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Overview of current and future targets of breast cancer medicines

Eri Amalia[1*],Ajeng Diantini1,Anas Subarnas[1]

[1]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Jl. Raya Bandung-Sumedang km.21 Jatinangor - 45363, Indonesia.*

Abstract

Nowadays, many drugs are available for the treatment of breast cancer disease. Particular drugs are applied depending on the types of cancer cells known as Luminal-A, Luminal-B, Basal-like, HER2+, and Triple Negative Breast Cancer. This study summarizes those specific actions, also reveal one of new validated target therapy which beneficial for drug discovery of breast cancer medicine.

Method: We did an analysis of breast cancer medicines from first approved by the US FDA in 1953 until May 2019. The drugs were identified in term of its mechanism of action and binding site to inhibit cancer cell proliferation.

Result: To date, 33 drugs had been approved by the US FDA for breast cancer treatment. Thirteen drugs are known as chemotherapy drugs, with the mechanism of action as an alkylating agent, Dihydrofolate reductase– thymidylate synthetase inhibitor, and microtubulin inhibitor. Twelve drugs are known as targeted therapy including three monoclonal antibody targeting HER2/ErbB2 and a monoclonal antibody inhibit the interaction of programmed cell death protein-1/programmed cell death-ligand 1, and eight small molecule inhibitors are targeting intracellular enzyme including a tyrosine kinase, Cyclin-Dependent Kinase 4/6, Poly(Adenosine Diphosphate-Ribose) Polymerase-1. Eight drugs are hormonal therapy to reduced estrogen level and block the growth of cancer cell.

Conclusion: We found that the roadmap of the breast cancer treatment approach is changing from chemotherapy into targeted therapy both as small molecule inhibitor and monoclonal antibody. Meanwhile, hormonal treatment remains useful for cancer women with hormonal dysregulation. Alternatively, the discovery of breast cancer drugs targeting Dihydroorotate dehydrogenase is promising to be developed in the future.

Keywords: Breast cancer, Dihydroorotate dehydrogenase, CDK4/6, PARP, HER2/ErbB2, Tyrosine kinase, mTOR, Aromatase, monoclonal antibody, PD-L1.

BACKGROUND

The number of new cases of Breast cancer patients increasing each year reported as 1 in 8 women in their life. The risk is due to increase in life expectancy and affected by several factors including inherited genetic alteration of BRCA1/BRCA2, and breast cancer recovered history, a woman with 25-50% or more breast density, taller woman, menstrual cycles, higher bone mineral density in postmenopausal women and high level of endogenous hormone levels. Other risks are related to reproductive factors including pregnancy, use of fertility drugs, breastfeeding history, use of hormonal birth control, and postmenopausal hormones. Obesity, less physical activity, alcohol intake, tobacco consumption as well as the environmental condition including a residue of atomic bomb radiation exposure or other sources of radiation including medical treatment, diethylstilbestrol exposure, and night shift work due to reduction of melatonin production, also may induce risk factors [1,2,3].

The disease is classified as five significant subtypes are known as Luminal-A (ER+/HER2-), Luminal-B (ER+/HER2+), Basal-like, HER2+ and Triple Negative Breast Cancer/TNBC (ER-/HER-) based on the gene expressions of the cancer cells [4, 5]. Local treatment such as surgery and/or radiation followed by one of the systemic treatment drugs known as chemotherapy, hormone therapy, and targeted therapy is applied [1].

The FDA has approved more than 150 anticancer drugs for several cancer treatment drugs work as cytotoxic based drugs and target-based drugs [6]. Among 150 drugs, 33 drugs have been approved by the FDA in breast cancer treatment from 1953 to 2019 [1, 7, 8]. Of the 33 drugs, 13 drugs are identified as chemotherapy used in first-line therapy of TNBC. The compounds are exerting its action as an alkylating agent, microtubulin inhibitor and Dihydrofolate Reductase (DHFR) – thymidylate synthetase (TS) inhibitor. Twelve drugs are targeted therapy, including monoclonal antibody proteins and small molecule inhibitors which cause less adverse effect than chemotherapy. Three monoclonal antibody drugs specifically inhibit extracellular human epidermal growth factor receptor 2 HER2, and a new monoclonal antibody inhibits programmed cell death-ligand 1 (PD-L1). Meanwhile, 8 small molecule inhibitors having role in intracellular enzyme including tyrosine kinase, Cyclin-Dependent Kinase 4/6 (CDK4/6), Poly(ADP-Ribose) Polymerase-1 (PARP1) and cause inhibition of downstream cell proliferation process Eight drugs are used in hormonal therapy for patients expressing estrogen and/or progesterone receptor to reduced estrogen level and block the growth of cancer cell, and (table 1).

1. CHEMOTHERAPY DRUG TARGET

CDK 4 and CDK6

CDK 4 and CDK6 are family of proline-directed serine/threonine kinases involved in the regulation of eukaryotic cell cycle in the G1 phase. CDK4 localization is in nuclear or nuclear/cytoplasmic [9]. Meanwhile, CDK6 is localized predominantly in the cytoplasm, nevertheless complex of CDK6 and cyclin D (CDK6/cycD) is also found in the nucleus [10]. Similar with other 18 members of CDKs, its catalytic core comprises of ATP-binding pocket, PSTAIRE-like cyclin binding domain, and an activating T-loop motif [11].

Table 1. Therapeutic approach of current breast cancer treatment

Therapeutic approached	Mechanism of Action	Binding Site	Compound name	USFDA Approval	Brand name & company
Chemotherapy	Alkylating agent	DNA strands at N7 position of guanine base	Cyclophosphamide	1959	Cyclophosphamide, Baxter
			Thiotepa	1994	Tepadina®, Adienne S.r.l S.U
			Doxorubicin	1974	Adriamycin®, Carlo Erba Farmitalia
			Epirubicin HCl	1999	Ellence®, Pharmacia & UpJohn
	Microtubulin depolimerization	Vinca alkaloid site of TUBA1, TUBB, TUBD1, TUBE1and TUBG1	Vinblastin SO4	1965	Velban®, Eli Lilly
	Microtubulin stabilizer	Taxane site of TUBA4A and TUBB1	Paclitaxel	1992	Taxol®, Bristol-Myers Squibb
			Docetaxel	1996	Taxotere®, Bristol-Myers Squibb
			Erybuline mesylate	2010	Halaven®, Eisai Co.
		Taxane site of TUBB3	Ixabepilon	2007	Ixempra®, Bristol-Myers Squibb
	DHFR-Thymidilate synthetase inhibitor	DHFR active site (a loop at amino acid residues 9–24)	Methotrexate	1953	Methotrexate, Pd.Rx Pharmaceuticals, Inc.
		Binding site of Thymidilate synthetase	5-Fluorouracyl	1970	Adrucil®, Hoffman la Roche
			Gemcitabine HCl	1996	Gemzar®, Eli Lilly
			Capecitabine	1998	Xeloda®, Genetech, Inc
Targeted therapy	CDK4 and CDK6 inhibitor	ATP-binding pocket of the CDK4 and CDK6	Palbociclib	2015	Ibrance®, Pfizer
			Ribociclib	2017	Kisqali®, Novartis
			Abemaciclib	2017	Verzenio®, Eli Lilly
	PARP1 inhibitor	Nicotinamide binding pocket	Olaparib	2014	Lynparza®, Astrazeneca
	PARP1 and PARP2 inhibitor		Talazoparib	2019	Talzenna®, Pfizer
	mTOR	The everolimus binds to FKBP-12, furthermore binds to mTOR complex 1.	Everolimus	2009	Afinitor®, Novartis
Targeted therapy	HER2 inhibitor	ATP binding pocket of the EGFR family	Lapatinib ditosylate	2007	Tykerb®, Novartis
			Neratinib maleat	2017	Nerlynx®, Puma Biotechnology, Inc
	HER2 inhibitor	domain IV of HER2	Trastuzumab	1998	Herceptin®, Hoffman la Roche
		Near domain I, II and III of HER2	Pertuzumab	2012	Perjeta®, Genetech, Hoffman la Roche
		domain IV of HER2 and as anti-microtubule agent.	Ado-Trastuzumab Emtansin	2013	Kadcyla®, Genetech-Hoffman la Roche
	PD-L1	residues (E58, R113) of PD-L1	Atezolizumab	2019	Tecentriq® Genetech-Rosche
Hormonal Therapy	Estrogen reseptor inhibitor	Estrogen binding pocket	Tamoxifene	1977	Novaldex®, Astra Zeneca
			Toremifene	1997	Fareston®, Kyowa Kirin, Ltd
			Fulvestrant	2002	Faslodex®, Astra Zeneca
	Aromatase inhibitor	Substrate binding site of CPR	Exemestane	1999	Aromasin®, Pharmacia & Upjohn Inc.
		Heme iron group of active site of CPR	Letrozole	1997	Femara®, Novartis
			Anastrozole	1995	Arimidex®®, AstraZeneca
	LHRH analog	Binding site of GnRH receptor	Goserelin acetate	1989	Zoladex®, Astra Zeneca
			Megestrol Acetate	1993	Megace®, Bristol-Myers Squibb

These kinases require a protein called Cyclins to form an enzyme complex and undergo phosphorylation of specific downstream protein activation in each phase. Among CDK's proteins, CDK 1, CDK2, CDK4, and CDK6 mechanism are well known to regulate the function of the cell cycle. In the G1 phase, cycD bind to CDK4 or CDK6 to form a complex of CDK4/cycD or CDK6/cycD to phosphorylate Retinoblastoma (Rb) protein, which is known as essential protein as a tumor suppressor. The phosphorylation will release the E2F, a group of genes that codify a family of the transcription factor, and undergo the gene coding transcription in S phase for cell proliferation [12, 13].

G1 to S-phase cell cycle is understood as a rate-limiting step of cell progression. Deregulation of the CDK4/cycD pathway has been identified to be responsible for cancer progression. U.S FDA approves three small molecules of CDK4 and CDK6 inhibitors for breast cancer therapy. The first compound approved in 2015 was Palbociclib which was developed by Pfizer, followed by Ribociclib (developed by Novartis) and Abemaciclib (developed Ely lily) in 2017. Palbociclib and Ribociclib has a similar structure and exhibits similar inhibitory activity against CDK4 and CDK6. Meanwhile, Abemaciclib exhibits better selectivity for CDK4 than to CDK6 [13, 14]. These inhibitors bind in the ATP-binding pocket of the CDK4 and CDK6 to inhibit the Rb phosphorylation, stabilized the complex of phosphorylating Rb-E2F and retain release of E2F, therefore lead to inhibition of further gene expression program. This action will block the downstream cell cycle progression and leads to tumor growth arrest at G0/G1 [9, 10, 14, 15, 16].

PARP1 ad PARP2

PARP-1, as a member of the nuclear enzyme PARP family of proteins, incorporated NAD+ for post-translational modification of proteins to maintain the sustainability of the cell in DNA repair mechanism. There are 17 members of PARPs identified and characterized by homology to the catalytic domain of PARP1. PARP-1 comprises of six-domain known as F1 (Zn1) and F2 (Zn2) know as zinc-finger domains which bind to DNA structures, the F3 (Zn3) is zinc-binding domain which also contributes to DNA binding, PARP-1 interdomain contacts, auto-modification domain (AD) and WGR domain is present in sequence from N-terminal to C-terminal. AD domain contains a BRCA1 C-terminus fold and linker regions, and the WGR domain will participate in DNA binding and forms DNA interdomain contacts essential for DNA damage-dependent activation. The catalytic domain contains two subdomains: a helical domain that is conserved in DNA damage-dependent PARPs 1/2/3, and the active site ADP-ribosyltransferase domain [17, 18, 19]. The F1 and F2 domain of PARP1 will rapidly bind to the specific breaks of DNA and activated the Single-Strand Breaks (SSBs). This binding mechanism will induce synthesis of the poly (ADP-ribose) polymerase or PAR chain, and other 2 PARP-dependent DNA damage repair proteins for the further mechanism are resealing the DNA to SSBs DNA repair completion. After repairing, the PAR chains are degraded via Poly(ADP-ribose) glycohydrolase. Instead of SSBs DNA repair, a normal healthy cell will utilize Double Strand Breaks DNA repair through activation of BRCA1and BRCA2. However, cancer cell with BRCA gene mutation will lose its ability of this DSBs pathway and entirely rely on SSBs DNA repair pathway [20, 21].

Olaparib (Lynparza®-Astra Zeneca) and Talazoparib (Talzenna®-Pfizer) are both PARP inhibitors approved in 2014 and 2018, consecutively, as a breast cancer therapy in a solid dosage form. The pyrimidine ring of Olaparib binds within nicotinamide binding pocket of PARP1 and block its activity to repair of DNA SSBs, the increment of PARP-DNA complex causing toxic PARP trapping in DNA disrupting cellular homeostasis and cell death [17, 19, 20, 21, 22]. PARP1 is activated once DNA damage identified in G1/S checkpoint. Thus the inhibitor of this enzyme resulting in G0/G1 cell cycle arrest [23]. Different with Olaparib, Talazoparib has dual-mechanism as PARP1, and PARP2 inhibitor for DNA repair in cancer cell expressed BRCA1 and BRCA2 [24].

TUBULIN

Microtubule is a cytoskeletal element formation of protofilament arrangement composed of regular beta-tubulin subunit and alpha-tubulin subunit heterodimers assembly. Microtubule is essential for intracellular transport, also involved in the movement and attachment of the chromosomes in various stages of M phase. An initial mitotic spindle of microtubule begins to form in prophase. It attached kinetochore of the chromosome and underwent several growing and shortening periods during prometaphase and metaphase. The microtubules remain attached to the chromosomes and regulated shortening and lengthening process during anaphase for cell dividing completion [25].

α-Tubulin (consist of 8 subunits) and β-Tubulin (consist of 9 subunits) are a member of tubulin superfamily. Along with others families of tubulins: γ-Tubulin, Delta (δ), and epsilon (ε) tubulin, the human tubulin genes and pseudogenes are distributed over all chromosomes and localize at centrioles for maintaining the centriole structure and its function [26].

The monomer structure of alpha- and beta-tubulin are identical, and it composes of three functional domains: the amino-terminal domain containing the guanine nucleotide (GTP) binding region, an intermediate domain containing the inhibitor binding site, and the carboxy-terminal domain which probably constitutes the binding surface for motor proteins. Vinblastin SO4 is the first antimitotic agent approved by the US FDA since 1965 for breast cancer treatment. This compound is antitumor alkaloid derived from the isolation of Madagascar periwinkle Catharanthus roseus, and known as TUBA1; TUBB; TUBD1; TUBE1; TUBG1 inhibitor. This vinca alkaloids bind to the microtubule directly at the vinca alkaloid site of tubulin, to β-subunit which surrounding GTP-binding site on tubulin. This action leads to alter the dimer

conformation in tubulin in connection with tubulin self-association, inhibit tubulin-dependent GTP hydrolysis, and GDP–GTP exchange, and to prevent the cell from making the spindles, therefore arresting mitosis at prometaphase. Vinblastine also causes inhibition of cross-links formed between the β-subunits of tubulin cys12–cys201/211 resulting in microtubule destabilizers, which cause the shortening (depolymerization) of microtubulin [27].

Different from vinca alkaloid, Paclitaxel, Docetaxel, and Eribulin mesylate, which was approved at 1992, 1996, and 2010 respectively, are TUBA4A and TUBB1 inhibitor. These compounds will bind at taxane site, binds to one side of β-tubulin keeping contact with the next protofilament within the microtubular lumen thus mimicking the GTP bind mechanism. Intervene of tubulin by Paclitaxel, resulting in permanent stabilization of microtubule dynamics. The mechanism is known as microtubule stabilizers, which lead to inhibition of cell division and tumor cell death. Ixabepilone (approved since 2007), is an epothilone B analog developed by Bristol-Myers Squibb known as TUBB3 inhibitor, this compound will also bind at taxane site and act similarly with Paclitaxel.

Interestingly most of the antimitotic tubulin inhibitors known as naturally derived compounds. Paclitaxel derived from the isolation of bark of the Pacific yew tree Taxus brevifolia Nutt. Meanwhile, Eribulin mesylate was first manufactured as a natural compound isolated from the marine sponge Halichondria okadai. Docetaxel is a derivative of taxane derivatives [28, 29].

Dihydrofolate Reductase (DHFR) – Thymidylate synthetase (TS)

Nucleotides are biomolecule required as the building block for nucleic acid formation, both RNA and DNA synthesis. Nucleotide composed of three chemical subunit known as a five-carbon sugar, a nitrogenous base, and a phosphate group, and classified as Purin or Pyrimidine nucleotide based on the structure of aromatic heterocycle of its nucleotide base. Purin nucleotide base includes Adenine and Guanine will have pyrimidine and imidazole ring, while pyrimidine base including Cytosine, Uracil (in RNA) and Thymine (in DNA) will have pyrimidine ring only[30]. Purine and Pyrimidine biosynthesis are known to have different biosynthesis pathway which involves multiple enzymes to work correctly.

Two of the enzyme involved in the pathways are DHFR and TS. DHFR monomer is an essential enzyme to catalyzes reduction of Dihydrofolate into tetrahydrofolate (THF). This reaction utilizing a molecule of NADPH as the electron donor, and subsequently lead to the formation of N5, N10-methylene-THF. Activation of DHFR simultaneously activates thymidylate synthetase to catalyze reductive methylation of Deoxyuridine monophosphate (dUMP) into dTMP (deoxythymidine monophosphate). Thus N5N10- methylene-THF will be oxidized and release a methyl group back to THF. The methyl group obtained will be attached to dUMP structure and form dTMP. Methotrexate is the first compound approved by the US FDA for breast cancer treatment in 1953, known as an antifolate with the structure similar to folic acid. Due to its structure, it acts as a DHF analog and competitively bind to DHFR active site (a loop at amino acid residues 9–24) and inhibit its activity. Inhibition of this enzyme reduced THF pool in the intracellular cell causing lack of thymidine nucleoside, also affecting many biosynthesis disruptions including retardation of de novo synthesis of purine and pyrimidine base and resulting in cell apoptosis [31, 32].

Thymidylate synthetase is a homodimer enzyme engage in thymidine nucleoside. Inhibitors of this enzyme, 5-Fluorouracil, Gemcitabine HCl and Capecitabine was approved since 1970, 1996 and 1998 respectively. These compounds bind to the active site of thymidylate synthetase and inhibit its activity [33].

5-Fluorouracil (5-FU) is known as uracil analog with a fluorine atom at the C-5 position. As pyrimidine analog, 5-FU rapidly enters cells using the same transport mechanism as uracil and converted into fluorouridine triphosphate (FUTP), fluorodeoxyuridine triphosphate (FdUTP) and fluorodeoxyuridine monophosphate (FdUMP) active metabolites. FdUMP will inhibit the activity of TS by forming the ternary complex with TS and 5,10-methylene tetrahydrofolate (CH2THF), thus blocking the conversion of deoxyuridylic acid to thymidylic acid, retain the downstream process of biomolecule and lead DNA damage Capecitabine known as a prodrug, in the system it will be metabolized into 5-FU hence exhibit a similar mechanism with 5-FU to inhibit cell proliferation [34, 35].

Gemcitabine, also known as 2’,2’-Difluoro-2”-deoxycytidine (dFdC), is a cytidine analog developed and marketed as Gemzar® by Eli Lilly. dFdC and its active metabolite difluorodeoxyuridine monophosphate (dFdUMP) has a similar structure with native dUMP, and these metabolite binds at dUMP binding site to inhibit TS activity and arrests DNA synthesis. Thus inhibition of thymidylate synthase-DHFR bifunctional enzyme renders obstruction in the downstream process of nucleobase formation, resulting in lack of biomaterial and lead to arrest the cell cycle in S phase [36, 37].

DNA STRANDS (ALKYLATING AGENT)

Several alkylating agents approved for breast cancer treatment are Cyclophosphamide, Thiotepa (derivative of N,N', N"-triethylenephosphoramide (TEPA)), Doxorubicin and Epirubicin HCl in 1959, 1994, 1974 and 1999 respectively. These compounds incorporated bind to DNA strand at the N7 position of guanine base thus render the topoisomerase II (TOP2A) activity to initiate DNA synthesis. After administering, Cyclophosphamide undergoes to form active metabolite phosphoramide mustard. In alkaline or neutral pH of the system, the phosphoramide mustard is converted to carbonium ion which forms an irreversible covalent linkage with the N7 of guanine base in a DNA strand. The second functional group of carbanium ion creates another link with guanine base in the same strand (intrastrand cross-linkages) or with the opposite double strand DNA (interstrand) which forms irreversible DNA interstrand and intrastrand cross-

linkages at the guanine N-7 position of double-stranded DNA strand [38]. Similarly, the highly reactive ethylenimine groups of Thiotepa, induced crosslinking of alkylated guanine of DNA strand at the N7 position of guanine bases [39].

Doxorubicin is a cytotoxic anthracycline drug derived from isolated cultures of Streptomyces peucetius var. caesius. Another anthracycline antibiotic compound found is Epirubicin known having a similar structure to doxorubicin, distinct at epimerization of hydroxyl (-OH) groups in 4'aminosugar. Cytotoxic effect of these compounds are known in two mechanisms, first is bind at guanine base of double-stranded DNA through intercalation mechanism and thus prevent TOP2A activity from releasing supercoil DNA for initiation of DNA replication and synthesis. Secondly, it is due to superoxide ions and hydrogen peroxide formation during the transformation of semiquinone into quinone form of doxorubicin and Epirubicin. The reactive oxygen species formed will further cause oxidative stress, induced activation of Nuclear Factor Kappa B1, and lead to cell death [40, 41, 42, 43].

2. TARGETED THERAPY DRUG TARGET

Two classifications of tyrosine kinases are determined. First are receptor tyrosine kinase, including EGFR (Epidermal growth factor receptor), PDGFR (Platelet-derived growth factor receptor), FGFR (Fibroblast growth factor receptor) and Insulin Receptor. The second class is known as non-receptor tyrosine kinase, including dual kinase complex SRC (Steroid receptor coactivator)-FAK (Focal adhesion kinase), ABL, and Janus Kinase [44, 45].

Tyrosine kinases are family of enzymes, have an essential role in activating signal transduction for cell proliferation, differentiation, cell metabolism, and cell death. The enzymes catalyze the phosphorylation of tyrosine residues of the target protein in the presence of an ATP molecule.

EGFR family comprises four distinct members EGFR/HER1/ErbB-1, HER2/ErbB-2, HER3/ErbB-3, and HER4/ErbB-4. Different with HER1, HER3 and HER 4, which are present as a dimer transmembrane protein, HER2 is present as a monomer transmembrane protein which forms homodimer or heterodimers with other EGFR proteins for its activation. Activation of HER2 is due to homo-/hetero-dimerization of HER2 leads to autophosphorylation and/or transphosphorylation of specific tyrosine residues. The phosphorylation induced cross-phosphorylation triggers multiple signaling pathways such as phosphatidylinositol-3 kinase (PI3K) for cell survival, mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK1/2) for cell proliferation, and other multiple cellular responses (figure 2) [46, 47].

Monoclonal antibody targeting Tyrosine kinases

Trastuzumab, Pertuzumab, and Ado-Trastuzumab Emtansine are a monoclonal antibody (MAB) known as tyrosine kinases inhibitor, explicitly targeting human EGFR 2/HER2/ErbB2. This MAB was approved for breast cancer treatment is since 1998, 2012, and 2013, respectively [8].

HER2 has an extracellular region composed of 630 amino acids, a single membrane-spanning region, and a cytoplasmic tyrosine kinase. The extracellular region is composed of four domains. These four domains are arranged as tandem repeats of a two-domain unit consisting of an L domain (domains I and III; B190 amino acids each) and a cysteine-rich domain (domains II and IV; B120 amino acids each). Trastuzumab was introduced as a recombinant IgG1 kappa, humanized monoclonal antibody that selectively binds to a region in domain IV of HER2 [48, 49]. The primary inhibition mechanism is known through blockage of tyrosine kinase SCR signaling lead to increases PTEN level and activity, thus affect to inhibition of the MAPK and PI3K/Akt pathways give rise to the suppression of cell growth and proliferation[41, 48].

Ado-Trastuzumab Emtansine (Kadcyla®), also known as Trastuzumab-DM1 (T-DM1) is HER2 antibody-drug conjugate comprised of MAB trastuzumab covalently linked to cytotoxic agent maytansine derivative agent, emtansine (DM1). This drug arrest cell proliferation by a similar mechanism with Trastuzumab, extensively this compound exert activity targeting intracellular delivery of the potent anti-microtubule agent, leads to apoptosis [50, 51].

Different with Trastuzumab, Pertuzumab is a humanized MAB to prevent HER2-HER3 dimerization and reducing signaling via PI3K/Akt pathway by binding near to domain I, II and III of HER2, and known will block downstream process inhibit cell proliferation. However, its activity more superior when used in combination with Trastuzumab than a single application [52, 53].

Monoclonal antibody targeting programmed death ligand-1 (PD-L1)

Programmed cell death protein 1 (PD-1) is a protein on the surface of immune cells, including T-cells, B cells, and dendritic cells. In breast cancer cell condition, increasing of programmed death ligand-1 (PD-L1) expression occurs. The PD-1/PDL-1 complex will attract interaction of tyrosine phosphatase (SHP-1 and SHP-2) and thus inactivate the T-lymphocytes mediated immune response. Moreover, it leads to inhibition of the RAS- MEK-ERK and PI3K-AKTmTOR pathways, resulting in inhibition of T-lymphocytes mediated immune response. This mechanism leads to cancer cellular survival [54, 55].

Atezolizumab (Tecentriq®) is a non-glycosylated IgG1 kappa immunoglobulin, a monoclonal antibody recently approved by the FDA in March 2019 [8]. Atezolizumab binds at the same site with native PD-1, at residues (E58, R113) of PD-L1 thus blocks PD-1/PD-L1 interaction. This mechanism will activate the T-lymphocytes immune system and induce apoptosis in a cancer cell [55].

Small molecule targeting Tyrosine kinase

Lapatinib ditosylate (Tykerb®) and Neratinib maleate (Nerlynx®) are approved as small molecule tyrosine kinase inhibitors since 2007 and 2017 respectively. These compounds are non-peptide anilinoquinazolone compounds homologous of the adenosine triphosphate (ATP) with a similar mechanism of action, thus compete

with ATP to bind inside the ATP binding pocket of the EGFR [56]. These agents also affect to inhibition of intracellular signaling pathways such as PI3K/AKT, mitogen-activated protein kinases (MAPKs), PLCγ, and STAT. However, due to its structure, especially the presence of the aromatic group in the structure, affecting the differences dissociation constant (Kd) and selectivity to EGFR. Thus resulting in different molecular interactions and its response [56]. These agents also affect to inhibition of intracellular signaling pathways such as PI3K/AKT, mitogen-activated protein kinases (MAPKs), PLCγ, and STAT. However, due to its structure, especially the presence of the aromatic group in the structure, affecting the differences dissociation constant (Kd) and selectivity to EGFR. Thus resulting in different molecular interactions and its response [57].

Lapatinib and neratinib have aromatic in their structure, binds into the ATP pocket of the EGFR family in a closed conformation and reducing its flexibility for dimerization. Lapatinib binds reversibly at ATP binding site, inhibit the activation of the EGFR, HER2, and HER3 as well as its downstream signaling pathways, MAPK, PI3K-AKT leads to retardation activation of nuclear factor κB (NF-κB) and inhibit PLCg. This also affects to inhibition of the Raf, ERK, AKT, and PLCγ1 proteins resulting inhibition p95-HER2. Moreover, Lapatinib known inhibits insulin-like growth factor I (IGF-I) thus blocked cross-talk of IGF-I receptor and HER2 resulting suppression of HER2 phosphorylation. Besides the phosphorylation inhibition, Lapatinib enhances its apoptosis potency through increased of p38 MAPKs that are responsive to stress stimuli and involved in apoptosis, the subG1 phase of the cell cycle, CDK inhibitor proteins p21 and p27. Lapatinib binding increased the pro-apoptotic protein BIM and reduced an apoptosis inhibitor protein-survivin commonly express in 90% of all breast cancer cases. This compound also increased fragmentation of PARP. Neratinib is irreversible tyrosine kinase inhibitor covalently binds cysteine residues in ATP binding pocket of HER1 (at Cys-773) Cys-805 in, HER2 (at Cys-805), and HER4, moreover blocks their downstream signaling pathways, including inhibiting AKT and MEK phosphorylation leads to arrest at the G1-S phase [57].

mTOR

mTOR (mammalian target of Rapamycin) is a serine/threonine protein kinase belonging to the phosphoinositide 3-kinase-related kinase (PIKK) family, which is involved in downstream of PI3K and Akt pathway (PI3K/Akt/mTOR pathway). It also is known as the mechanistic target of rapamycin and FK506-binding protein 12-rapamycin-associated protein 1 (FRAP1) [58]. There are two distinct complexes of mTOR known as mTORC1 and mTORC2 which regulate different function. The mTORC1 complex consists of Raptor,mLST8, and proline-rich Akt substrate 40(PRAS40) and Deptor. It has a crucial role in regulating protein synthesis and autophagy and as the target of rapamycin and rapamycin analogs. mTORC1 activation is initiated by Akt phosphorylation of TSC2 at the serine 939 and threonine 1462 sites, thus inhibiting TSC1/2 known as a tumor suppressor and heterodimer of tuberin anhamartin; it also phosphorylates PRAS40, thus stimulating mTORC1. Activation of mTORC1 affect Human Inducible Factor-1 (HIF-1) activation, leads to angiogenesis formation, Glucose Transporter 1 (GLUT1) and Lipin-1 activation to induce metabolism.

Meanwhile, mTORC2 complex consists of requisite mLST8 as well as in mTORC1, and two subunits Rictor and Deptor to involved in regulating kinases of the AGC family [59]. The mTORC2 is not a target of Rapamycin and rapalog. This enzyme promotes the activation of insulin receptors and insulin-like growth factor 1 receptors,also responsible for activation of IGF-IR/InsR in vivo, as a crucial regulator of IGF-IR and InsR signaling pathways, has also been implicated in the control and maintenance of the actin cytoskeleton [60]. Everolimus (Afinitor ®) is an oral mTOR inhibitor approved by the US Food and Drug Administration (FDA) in 2009 for postmenopausal women with HR-positive breast cancer. Everolimus is a Macrolide lactam, a derivative of Rapamycin (sirolimus), developed by Novartis Pharma Stein AG- Stein, Switzerland. The compound binds to an intracellular protein FK506 binding protein 12 kDa (FKBP-12). Moreover, the everolimus–FKBP-12 complex binds to mTOR complex 1 and inhibits mTOR1 protein kinase activity. The inhibition reduced the activity of S6 ribosomal protein kinase (S6K1) and eukaryotic elongation factor 4E-binding protein (4E-BP1), which are involved in protein synthesis. S6K1 is a substrate of mTORC1 and also has a role to phosphorylates the activation domain 1 of the estrogen receptor, which results in ligand-independent activation of the receptor. Everolimus has been shown to inhibit the expression of hypoxia-inducible factor (e.g., HIF-1) and reduced the expression of vascular endothelial growth factor (VEGF) thus reducing cell proliferation, angiogenesis, and glucose [6, 58, 61, 62].

3. HORMONAL THERAPY DRUG TARGET

Hormonal therapy is applied to hormone receptor positive and estrogen-responsive breast cancer. Three mechanisms of hormonal therapy currently used in the treatment, known by inhibition of estrogen receptor activation, inhibition of estrogen binding to the CPR- cytochrome P450, and inhibition production of estrogen by interaction with LnGHR (Luteinizing growth hormone receptor).

ESTROGEN RECEPTOR

There are three main natural estrogens in women include estrone (E1), estradiol (E2) or 17β-estradiol, and estriol (E3). Estradiol is a significant form of estrogens, essential to normal women in the regulation of the musculoskeletal system, brain, and cardiovascular system. Moreover, estrogen signaling pathway also required in the initiation of cell proliferation and cell survival. Estrone and estriol are a form of estrogens predominantly found in postmenopausal and during pregnancy, respectively. Estrogen receptors (ERs) is a member of the nuclear hormone receptor (NR) family. ER is found in the cytosol

bound to chaperone proteins such as HSP90 and HSP70. The ER is a ligand-activated transcription factor composed of DNA binding domain, a dimerization region, a hormone binding domain, and two transactivation domains—one located near the N-terminus (AF-1) and another near the C-terminus (AF-2). Based on its localization, ER known as nuclear estrogen receptors including ERα and ERβ; others are known as membrane estrogen receptors (mERs) including GPER (GPR30), ER-X, and Gq-mER which are mostly G protein-coupled receptors [63]. Estrogen binds to the Estrogen Receptor (ER) and activates genomic or non-genomic pathway of the transcription factor in the DNA synthesis process. Three mechanisms of the genomic pathway are known. First, upon binding of E2 at C-terminus transactivation function 2 (AF2) and an N-terminus transactivation domain (AF1) of ER, result in further phosphorylation and dimerization. The complex translocates from the cytoplasm into the nucleus, and bind the ERE in the promoter regions initiate the transcriptional activation or repression. Second, due to the interaction of ER with other transcription factors such as activator protein 1 (AP1) and specificity protein 1 (SP1) allows it to bind DNA indirectly and cause the activation or repression of target genes. This mechanism is known as ERE-independent genomic action. Third, through activation of ligand-independent ER at the AF1 domain affected by kinase stress-related kinases pathway e.g. p38 MAPK or JNK; p44/42 MAPK; PI3K/Akt.

Meanwhile, in the non-genomic pathway, the ER is usually bound to the membrane proteins in regions known as lipid rafts. These ERs also resulting cross-talk, interact with proteins of various kinase signaling pathways, such as PLC/PKC, Ras/Raf/MAPK, PI3K/AKT, and cAMP/PKA. It is distributed between the cell and intracellular membranes and activates EGFR [64, 65].

Tamoxifen is the first hormonal therapy approved in 1977 and used as cytostatic selective estrogen receptor modulators (SERM). The drug has triphenylethylene group in the structure and very similar to estrogen, thus act competitively with native estradiol binds within the same site of estrogen binding pocket in the ER, inhibits its activity and arrest at G0/G1 phases of the cell cycle [65, 66].

Later on, Toremifene and Fulvestrant were approved as estrogen receptor inhibitor at 1997 and 2002, respectively. Toremifene is a first generation nonsteroidal SERM that is structurally related to tamoxifen. Similar to tamoxifen, it is an estrogen agonist for bone tissue and cholesterol metabolism but is antagonistic on mammary and uterine tissue. Fulvestrant is an estrogen receptor (ER) antagonist inhibiting ESTI INJ, act both down-regulating and degrading the estrogen receptor, it competitively binding to estrogen receptors on tumor and other tissue targets, producing a nuclear complex that decreases DNA synthesis. Fulvestrant has demonstrated activity against tamoxifen-resistant breast cancers [66].

AROMATASE

In premenopausal women, estrogens are synthesized from androgens by the granulosa cells in the ovaries. The primary source of steroids in the ovaries is cholesterol. Nevertheless, when ovaries are no longer functional, the source of estrogens in postmenopausal women comes from the peripheral conversion of androgens by the aromatase enzyme.

Aromatase is an estrogen synthetase plays a vital role in breast cancer development. Human aromatase belongs to the cytochrome P450 family and is encoded by the CYP19 gene. It is a heterodimer of a cytochrome 450 aromatase (aromatase) and a ubiquitous NADPH cytochrome P450 reductase (CPR). This heterodimer enzyme embedded in the membrane of endoplasmic reticulum in which the FMN, FAD, and NADP binding sites are facing towards the cytoplasmic side. The catalytic portion of cytochrome P450 aromatase contains a heme group as well as a steroid binding site [67] . CPR composed of four domains, known as the FMN-binding domain, connecting domain, FAD-binding domain, and the NADP binding domain. Androgen molecule binds at the androgen-specific cleft of aromatase active site. During the synthesis of estrogen, electrons are transferred from NADPH through the FAD and FMN domains of CPR to the heme of aromatase. Upon receiving electrons from CPR, aromatase synthesizes estrogen from androgen by three hydroxylation steps. The first and second hydroxylations occur at the 19-methyl group of androgen, while the third hydroxylation results in the cleavage of the C10-C19 bond and the aromatization of the A ring. Resulting in the change in the structure of non-aromatic six-carbon A ring into a planar aromatic A ring of estrogen [68, 69].

Aromatase inhibitor classifies as steroidal aromatase inhibitor exemestane (Aromasin®, 1999) and nonsteroid aromatase inhibitor is known as letrozole (Femara®, 1997) and anastrozole (Arimidex®, 1995). Exemestane, as androgen derivatives, has a similar structure to the natural substrate androstenedione. It binds irreversibly to a similar binding site of androgen, causes permanent inactivation of the enzyme and estrogen deprivation lead. This condition leads to suppression of cell growth and apoptosis [64].

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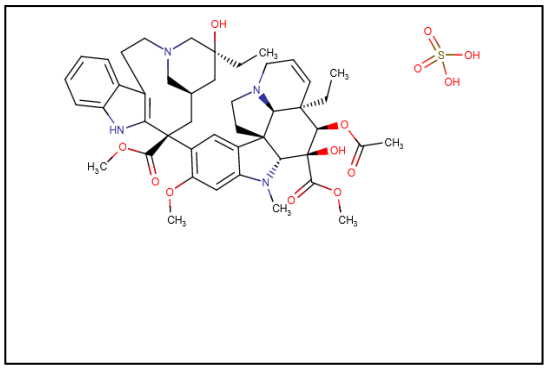
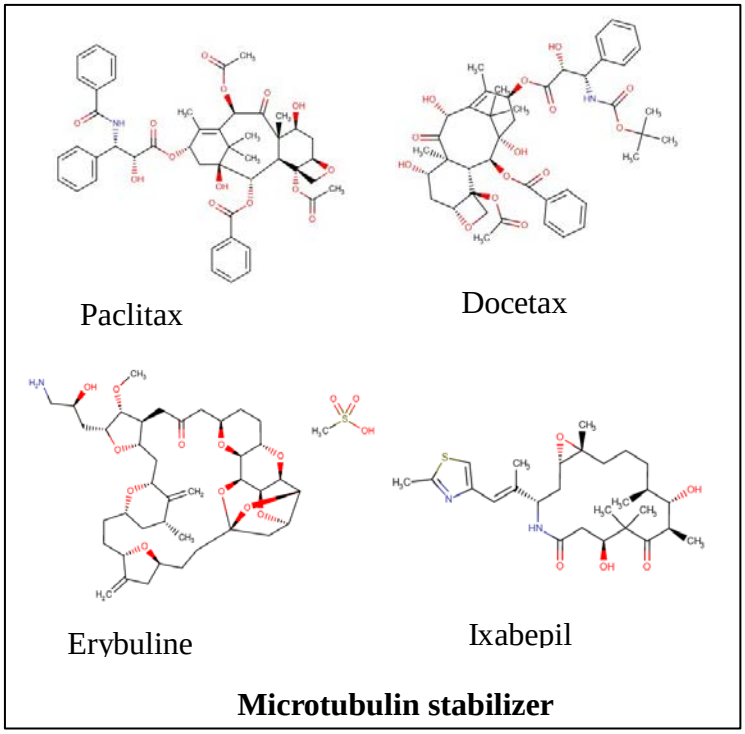
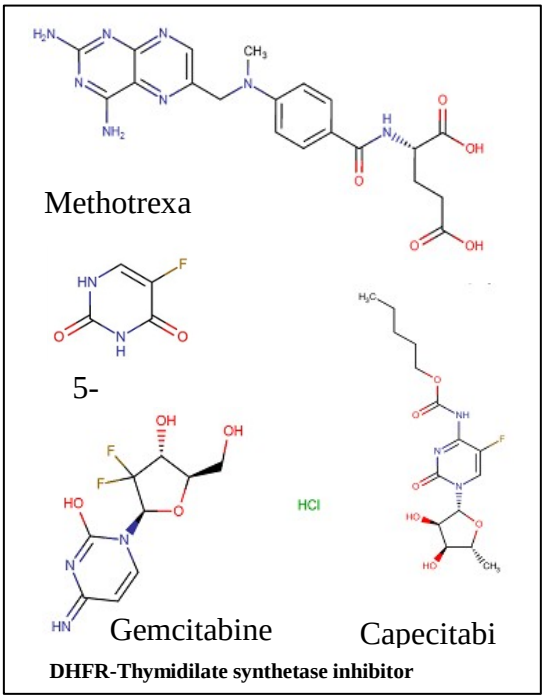
LHRH

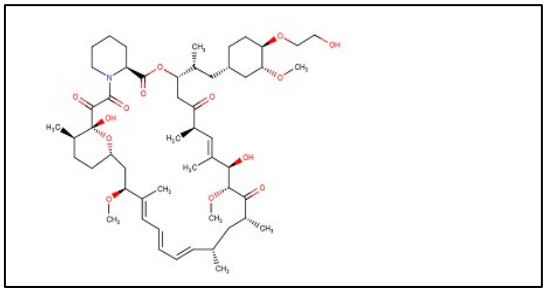
Goserelin acetate was approved in 1989 as a synthetic analog of luteinizing hormone-releasing hormone (LHRH)/gonadotropin releasing hormone (GnRH). While Megestrol Acetate (1993) is a synthetic progestin and has the same physiologic effects as natural progesterone. As

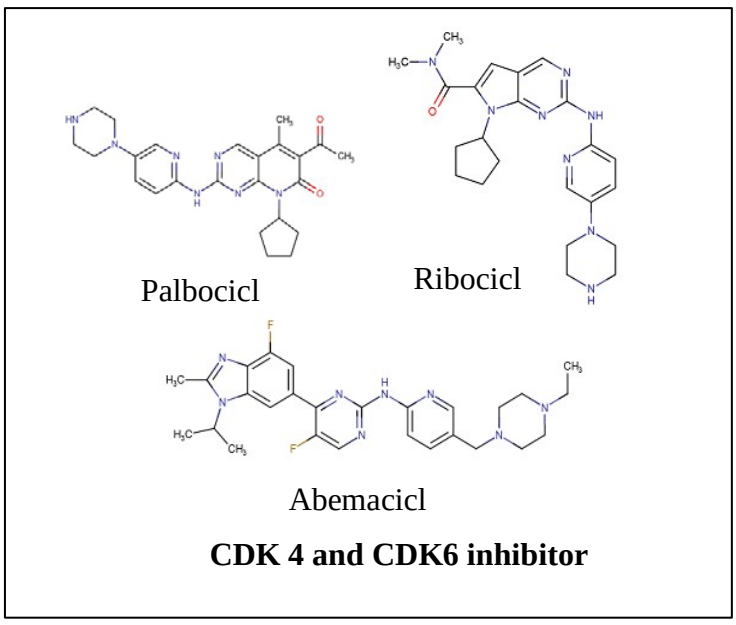
an LHRH agonist (LHRHa), it binds to the GnRH receptor cells in the pituitary gland. this leading to an initial increase in production of luteinizing hormone (LH) and stimulate the ovary to produce estrogen. Formation of estrogen gives negative feedback to hypothalamus and pituitary gland. LHRHa bind to pituitary GnRH receptors with superior affinity and a longer half-life than endogenous LHRH. It also is known 50-100 times more potent than natural LHRH and has more excellent resistance to enzymatic breakdown. During treatment of LHRHa, excessive release of LH and estrogen production occurs. The sudden elevation of estrogen level resulting in undesired adverse effect and sometimes worsen the breast cancer symptoms. LHRHa have prolonged half-life compared to native GnRH, thus will continuously stimulate the LH, this condition may lead to desensitized to GnRH and block the releasing of LH and inhibit estrogen production and reduce cancer cell growth and proliferation [70, 71].

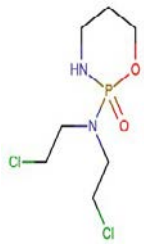






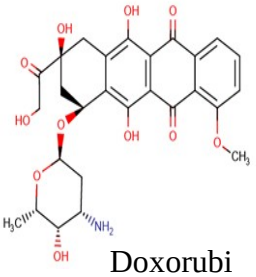




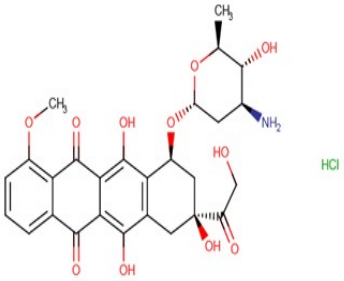


Cyclophospha

Thiote

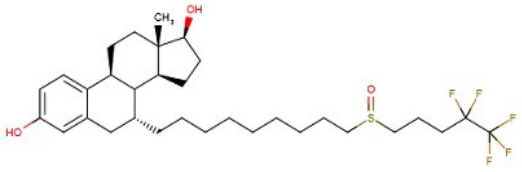


Doxorubi



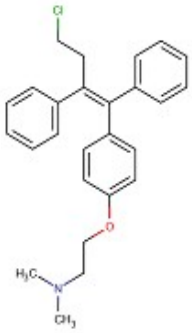
Epirubicin

Alkylating agent

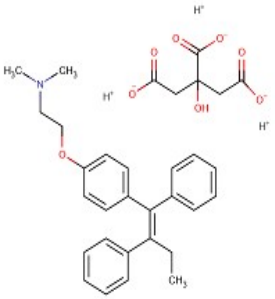


Fulvestrant

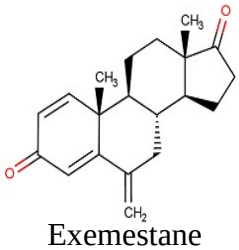
Estrogen receptor inhibitor



Toremife

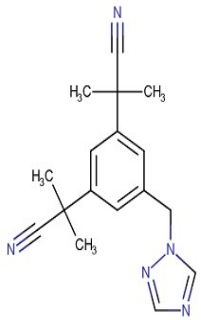


Tamoxifen

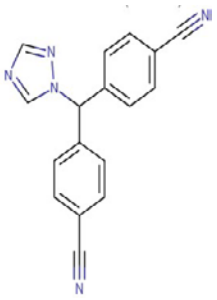


Exemestane

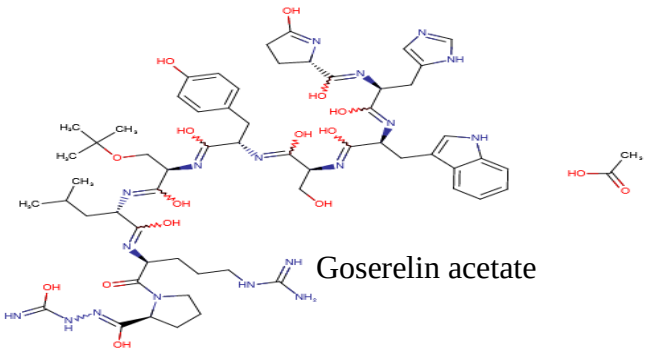
Aromatase inhibitor



Anastroz

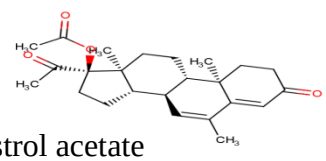


Letrozo

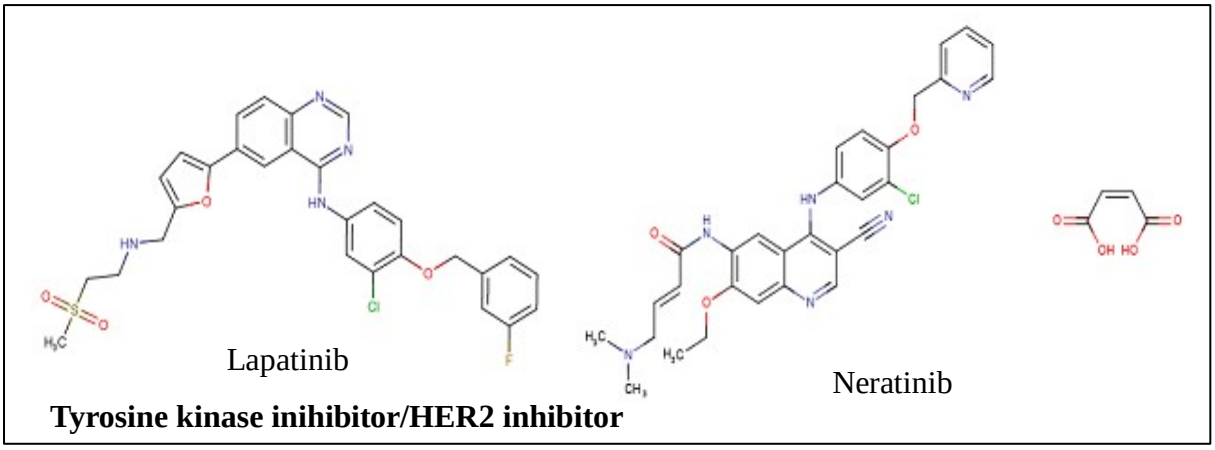


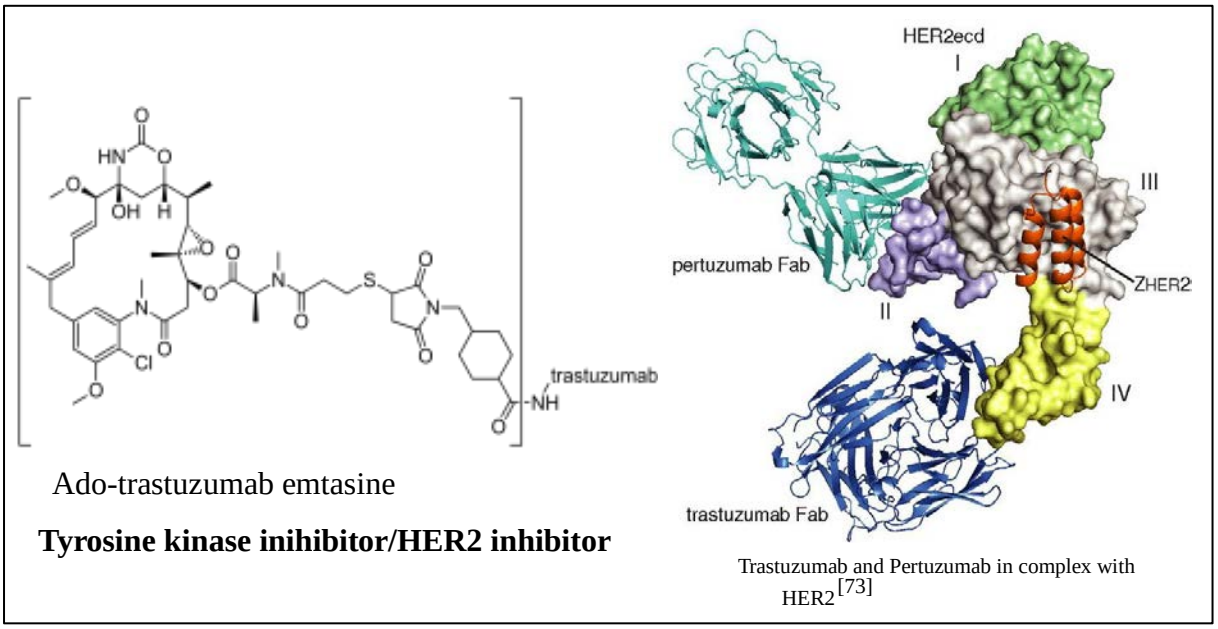
Goserelin acetate

LnRH analog



Megestrol acetate





Trastuzumab and Pertuzumab in complex with HER2 [73]

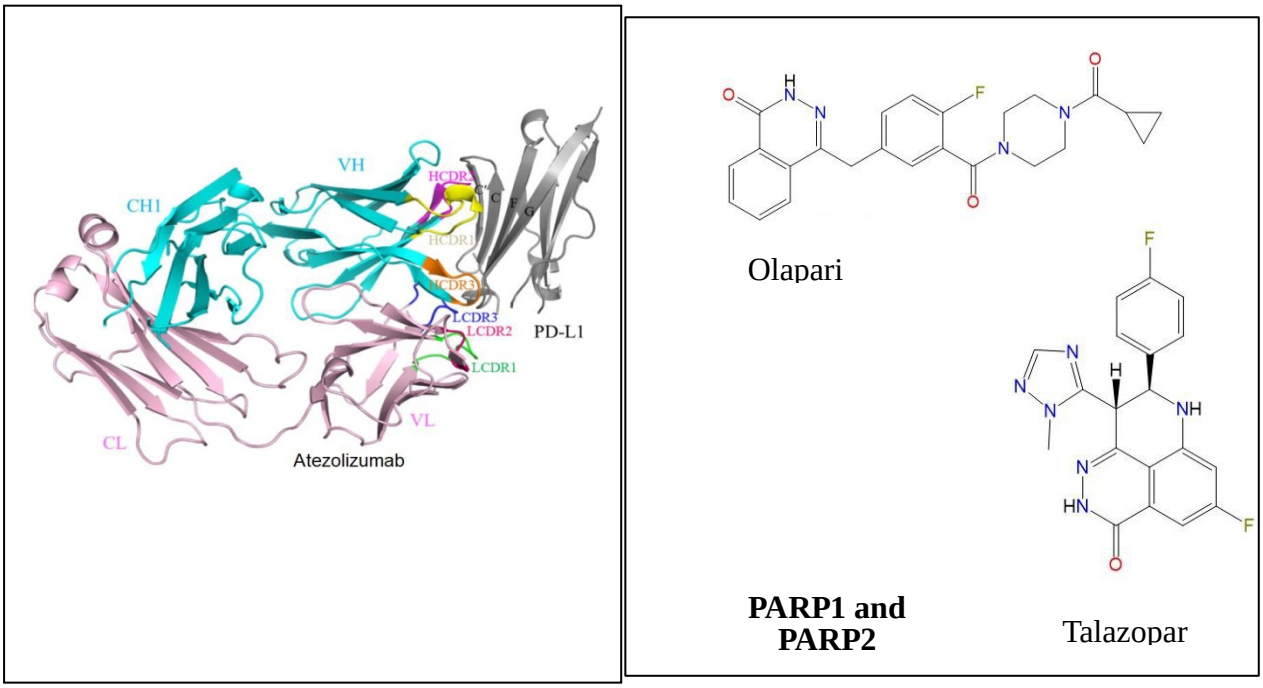


Figure 1. Structure of compounds approved for breast cancer therapy (continued)

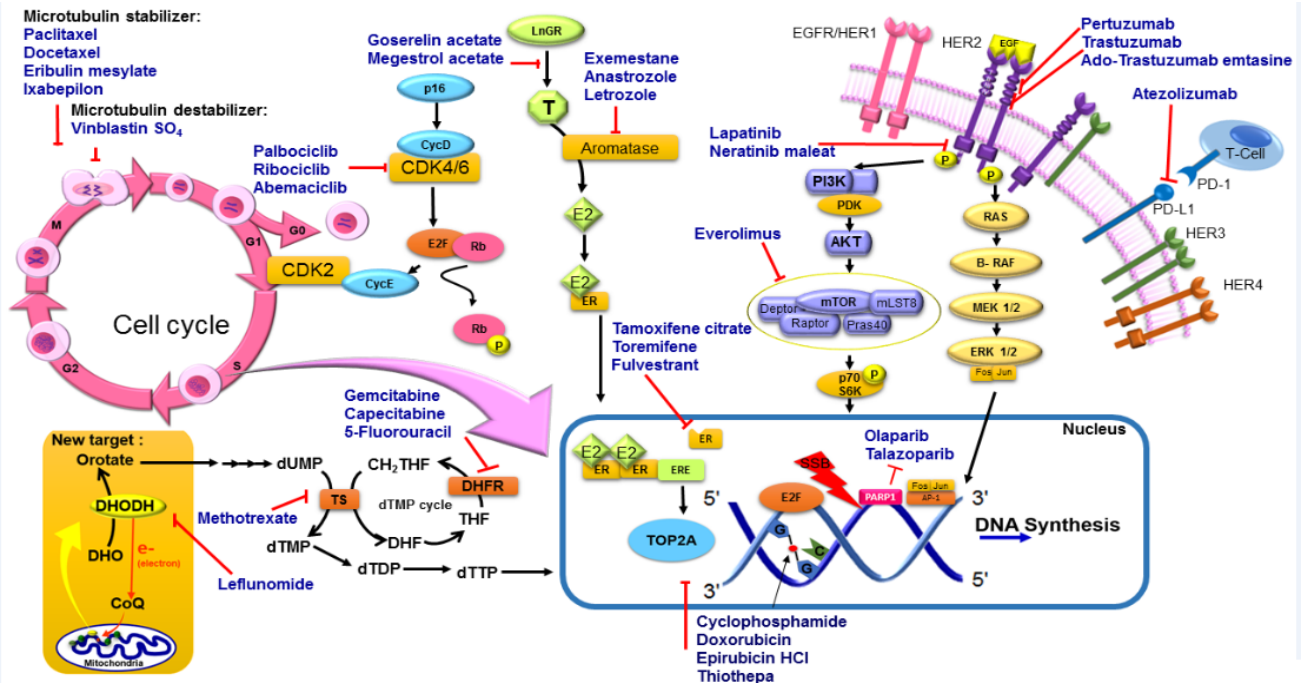


Figure 2. Mechanism of action of approved breast cancer drugs including chemotherapy drug targeting bifunctional enzyme Thymidylate synthetase (TS)-Dihydrofolate reductase (DHFR), Topoisomerase II (TOP2A) activity through alkylating action at DNA strands, microtubulin formation; targeted therapy specifically inhibit activity of Cyclin-Dependent Kinase 4/6 (CDK4/6), Poly(ADP-Ribose) Polymerase-1 (PARP1), mammalian target of rapamycin-1 (mTOR1), human epidermal growth factor receptor 2 (HER2) ; Hormonal therapy targeting LnGR (Luteinizing growth hormone receptor), Cytochrome P450 reductase (CPR), Estrogen Receptor (ER). Alternatively, new target therapy Dihydroorotate dehydrogenase (DHODH) is validated as a potential target of future cancer treatment therapy

4. FUTURE POTENTIAL DRUG TARGET

Currently, the researcher attempt to discover new drugs effective to treat breast cancer with low side undesired adverse effect. Development of new compounds, optimizing the structure of an approved compound, optimizing pharmaceutical formulation or research in the discovery of a new target could be an alternative way to discover new medicine. Several enzymes have been identified as a potential target in the future for breast cancer treatment. Amongst them, one of the potential targets of treatment is Dihydroorotate dehydrogenase (DHODH). DHODH is membrane protein enzyme located on the mitochondrial inner membrane. This enzyme engages in the fourth step of de novo pyrimidine biosynthesis, presented as a rate-limiting enzyme within this pathway to catalyzes the oxidation of dihydroorotate into orotate leading to the downstream of pyrimidine nucleotide synthesis. Along with this oxidation, DHODH simultaneously catalyzes the electron transfer from dihydroorotate to flavin mononucleotide and from reduced it to ubiquinone pool. The electron transferred to bc1 complex (complex III of mitochondrial), and finally to oxygen via cytochrome c and complex IV in a process known as oxygen respiration. Thus, DHODH importantly contributes directly to de novo pyrimidine biosynthesis and respiratory chain [72]. The human can utilize both salvage and de novo pyrimidine biosynthesis pathway [30]. In a normal cell, inhibition of DHODH will be managed by alternative salvage pathway. However, rapidly proliferating cancer cells have a limited supply of pyrimidine by salvage pathway and make it rely on a de novo pathway. Therefore, inhibition of DHODH not only will block the DNA/RNA biosynthesis but also known to decrease the mitochondrial membrane potential and mitochondrial complex III activity and increased reactive oxygen species production lead to arrest cell cycle at G2/M. Leflunomide is known as DHODH inhibitor for Rheumatoid arthritis treatment. This compound binds at Ubiquinone binding site and inhibits its activity, lead to suppression of cell proliferation. Currently, a natural compound produced by Acremonium sclerotigenum known as Ascofuranone is developed as DHODH inhibitor for several diseases including for breast cancer treatment. It exhibits Human DHODH at IC50 37.5 ± 12 nM. Moreover, it shows GI50 9.0 µM and 7.4 µM in MCF-7 and MDA-MB-23, respectively [72].

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Abbreviations: EGR : Extracellular Growth Factor; DHO : dihydroorotate; CoQ : Coenzyme Q10/ubiquinone; dUMP : Deoxyuridine monophosphate; dTMP : deoxythimidine monophosphate; dTDP : deoxy-thymidine-5-diphosphate; dTTP: deoxy-thymidine-5-

triphosphate.; CH2THF = 5,10-methylene tetrahydrofolate; THF : tetrahydrofolate; DHF : dihydrofolate; E2 : Estradiol; ER : Estrogen Receptor; ERE : estrogen response element ; E2F : group of genes that codifies a family of transcription factor; G : Guanine; C : Cytosine; SSB : Single Strand Break; Rb : retinoblastoma protein; CycE : Cyclin E; CDK2 : Cyclin Dependent Kinase 2; T : Testosteron; PI3K : phosphatidylinositol-3 kinase; AKT : protein kinase B; PDK : phosphoinositide-dependent kinase; p16 : p16INK4a, cyclin-dependent kinase inhibitor 2A, multiple tumor suppressor 1; AP-1: Activator Protein-1 a transcription factor); p70S6K : 70 kDa ribosomal protein S6 kinase ; RAF : Rapidly Accelerated Fibrosarcoma; MEK 1/2 : Mitogen-activated protein kinase kinase 1/2; ERK : extracellular signal-regulated kinases; EGFR/HER1 : Epitelial growth factor receptor/human epidermal growth factor receptor 1; HER3 : human epidermal growth factor receptor 3; Her4 : human epidermal growth factor receptor 4; PD-1 : Programmed cell death -1; PD-L1 : Programmed cell death ligand 1

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