-conjugated chemicals seem promising tools for this goal.

Combinations of several molecules, free (like the combination Vorinostat-Tam in patient with hormone-resistant BC [109] **or that of Tam with a Src inhibitor [110]**) or encapsulated in stealth or tumor recognizing **nanosystems**, are in clinical trials, but the doses

and sequence of administrations still need to be defined, because some combinations are incompatible, when these conditions are not precisely optimized. This is particularly true in case of HDACis injected in combinations with Hsp90 inhibitors (our unpublished results). We think that the development of combinations associating tumor-piloted nanosystems carrying anticancer agents should be undertaken to circumvent hormone-resistance in BC.

The development of gene strategies is also promising for BC treatment, as both positive re-activation of tumor suppressors, such as like ER β , LKB1 or wild-type p53 and also stopping expression of genes involved in tumor growth can be envisaged. This could be accomplished by the use of shRNA to silence, for example AKT, AIB-1, Bcl-2, or VEGF. Indeed, many trials are underway to study the use of antibodies targeting growth factor receptors and various inhibitors (TK, or HDAC, or others). However, we believe that effective treatments are more likely to emerge from the development of targeted chemical molecules, whether encapsulated in nanocarriers or linked to antibodies against proteins over-expressed by tumors for specific delivery to the tumor sites.

Acknowledgements: We thank V. Ogryzko, A. Campbell, J. Pierre and past and current members of our laboratories for helpful discussions and current comments on the manuscript. We also apologize to all those whose important work could not be cited owing to space limitations.

Legends of figures:

Figure 1: Classical mechanisms of ligand and phosphorylation-induced transcription in breast cancer cells and antiestrogen activities.

Estrogens like estradiol (E_2) and SERMs (like tamoxifen -Tam-) bind to ER associated in the HSP90-containing multi-chaperone complex, leading to the release of HSP90, dimerisation of ER and binding to DNA sequences. According to the “canonical model”, following E_2 -binding, ER dimers bind to specific 13bp DNA sequences of the estrogen receptor response element ERE (CGGGTCAnnnTGACCTG) characteristic of specific E_2 -regulated genes like *pS2*. DNA-bound ER-agonist complexes recruit several coactivators, some with histone acetyltransferase (HAT) activities, which allow repositioning of nucleosome and chromatin opening, then interaction with the general transcription machinery organized around RNA polymerase II; then transcription starts. Note that other sequences recognized by intermediate transcription factors (such as AP-1, SP-1) could serve as ER binding sites [111]. DNA-bound SERM-ER complexes (like Tam-ER) recruit corepressors and histone deacetylases (HDACs) promoting re-compaction of chromatin and arrest of transcription. However, corepressors may be recruited also by ER-agonist complexes. This is particularly the case of SMRT [112], showing that the quality of comodulators which contribute to enhancement of E_2 -induced transcription depends of the cellular and promoter context. Binding of pure anti-estrogens (like Fulvestrant and RU) leads to relocalisation of ER in the endoplasmic reticulum, where ER is ubiquitinated and rapidly degraded by the proteasome.

Figure 2: membrane emanating signals modifying estradiol responses.

E_2 may bind to ER localized at the membrane in interaction with Src and PI3K, which are thereby activated and in turn, activate mTOR through AKT phosphorylation by PI3K (a). In addition, activated AKT may phosphorylate both E_2 -free and -bound ER and enhance classical

genomic transcription. These effects enhance transcription of genes involved in cell cycle progression and survival. The E₂-activated GPER can trigger the PI3K/AKT/mTOR pathway (b), which is also activated through the enhancement of TK activity of homo- and hetero-dimers of growth factor receptors (b'). Another crosstalk has been established between GPER and EGFR (c), which enhances the activation of the MAPK pathway. The IGF axis (d) participates in the activation of cell proliferation, mainly in case of resistance to treatments and ER α is specifically activated by IGF-1R-induced phosphorylation (see text for details). The *SDF1* gene is activated by E₂ and the excreted SDF1 protein activates the chemokine receptor CXCR4 (e), a consequence also occurring from tamoxifen (Tam) binding to ER α . As a consequence, the MAPK pathway is activated and ER-mediated transcription may be enhanced in the absence of E₂. Inhibitions of some of the targets by chemical molecules currently in clinical trials (red marked labels) and antibodies (blue arrows), involved in these strongly interacted pathways are indicated. LY = LY294002.

Legends of Supplemental figures

Supplemental figure S1: Molecular structure of NR, GPER ligands, and aromatase inhibitors

Supplemental figure S2: IGF-1R tyrosine kinase inhibitors

Supplemental figures S3: Erb-B2/HER tyrosine kinase inhibitors

Supplemental figure S4: Structures of Gossypol (SRC 1-3 inhibitor), AMI1 (PRMT1 inhibitor) and AMD3100-Plerixafor (CXCR4 inhibitor)

Supplemental figure S5: Structures of HDAC inhibitors

Supplemental figure S6: Structures of some Src (a, b), PI3K/AKT (c, d, e) and mTOR (f) inhibitors. Supplemental figure 7: Hsp90 inhibitors

Legend of table

Table 1: targets impacting ER-mediated functions in breast cancer cells.

Bibliography

1. Ascenzi, P., A. Bocedi, and M. Marino, *Structure-function relationship of estrogen receptor alpha and beta: impact on human health*. Mol Aspects Med, 2006. **27**(4): p. 299-402.
2. Ali, S. and R.C. Coombes, *Estrogen receptor alpha in human breast cancer: occurrence and significance*. J Mammary Gland Biol Neoplasia, 2000. **5**(3): p. 271-81.
3. Helguero, L.A., et al., *Estrogen receptors alpha (ERalpha) and beta (ERbeta) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11*. Oncogene, 2005. **24**(44): p. 6605-16.
4. Nagata, Y., et al., *PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients*. Cancer Cell, 2004. **6**(2): p. 117-27.
5. Hynes, N.E. and H.A. Lane, *ERBB receptors and cancer: the complexity of targeted inhibitors*. Nat Rev Cancer, 2005. **5**(5): p. 341-54.
6. Le Romancer, M., et al., *Cracking the Estrogen Receptor's Posttranslational Code in Breast Tumors*. Endocr Rev, 2011. **32**(5): p. 597-622.
7. Pratt, W.B. and D.O. Toft, *Steroid receptor interactions with heat shock protein and immunophilin chaperones*. Endocr Rev, 1997. **18**(3): p. 306-60.
8. Heldring, N., et al., *Estrogen receptors: how do they signal and what are their targets*. Physiol Rev, 2007. **87**(3): p. 905-31.
9. Metivier, R., et al., *Estrogen receptor-alpha directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter*. Cell, 2003. **115**(6): p. 751-63.
10. Lonard, D.M. and B.W. O'Malley, *Nuclear receptor coregulators: judges, juries, and executioners of cellular regulation*. Mol Cell, 2007. **27**(5): p. 691-700.
11. Ogryzko, V.V., et al., *The transcriptional coactivators p300 and CBP are histone acetyltransferases*. Cell, 1996. **87**(5): p. 953-9.
12. Yang, X.J., et al., *A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A*. Nature, 1996. **382**(6589): p. 319-24.
13. O'Malley, B.W., *Coregulators: from whence came these "master genes"*. Mol Endocrinol, 2007. **21**(5): p. 1009-13.
14. Musgrove, E.A., et al., *Cyclin D as a therapeutic target in cancer*. Nat Rev Cancer, 2011. **11**(8): p. 558-72.
15. Paruthiyil, S., et al., *Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest*. Cancer Res, 2004. **64**(1): p. 423-8.
16. Strom, A., et al., *Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast cancer cell line T47D*. Proc Natl Acad Sci U S A, 2004. **101**(6): p. 1566-71.
17. Gougelet, A., et al., *Estrogen receptor alpha and beta subtype expression and transactivation capacity are differentially affected by receptor-, hsp90- and immunophilin-ligands in human breast cancer cells*. J Steroid Biochem Mol Biol, 2005. **94**(1-3): p. 71-81.
18. Grober, O.M., et al., *Global analysis of estrogen receptor beta binding to breast cancer cell genome reveals an extensive interplay with estrogen receptor alpha for target gene regulation*. BMC Genomics, 2011. **12**: p. 36.
19. Matthews, J. and J.A. Gustafsson, *Estrogen signaling: a subtle balance between ER alpha and ER beta*. Mol Interv, 2003. **3**(5): p. 281-92.
20. Bouclier, C., et al., *Coadministration of nanosystems of short silencing RNAs targeting oestrogen receptor alpha and anti-oestrogen synergistically induces tumour growth inhibition in human breast cancer xenografts*. Breast Cancer Res Treat, 2010. **122**(1): p. 145-58.
21. Pequeux, C., et al., *Stromal Estrogen Receptor-alpha Promotes Tumor Growth by Normalizing an Increased Angiogenesis*. Cancer Res, 2012. **72**(12): p. 3010-9.
22. Levin, E.R. and R.J. Pietras, *Estrogen receptors outside the nucleus in breast cancer*. Breast Cancer Res Treat, 2008. **108**(3): p. 351-61.
23. Simoncini, T., et al., *Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase*. Nature, 2000. **407**(6803): p. 538-41.

24. Sotgia, F., et al., *Caveolin-1, mammary stem cells, and estrogen-dependent breast cancers*. *Cancer Res*, 2006. **66**(22): p. 10647-51.
25. Bjornstrom, L. and M. Sjoberg, *Estrogen receptor-dependent activation of AP-1 via non-genomic signalling*. *Nucl Recept*, 2004. **2**(1): p. 3.
26. Marino, M., et al., *Distinct nongenomic signal transduction pathways controlled by 17beta-estradiol regulate DNA synthesis and cyclin D(1) gene transcription in HepG2 cells*. *Mol Biol Cell*, 2002. **13**(10): p. 3720-9.
27. Kang, L., et al., *Involvement of estrogen receptor variant ER-alpha36, not GPR30, in nongenomic estrogen signaling*. *Mol Endocrinol*, 2010. **24**(4): p. 709-21.
28. Filardo, E.J. and P. Thomas, *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): p. 2953-62.
29. Filardo, E.J., et al., *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): p. 70-84.
30. Dennis, M.K., et al., *Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity*. *J Steroid Biochem Mol Biol*, 2011. **127**(3-5): p. 358-66.
31. Lappano, R., et al., *MIBE acts as antagonist ligand of both estrogen receptor alpha and GPER in breast cancer cells*. *Breast Cancer Res*, 2012. **14**(1): p. R12.
32. Jordan, V.C., *SERMs: meeting the promise of multifunctional medicines*. *J Natl Cancer Inst*, 2007. **99**(5): p. 350-6.
33. Lonard, D.M., et al., *The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation*. *Mol Cell*, 2000. **5**(6): p. 939-48.
34. Marsaud, V., et al., *Various phosphorylation pathways, depending on agonist and antagonist binding to endogenous estrogen receptor alpha (ERalpha), differentially affect ERalpha extractability, proteasome-mediated stability, and transcriptional activity in human breast cancer cells*. *Mol Endocrinol*, 2003. **17**(10): p. 2013-27.
35. Ali, S. and R.C. Coombes, *Endocrine-responsive breast cancer and strategies for combating resistance*. *Nat Rev Cancer*, 2002. **2**(2): p. 101-12.
36. (EBCTCG), E.b.c.t.c.g., *Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials*. *Lancet*, 2005. **365**(9472): p. 1687-717.
37. Henson, E.S. and S.B. Gibson, *Surviving cell death through epidermal growth factor (EGF) signal transduction pathways: implications for cancer therapy*. *Cell Signal*, 2006. **18**(12): p. 2089-97.
38. Wang, Y., et al., *Small molecule inhibition of the steroid receptor coactivators, SRC-3 and SRC-1*. *Mol Endocrinol*, 2011. **25**(12): p. 2041-53.
39. Goodwin, P.J., *Insulin in the adjuvant breast cancer setting: a novel therapeutic target for lifestyle and pharmacologic interventions?* *J Clin Oncol*, 2008. **26**(6): p. 833-4.
40. Manning, B.D. and L.C. Cantley, *AKT/PKB signaling: navigating downstream*. *Cell*, 2007. **129**(7): p. 1261-74.
41. Hutcheson, I.R., et al., *Fulvestrant-induced expression of ErbB3 and ErbB4 receptors sensitizes oestrogen receptor-positive breast cancer cells to heregulin beta1*. *Breast Cancer Res*, 2011. **13**(2): p. R29.
42. Sutherland, R.L., *Endocrine resistance in breast cancer: new roles for ErbB3 and ErbB4*. *Breast Cancer Res*, 2011. **13**(3): p. 106.
43. Van de Velde, P., et al., *Exploration of the therapeutic potential of the antiestrogen RU 58668 in breast cancer treatment*. *Ann N Y Acad Sci*, 1995. **761**: p. 164-75.
44. Menendez, J.A., I. Mehmi, and R. Lupu, *Trastuzumab in combination with heregulin-activated Her-2 (erbB-2) triggers a receptor-enhanced chemosensitivity effect in the absence of Her-2 overexpression*. *J Clin Oncol*, 2006. **24**(23): p. 3735-46.

45. Musgrove, E.A. and R.L. Sutherland, *Biological determinants of endocrine resistance in breast cancer*. Nat Rev Cancer, 2009. **9**(9): p. 631-43.
46. Kaklamani, V.G., et al., *Pilot neoadjuvant trial in HER2 positive breast cancer with combination of nab-paclitaxel and lapatinib*. Breast Cancer Res Treat, 2012. **132**(3): p. 833-42.
47. Fereshteh, M.P., et al., *The nuclear receptor coactivator amplified in breast cancer-1 is required for Neu (ErbB2/HER2) activation, signaling, and mammary tumorigenesis in mice*. Cancer Res, 2008. **68**(10): p. 3697-706.
48. Lahusen, T., et al., *The role and regulation of the nuclear receptor co-activator AIB1 in breast cancer*. Breast Cancer Res Treat, 2009. **116**(2): p. 225-37.
49. Urbinati, G., et al., *Liposomes loaded with histone deacetylase inhibitors for breast cancer therapy*. Int J Pharm, 2010. **397**(1-2): p. 184-93.
50. Duong, V., et al., *ERalpha and ERbeta expression and transcriptional activity are differentially regulated by HDAC inhibitors*. Oncogene, 2006. **25**(12): p. 1799-806.
51. Zhou, Q., P. Atadja, and N.E. Davidson, *Histone deacetylase inhibitor LBH589 reactivates silenced estrogen receptor alpha (ER) gene expression without loss of DNA hypermethylation*. Cancer Biol Ther, 2007. **6**(1): p. 64-9.
52. Jang, E.R., et al., *The histone deacetylase inhibitor trichostatin A sensitizes estrogen receptor alpha-negative breast cancer cells to tamoxifen*. Oncogene, 2004. **23**(9): p. 1724-36.
53. Minucci, S. and P.G. Pelicci, *Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer*. Nat Rev Cancer, 2006. **6**(1): p. 38-51.
54. Margueron, R., et al., *Oestrogen receptor alpha increases p21(WAF1/CIP1) gene expression and the antiproliferative activity of histone deacetylase inhibitors in human breast cancer cells*. J Endocrinol, 2003. **179**(1): p. 41-53.
55. Duong, V., et al., *Specific activity of class II histone deacetylases in human breast cancer cells*. Mol Cancer Res, 2008. **6**(12): p. 1908-19.
56. Lafarga, V., et al., *A novel GRK2/HDAC6 interaction modulates cell spreading and motility*. Embo J, 2011. **31**(4): p. 856-69.
57. Hurtado, A., et al., *Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen*. Nature, 2008. **456**(7222): p. 663-6.
58. Hurtado, A., et al., *FOXA1 is a key determinant of estrogen receptor function and endocrine response*. Nat Genet, 2011. **43**(1): p. 27-33.
59. Ross-Innes, C.S., et al., *Differential oestrogen receptor binding is associated with clinical outcome in breast cancer*. Nature, 2012. **481**(7381): p. 389-93.
60. Ramamoorthy, S., et al., *Overexpression of ligase defective E6-associated protein, E6-AP, results in mammary tumorigenesis*. Breast Cancer Res Treat, 2012. **132**(1): p. 97-108.
61. Le Romancer, M., et al., *Regulation of estrogen rapid signaling through arginine methylation by PRMT1*. Mol Cell, 2008. **31**(2): p. 212-21.
62. Zeng, P.Y. and S.L. Berger, *LKB1 is recruited to the p21/WAF1 promoter by p53 to mediate transcriptional activation*. Cancer Res, 2006. **66**(22): p. 10701-8.
63. Zhuang, Z.G., et al., *Enhanced expression of LKB1 in breast cancer cells attenuates angiogenesis, invasion, and metastatic potential*. Mol Cancer Res, 2006. **4**(11): p. 843-9.
64. Hardie, D.G., *AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy*. Nat Rev Mol Cell Biol, 2007. **8**(10): p. 774-85.
65. Nath-Sain, S. and P.A. Marignani, *LKB1 catalytic activity contributes to estrogen receptor alpha signaling*. Mol Biol Cell, 2009. **20**(11): p. 2785-95.
66. Linher-Melville, K., S. Zantinge, and G. Singh, *Liver kinase B1 expression (LKB1) is repressed by estrogen receptor alpha (ERalpha) in MCF-7 human breast cancer cells*. Biochem Biophys Res Commun, 2012. **417**(3): p. 1063-8.
67. Brown, K.A., et al., *Metformin inhibits aromatase expression in human breast adipose stromal cells via stimulation of AMP-activated protein kinase*. Breast Cancer Res Treat, 2010. **123**(2): p. 591-6.

68. Taliaferro-Smith, L., et al., *LKB1 is required for adiponectin-mediated modulation of AMPK-S6K axis and inhibition of migration and invasion of breast cancer cells*. *Oncogene*, 2009. **28**(29): p. 2621-33.
69. Wu, X., et al., *HOXB7, a homeodomain protein, is overexpressed in breast cancer and confers epithelial-mesenchymal transition*. *Cancer Res*, 2006. **66**(19): p. 9527-34.
70. Jin, K., et al., *The HOXB7 protein renders breast cancer cells resistant to tamoxifen through activation of the EGFR pathway*. *Proc Natl Acad Sci U S A*, 2012. **109**(8): p. 2736-41.
71. Holmes, K.A., et al., *Transducin-like enhancer protein 1 mediates estrogen receptor binding and transcriptional activity in breast cancer cells*. *Proc Natl Acad Sci U S A*, 2012. **109**(8): p. 2748-53.
72. Couse, J.F. and K.S. Korach, *Estrogen receptor null mice: what have we learned and where will they lead us?* *Endocr Rev*, 1999. **20**(3): p. 358-417.
73. Lazennec, G., et al., *ER beta inhibits proliferation and invasion of breast cancer cells*. *Endocrinology*, 2001. **142**(9): p. 4120-30.
74. Hartman, J., et al., *Estrogen receptor beta inhibits angiogenesis and growth of T47D breast cancer xenografts*. *Cancer Res*, 2006. **66**(23): p. 11207-13.
75. Lazennec, G., *Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis*. *Cancer Lett*, 2006. **231**(2): p. 151-7.
76. Hodges-Gallagher, L., et al., *Estrogen receptor beta increases the efficacy of antiestrogens by effects on apoptosis and cell cycling in breast cancer cells*. *Breast Cancer Res Treat*, 2008. **109**(2): p. 241-50.
77. Lindberg, K., et al., *Estrogen receptor beta represses Akt signaling in breast cancer cells via downregulation of HER2/HER3 and upregulation of PTEN: implications for tamoxifen sensitivity*. *Breast Cancer Res*, 2011. **13**(2): p. R43.
78. Castoria, G., et al., *PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells*. *Embo J*, 2001. **20**(21): p. 6050-9.
79. Hiscox, S., et al., *Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells*. *Breast Cancer Res Treat*, 2006. **97**(3): p. 263-74.
80. Zhang, X.H., et al., *Latent bone metastasis in breast cancer tied to Src-dependent survival signals*. *Cancer Cell*, 2009. **16**(1): p. 67-78.
81. Herynk, M.H., et al., *Cooperative action of tamoxifen and c-Src inhibition in preventing the growth of estrogen receptor-positive human breast cancer cells*. *Mol Cancer Ther*, 2006. **5**(12): p. 3023-31.
82. Hiscox, S., et al., *Src as a therapeutic target in anti-hormone/anti-growth factor-resistant breast cancer*. *Endocr Relat Cancer*, 2006. **13 Suppl 1**: p. S53-9.
83. Wander, S.A., B.T. Hennessy, and J.M. Slingerland, *Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy*. *J Clin Invest*, 2011. **121**(4): p. 1231-41.
84. Zhang, S., et al., *Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways*. *Nat Med*, 2011. **17**(4): p. 461-9.
85. Lazennec, G. and A. Richmond, *Chemokines and chemokine receptors: new insights into cancer-related inflammation*. *Trends Mol Med*, 2010. **16**(3): p. 133-44.
86. Bieche, I., et al., *CXC chemokines located in the 4q21 region are up-regulated in breast cancer*. *Endocr Relat Cancer*, 2007. **14**(4): p. 1039-52.
87. Chavey, C., et al., *Oestrogen receptor negative breast cancers exhibit high cytokine content*. *Breast Cancer Res*, 2007. **9**(1): p. R15.
88. Chavey, C., et al., *Interleukin-8 expression is regulated by histone deacetylases through the nuclear factor-kappaB pathway in breast cancer*. *Mol Pharmacol*, 2008. **74**(5): p. 1359-66.
89. Boudot, A., et al., *Differential estrogen-regulation of CXCL12 chemokine receptors, CXCR4 and CXCR7, contributes to the growth effect of estrogens in breast cancer cells*. *PLoS One*, 2011. **6**(6): p. e20898.

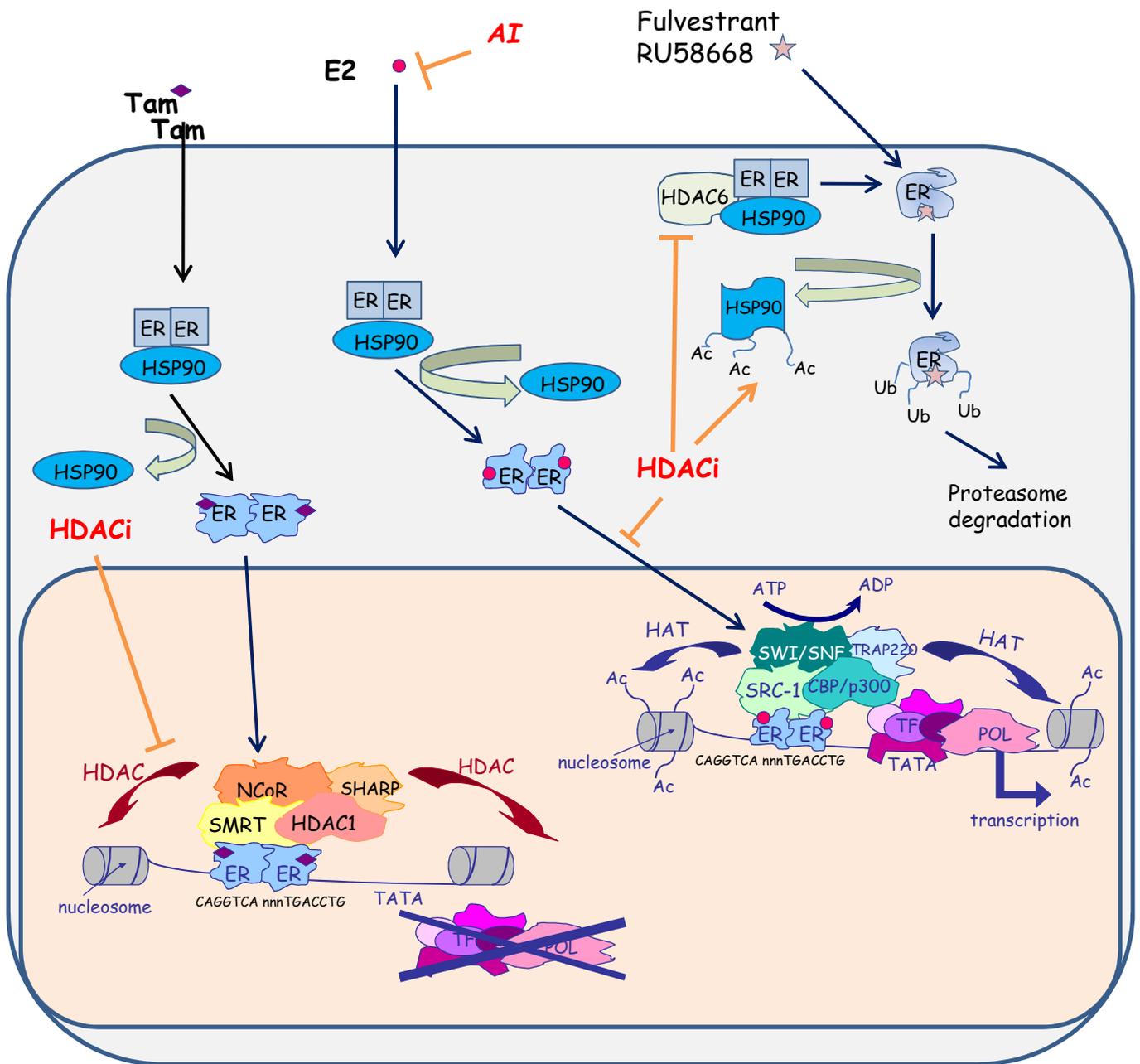
90. Kobayashi, T., et al., *Expression pattern of stromal cell-derived factor-1 chemokine in invasive breast cancer is correlated with estrogen receptor status and patient prognosis*. *Breast Cancer Res Treat*, 2010. **123**(3): p. 733-45.
91. Sengupta, S., R. Schiff, and B.S. Katzenellenbogen, *Post-transcriptional regulation of chemokine receptor CXCR4 by estrogen in HER2 overexpressing, estrogen receptor-positive breast cancer cells*. *Breast Cancer Res Treat*, 2009. **117**(2): p. 243-51.
92. Rhodes, L.V., et al., *Cytokine receptor CXCR4 mediates estrogen-independent tumorigenesis, metastasis, and resistance to endocrine therapy in human breast cancer*. *Cancer Res*, 2011. **71**(2): p. 603-13.
93. Sauve, K., et al., *Positive feedback activation of estrogen receptors by the CXCL12-CXCR4 pathway*. *Cancer Res*, 2009. **69**(14): p. 5793-800.
94. Pollak, M., *Insulin and insulin-like growth factor signalling in neoplasia*. *Nat Rev Cancer*, 2008. **8**(12): p. 915-28.
95. Jones, J.I. and D.R. Clemmons, *Insulin-like growth factors and their binding proteins: biological actions*. *Endocr Rev*, 1995. **16**(1): p. 3-34.
96. Law, J.H., et al., *Phosphorylated insulin-like growth factor-i/insulin receptor is present in all breast cancer subtypes and is related to poor survival*. *Cancer Res*, 2008. **68**(24): p. 10238-46.
97. Lisztwan, J., et al., *The aromatase inhibitor letrozole and inhibitors of insulin-like growth factor I receptor synergistically induce apoptosis in in vitro models of estrogen-dependent breast cancer*. *Breast Cancer Res*, 2008. **10**(4): p. R56.
98. Gao, J., et al., *Targeting the Insulin-like Growth Factor Axis for the Development of Novel Therapeutics in Oncology*. *Cancer Res*, 2012. **72**(1): p. 3-12.
99. Casa, A.J., et al., *Estrogen and insulin-like growth factor-I (IGF-I) independently down-regulate critical repressors of breast cancer growth*. *Breast Cancer Res Treat*, 2012. **132**(1): p. 61-73.
100. Renoir, J.M., et al., *The non-DNA-binding heterooligomeric form of mammalian steroid hormone receptors contains a hsp90-bound 59-kilodalton protein*. *J Biol Chem*, 1990. **265**(18): p. 10740-5.
101. Freeman, B.C., et al., *The p23 molecular chaperones act at a late step in intracellular receptor action to differentially affect ligand efficacies*. *Genes Dev*, 2000. **14**(4): p. 422-34.
102. Neckers, L., *Heat shock protein 90: the cancer chaperone*. *J Biosci*, 2007. **32**(3): p. 517-30.
103. Blagg, B.S. and T.D. Kerr, *Hsp90 inhibitors: small molecules that transform the Hsp90 protein folding machinery into a catalyst for protein degradation*. *Med Res Rev*, 2006. **26**(3): p. 310-38.
104. Radanyi, C., et al., *Antiproliferative and apoptotic activities of tosylcyclonovobiocic acids as potent heat shock protein 90 inhibitors in human cancer cells*. *Cancer Lett*, 2009. **274**(1): p. 88-94.
105. Audisio, D., et al., *Discovery and biological activity of 6BrCaQ as an inhibitor of the Hsp90 protein folding machinery*. *ChemMedChem*, 2011. **6**(5): p. 804-15.
106. Yu, X., et al., *Induction of cell proliferation and survival genes by estradiol-repressed microRNAs in breast cancer cells*. *BMC Cancer*, 2012. **12**: p. 29.
107. Drummond, D.C., et al., *Enhanced pharmacodynamic and antitumor properties of a histone deacetylase inhibitor encapsulated in liposomes or ErbB2-targeted immunoliposomes*. *Clin Cancer Res*, 2005. **11**(9): p. 3392-401.
108. Krop, I.E., et al., *A Phase II Study of Trastuzumab Emtansine in Patients With Human Epidermal Growth Factor Receptor 2 -Positive Metastatic Breast Cancer Who Were Previously Treated With Trastuzumab, Lapatinib, an Anthracycline, a Taxane, and Capecitabine*. *J Clin Oncol*, 2012.
109. Munster, P.N., et al., *A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer*. *Br J Cancer*, 2011. **104**(12): p. 1828-35.
110. Anbalagan, M., et al., *KX-01, a novel Src kinase inhibitor directed toward the peptide substrate site, synergizes with tamoxifen in estrogen receptor alpha positive breast cancer*. *Breast Cancer Res Treat*, 2012. **132**(2): p. 391-409.

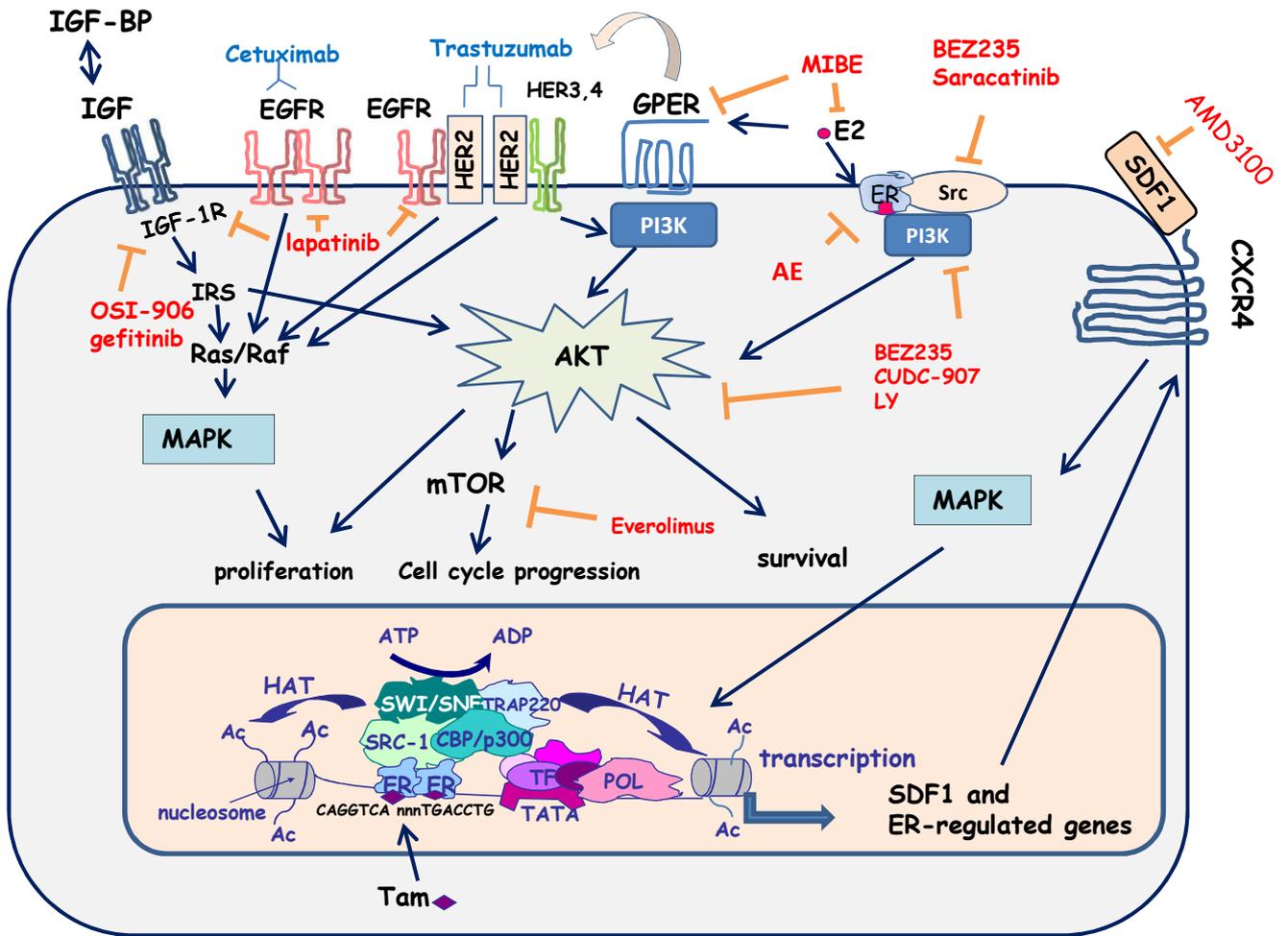
111. Paech, K., et al., *Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites*. *Science*, 1997. **277**(5331): p. 1508-10.
112. Peterson, T.J., et al., *The silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) corepressor is required for full estrogen receptor alpha transcriptional activity*. *Mol Cell Biol*, 2007. **27**(17): p. 5933-48.
113. Urbinati, G., et al. *Nanocarriers targeting breast cancers to deliver modulators of estrogen receptor*. IN: *Lipid nanocarriers in cancer diagnosis and therapy*. Akron, Ohio: iSmithers Creative, Souto E.B., ed. Publishing Solutions, 2011; **10**:279-30.

110. Urbinati, G., et al. *Nanocarriers targeting breast cancers to deliver modulators of estrogen receptor*. IN: *Lipid nanocarriers in cancer diagnosis and therapy*. Akron, Ohio: iSmithers Creative, Souto E.B., ed. Publishing Solutions, 2011; **10**:279-30.

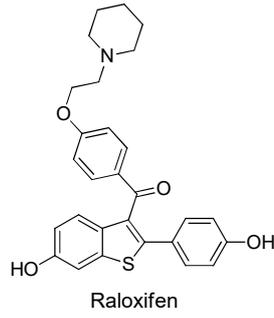
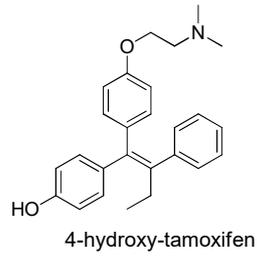
Targets	activity	Molecules/ Gene manipulation
ERβ	ER α dominant negative	No specific ligand known to arrest BC cell growth. Over-expression by gene transfer
HDAC	Histone deacetylase	TSA ; M344 ; BML210 ; Panabinstat ; Mocetinostat ; Docinostat ; Entinostat ; Resveratrol ; Romidepsin
SRC1-3	ER α Co-act	Gossypol
E6AP	ER α Co-act, E3-ubiquitin ligase	No drug known. Over-expression by gene transfer
PRMT1	Arginine methyl-transferase	No drug known; siRNA transfer
LKB1	ER α Co-act; ser/Thr kinase	Metformin, over-expression
HOXB7	Transcription factor	No drug known ; siRNA transfer
TLE1	Modulator of ER α -mediated transcription	No drug known; siRNA transfer
PAX2	Mediator of ER α -mediated repression of Erb-B2 by Tam	No drug known; siRNA transfer
FOXA1	Regulator of ER α -chromatin interaction	No drug known; siRNA transfer
PI3K/AKT	Ser/thr kinase,	LY2940002, BEZ235, CUDC-907
Src	Tyr kinase	Dasatinib, Bosotunib, Saracatinib, BEZ235
mTOR	Ser/thr kinase,	Everolimus, Temsirolimus
IGF-1R	Tyr kinase	OSI-906; ADW742; BMS554417; PQIP; BMS754807
GPER	Membrane E2-binding receptor	MIBE, G15, G16
HER	Growth factor membrane receptorTyr kinase,	Lapatinib, Gefitinib, Erlotinib
Hsp90	Molecular chaperone	17AAG; Radicicol; Ku 398; 4TCNA; 6BrCaQ
CXCR4/CXCL1 2 (SDF1)	ER α -enhanced mitogenic pathway	AMD3100; CXCR4 dominant negative over-expression or siRNA transfer

Table 1:

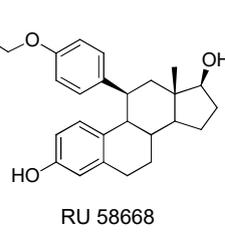
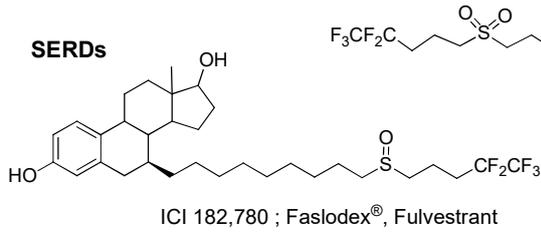




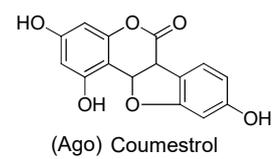
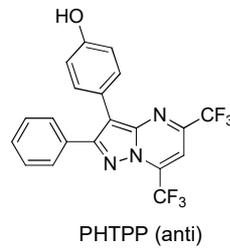
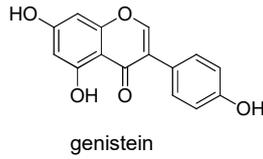
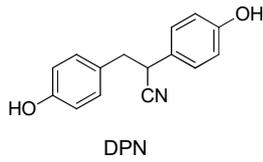
SERMs



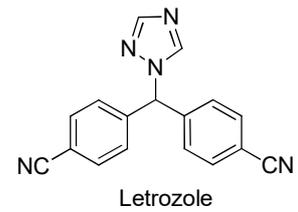
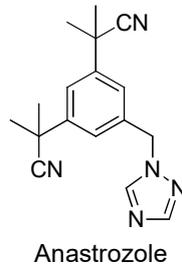
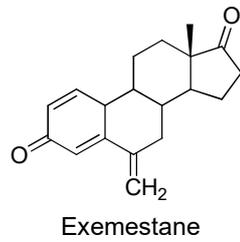
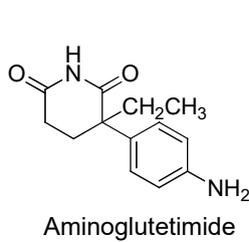
SERDs



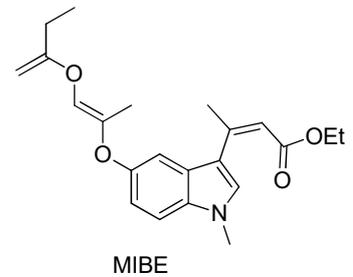
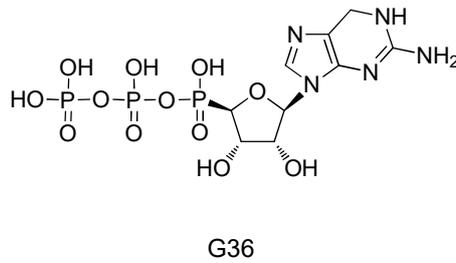
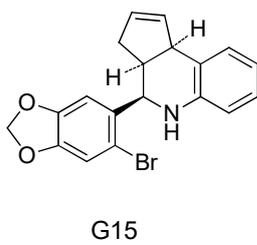
ER β Ligands



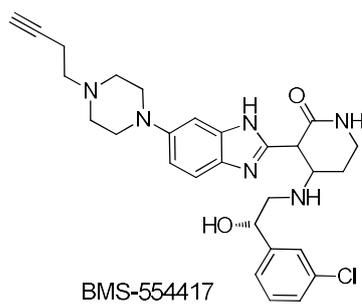
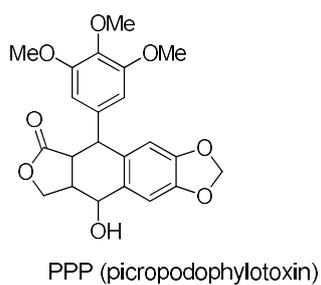
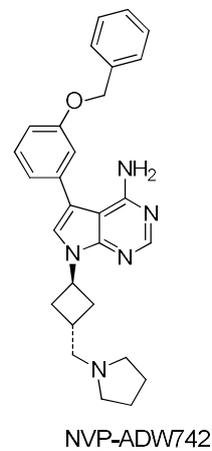
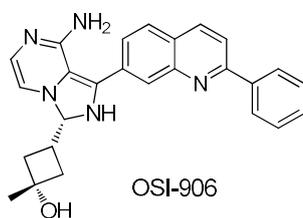
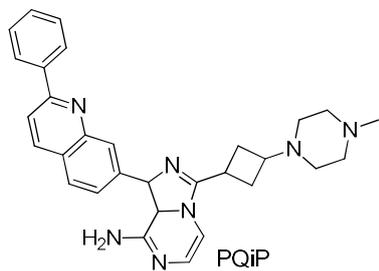
Aromatase inhibitors



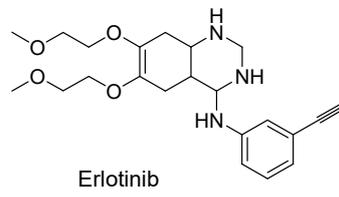
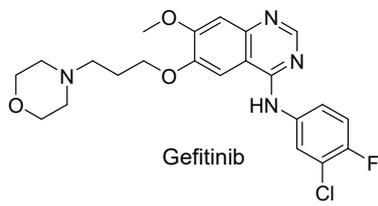
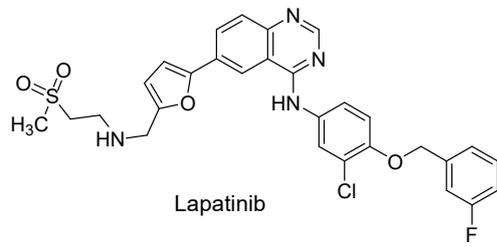
GPER ligands



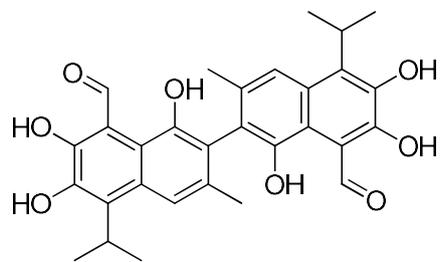
Supplemental figure 1:



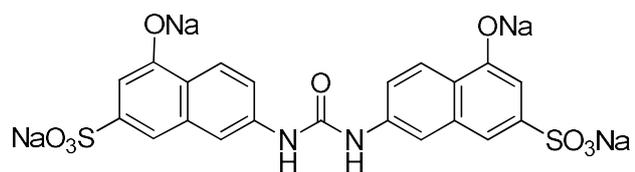
Supplemental figure 2:



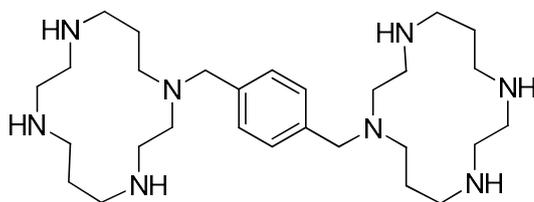
Supplemental figure 3:



Gossypol
(SRC1-3/AIB1)

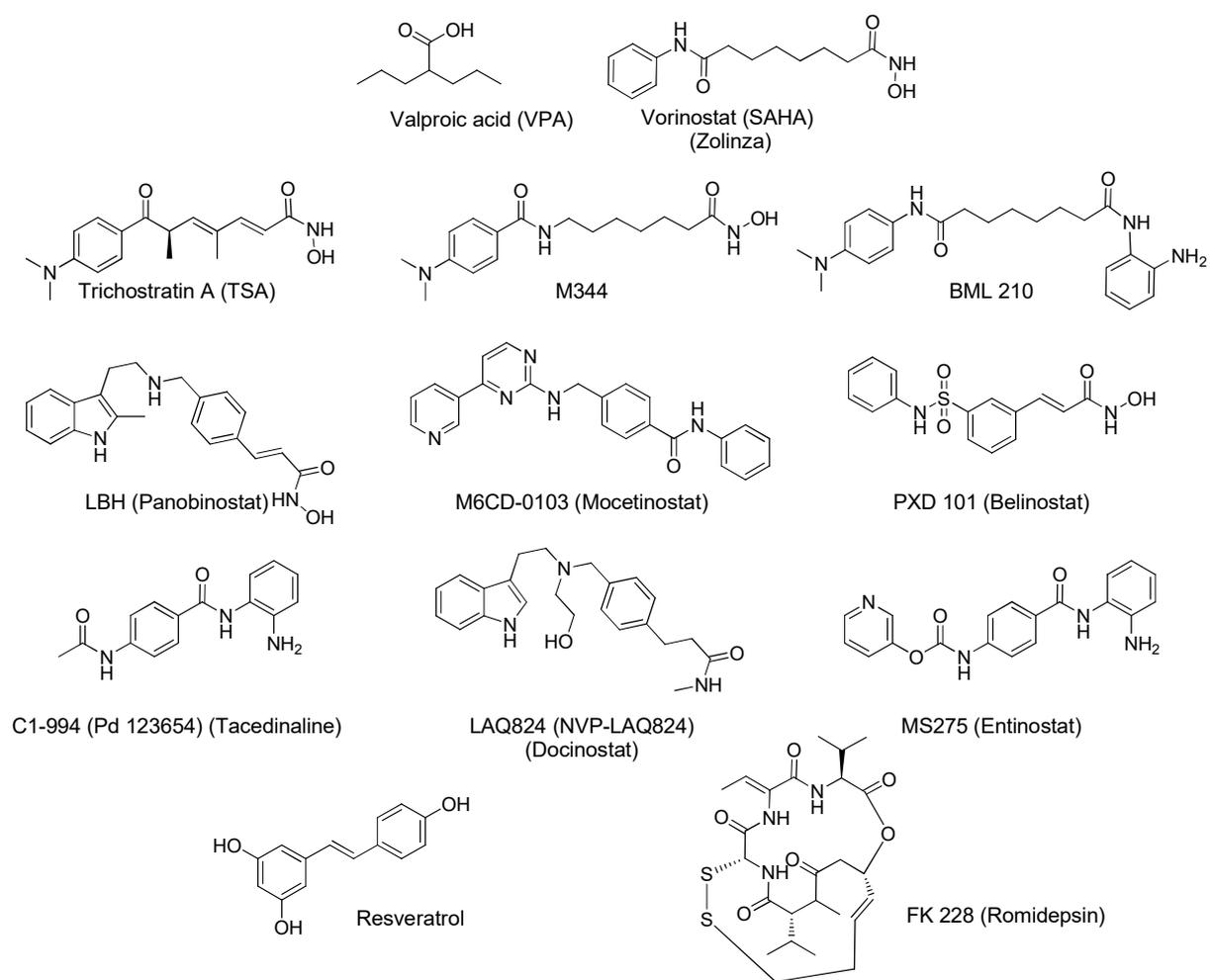


AMI1
(PRMT1)

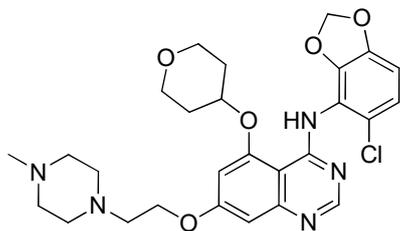


AMD3100
(CXCR4)

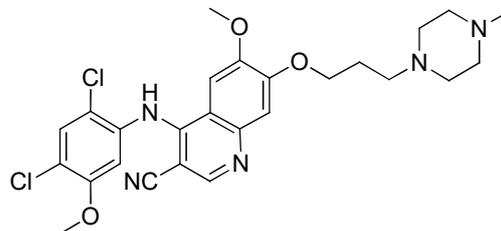
Supplemental figure 4:



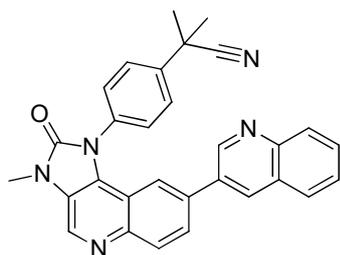
Supplemental figure 5:



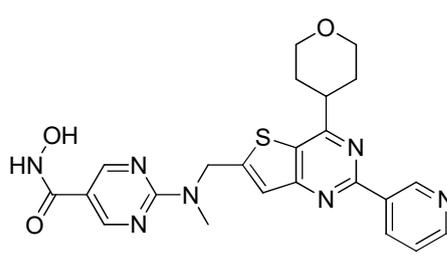
a) AZD 0530- Saracatinib
(Src/Bcr-Abl)



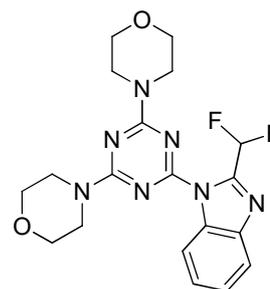
b) Bosotunib



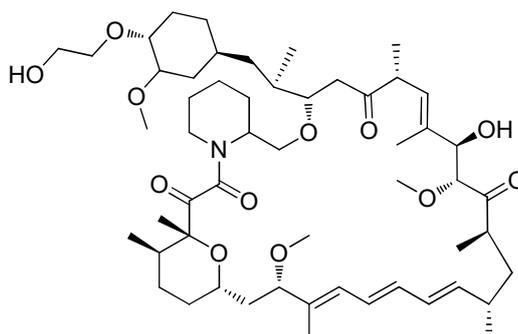
c) NVP - BEZ 235
(PI3K - mTOR)



d) CUDC907
(PI3K and HDAC)

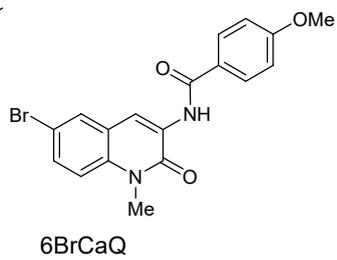
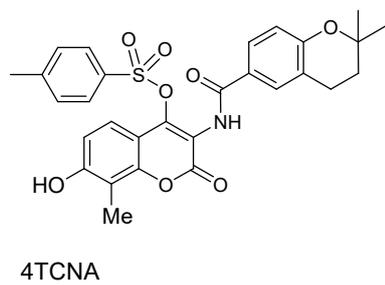
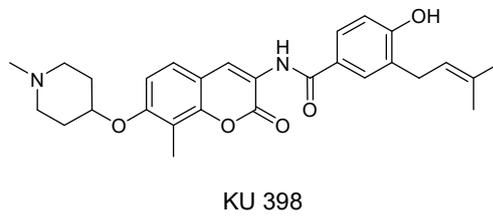
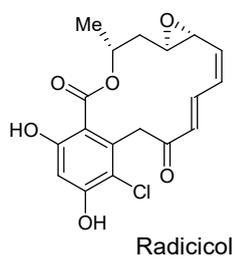
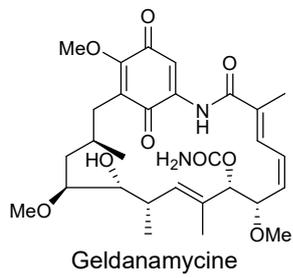


e) ZSTK474



f) Everolimus
(mTOR)

Supplemental figure 6:



Supplemental figure 7: