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Modern approaches to breast cancer treatment

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I, Davoud AHMADIMOGHADDAM, declare that this work is my original author's work and that all the information resources are presented in the list of references.

Hradec Králové, 2009 Davoud AHMADIMOGHADDAM

ABBREVIATIONS

ABC	ATP binding cassette
AC	Adriamycin (Doxorubicin) and Cyclophosphamide
AI	Aromatase Inhibitors
ANA	Anastrozole
BCRP	Breast cancer resistance protein
CDH1	E-Cadherin Gene
CDK	Cyclin-Dependent Kinase
CMF	Cyclophosphamide, Methotrexate, and 5-Fluorouracil
CS	Cowden Syndrome
DFS	Disease-Free Survival
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulfate
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
ERE	Estrogen-Responsive Element
ES	Estrone Sulfate
FAC	5-Fluorouracil, Doxorubicin, Cyclophosphamide
FAS	Fatty Acid Synthase
FEC	5-Fluorouracil, Epirubicin and Cyclophosphamide
FECD	FEC plus Docetaxel

FSH	Follicle-Stimulating Hormone
FUL	Fulvestrant
GnRH agonist	Gonadotropin-Releasing Hormone Agonist
HER	Human Epidermal growth factor Receptor
HIF-1	Hypoxia-Inducible Factor 1
IBC	Inflammatory Breast Cancer
IGF	Insulin-like Growth Factor
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone-Releasing Hormone
LKB1/STK11	Serine/Threonine Kinase Gene
LN	Lymph Node
LVEF	Left Ventricular Ejection Fraction
MA	Megestrol Acetate
MAPK	Mitogen-Activated Protein Kinases
MBC	Metastatic Breast Cancer
MPA	Medroxyprogesterone Acetate
MRI	Magnetic Resonance Imaging
MRP	Multidrug Resistance Protein
MDR	Multi Drug Resistance
MVD	Microvessel Density
OFS	Ovarian Function Suppression
OS	Overall Survival
PGE2	Prostaglandin E2

PR	Progesterone Receptor
PRE	Progesterone-Responsive Element
QOL	Quality Of Life
Qu	Quercetin
RAL	Raloxifene (RAL)
SERMs	Selective Estrogen Receptor Modulators
STS	Steroid Sulfatase
TAC	Taxotere, Adriamycin, Cyclophosphamide
TAF	Transcription Activation Function
TAM	Tamoxifen
TKI	Tyrosine Kinase Inhibitors
TTP	Time-To-Progression
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptors

ABSTRACT

Breast cancer is a malignant tumor that originates in the cells of the breast both in women and men. It is the second leading cause of cancer death in women today. Risk factors causing breast cancer in humans comprise, among others, prolonged exposure to estrogen, ionizing radiation, genetic predisposition (BRCA1, BRCA2, others), sedentary lifestyle, high-fat diet, alcohol, and tobacco smoking. Classical treatment strategies include chemotherapy, surgery and radiotherapy. The aim of this diploma thesis was to review recent developments in breast cancer treatment, such as endocrine therapy, molecular targeting therapy as well as breast cancer stem cells.

INTRODUCTION

Human cancer comprises in fact more than 200 different diseases which together account for one fifth of all deaths in the industrialized countries of the Western World [1]. Cancers fall into three large groups divided by the location of the organ. A first group of cancers are called “carcinomas” which arise from epithelial cells and comprise the lung, large intestine (colon and rectum), breast cancer in women and prostate cancer in men. The second group of cancers is not as prevalent as the first group and comprises carcinomas of the bladder, stomach, liver, kidney, pancreas, esophagus, and cervix and ovary in women. The third groups of cancers are created in the soft tissue like the brain, testes, bone, and other organs which are relatively rare.

Cancers are caused by exogenous chemical, physical, or biological carcinogens and endogenous processes. A different type of classification has been issued by the WHO. They classify carcinogens into five groups, each having a different definition. Group 1, the agent is carcinogenic in humans. The exposure circumstance entails exposures that are carcinogenic to humans. Group 2A, the agent is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to human. Group 2B, the agent is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans. Group 3, the agent is not classifiable as to carcinogenicity in humans. Group 4, the agent is probably not carcinogenic to humans. Chemical carcinogens comprise nickel, cadmium, arsenic, nitrosamines, trichloroethylene, arylamines, benzopyrene, aflatoxins, and reactive oxygen species. Physical carcinogens comprise UV irradiation (especially UVB), and ionizing radiation. Biological carcinogens comprise Human papilloma virus, Epstein-Barr virus, Hepatitis B virus, Helicobacter pylori, and Schistosoma mansoni. Endogenous processes consist of DNA replication, metabolic reaction generating reactive oxygen species, and chronic inflammation. The mechanisms of carcinogenesis in humans are complex and multifactorial. Nevertheless, precise elucidation of the mechanisms is helpful and insights from the molecular biology are beginning to contribute to improve prevention of cancer.

Typical demonstrations of human cancers are increased cell proliferation (often autonomous), insufficient apoptosis, altered cell and tissue differentiation, altered metabolism, genomic instability, invasion into different tissue layers and other tissues (with disturbed tissue architecture), metastasis into local lymph nodes and distant tissues, immortalization (growth beyond replicative senescence). Moreover, these demonstrations may be acquired step by step and become evident at various stages during the progression of cancer.

Breast tumors have been noted since antiquity and were probably first described in the Edwin Smith surgical papyrus originating from Egypt at around 2500 B.C.[2]. Sir George Thomas Beatson introduced oophorectomy as a treatment for breast cancer in the late 1890s and this is the first documented endocrine therapy for breast cancer [3]. Today, breast cancer is the most commonly occurring cancer among women in many countries. One in every eight women will develop breast cancer at some point in their lives. Breast cancer is both genetically and histopathologically heterogeneous, and the mechanisms underlying breast cancer development remain largely unknown [4]. In North American and Western European women, carcinoma of the breast is the most common malignancy and the second most common cause of cancer-related death [5]. It accounts for 22% of all female cancers and the estimated annual incidence of breast cancer worldwide is about one million cases [2]. Worldwide, breast cancer is the second most common type of cancer after lung cancer (10.4% of all cancer incidence, both sexes counted) and the fifth most common cause of cancer death [6]. In 2005, breast cancer caused 502,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths) [6].

AIM OF THE STUDY

The aim of the study is to evaluate the latest treatment strategies of treating breast cancer which has been used in clinical trials. In this study scientific articles and scientific bibliographical investigations were used. In fact, I was looking for the latest treatment strategies of treating breast cancer which have already been tested by clinical trials and were effective in patients. The goal was to collect successful therapies chosen from different sources and different countries. Despite the fact that there are still many questions with no answer as far as the treatment of breast cancer is concerned many new discoveries of combination therapy has shown good results.

METHODOLOGY

In this diploma thesis articles extracted from PubMed searches, World Health Organization (WHO), Society Cancer Institute, National Institute of Health, and National Comprehensive Cancer Network have been used. All of them were filtered for English language and published from 2000 to 2009. Publications included mainly journals, published articles and guideline manuals.

I. BACKGROUND

1. INTRODUCTION

Breast cancer is a malignant tumor that originates in the cells of the breast both in women and men. Estrogen plays an important role in the development and progression of breast cancer. Today, the biology of breast cancer has been improved and in result of that a treatment of the disease continues to change. Most breast cancers are carcinomas, i.e., malignant tumors of epithelia. Less than 1% of breast cancers are sarcomas that arise from connective tissue, bone, muscle or fat [4].

Breast cancer is the second leading cause of cancer death in women today [7]. Most cases occur in postmenopausal women, but many younger women with hereditary predisposition are afflicted too. Breast cancer occurs in males too, this is because the breast is composed of identical tissues in males and females. Incidences of breast cancer in men are approximately 100 times less common than in women, but men with breast cancer are considered to have the same statistical survival rates as women [8].

Cancer of the breast is common in developed countries where the lifetime risk ranges from 1 in 12 to 1 in 20 women. Among women living in developing countries the risk is lower but appears to be increasing. Risk factors for breast cancer include high socio-economic status, early menarche, late first birth, late menopause, and a family history of breast cancer [6].Breast cancer incidence (most recent) by country is shown in the table 1[6].

Table 1. Breast cancer incidence (most recent) by country. Source: [6]

Rank	Countries	Amount
1	Iceland	39.4 per 100.000 females
2	Denmark	30.4 per 100.000 females
3	Netherlands	28.7 per 100.000 females
4	Belgium	28.7 per 100.000 females
5	New Zealand	28 per 100.000 females
6	Ireland	27.5 per 100.000 females
7	Hungary	26.6 per 100.000 females
8	United Kingdom	26 per 100.000 females
9	Germany	23.5 per 100.000 females
10	Canada	22.6 per 100.000 females
11	Czech Republic	22.2 per 100.000 females
12	Italy	22 per 100.000 females
13	France	21.7 per 100.000 females
14	Australia	21.6 per 100.000 females
15	Austria	21.5 per 100.000 females
16	Norway	21.3 per 100.000 females
17	United States	21.2 per 100.000 females
18	Luxembourg	21 per 100.000 females
19	Spain	19.5 per 100.000 females
20	Portugal	19.3 per 100.000 females
21	Slovakia	19.2 per 100.000 females
22	Sweden	18.5 per 100.000 females
23	Finland	18.1 per 100.000 females
24	Poland	17.9 per 100.000 females
25	Greece	16.8 per 100.000 females
26	Japan	8.6 per 100.000 females
…	Weighted average	22.8 per 100.000 females

2. BREAST BIOLOGY

Breast tissue is different from other body tissue. In fact there are four growth phases. The first growth phase happens during the fetal development and the second one start during puberty. It is followed by the growth cycles and the last phase is cessation of growth in menopause. First of all, the organ does not develop fully before puberty, so there is one additional growth phase during the second decade of life. In puberty, immature ducts elongate to form 15-20 lobuli.

Then, up to 45 years of age, the ductular tissue undergo monthly cycles of proliferation and apoptosis. Up to 500 cycles may take place before menopause.

This cycling is interrupted by pregnancies. Estradiol and progesterone act on cells by binding to specific receptors. Both estrogen and progesterone receptors belong to a group of more closely related receptors which are termed Estrogen-Responsive Elements (ERE), Progesterone-Responsive Elements (PRE).

These cycles are controlled by a combination of hormones and locally produced growth factors. The monthly cycles are controlled by estradiol and progesterone, supported by insulin and further hormones.

Proliferation of epithelial cells in the breast is supported by the stroma [1]. Stromal cells and epithelial cells both produce paracrine growth factors, partly in response to steroid hormones [1].

2.1. ESTROGEN

Estrogens are female sex hormones which are steroid compounds and are named after their importance in the estrous cycle. Estradiol, estriol and estrone are three major naturally occurring estrogens in women (figure 1).

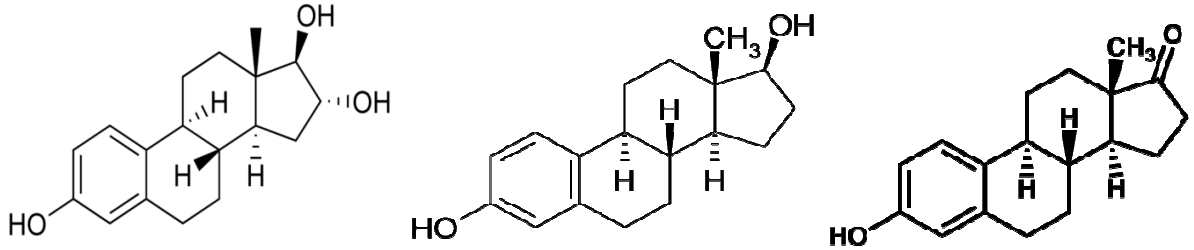

Figure1. Structures of three major estrogens in women

In the body these estrogens are produced by enzymatic conversion of androgens. Estrogens are present in higher level in women of reproductive age, but they are present in low level in men too. Their function includes development of female secondary sex characteristics, such as breasts and thickening of the endometrium and other aspects regulating the menstrual cycle.

Estrogens in blood have negative effect on FSH and LH because they decrease their level in blood. Therefore, estrogen is used along with synthetic progestin as oral contraceptive. In men, estradiol is involved in LH feedback and

not testosterone. Estrogen is used in hormone replacement therapy in postmenopausal women in order to prevent osteoporosis and treat the symptoms of menopause, such as hot flashes, vaginal dryness, urinary stress incontinence, chilly sensations, dizziness, fatigue, irritability, and sweating.

Estrogen receptors (ERs) are intracellular receptors which are activated by the hormone 17β-estradiol (estrogen). They mainly function as a DNA binding transcription factor which regulates gene expression. There are two different forms of estrogen receptors, α and β, which are encoded by gene ESR1 and ESR2 respectively. Both ERs are widely expressed in different tissue types. The ERα is found in endometrium, breast cancer cells, ovarian stroma cells, and in the hypothalamus[9]. The ERβ is located in the kidneys, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells. 17-beta-estradiol binds equally to both receptors and estrone binds preferentially to the alpha receptor and estriol to the beta receptor.

The DNA-binding domain of estrogen receptor α (ERα) is flanked on the N-terminal side by a transactivation domain, designated as activation function 1(AF-1). A hinge region on its C-terminal side connects a second transactivation domain, AF-2. The AF-2 domain binds the ligand and its activity is strongly dependent on ligand-binding. The AF-2 domain of the ERα is known to bind at least five different co-activator proteins specifically. Certain co-activators interacting with estrogen receptors are regulated by mitogen-activated protein kinases (MAPK) phosphorylation in response to growth factors of the endothelial growth factor (EGF) family.

A second mechanism of ERα action does not require binding of the receptor to DNA. In this fashion, the receptors can regulate the activity of genes that do not possess canonical receptor binding sites. Mimicking activation of MAPK pathway may be a main mechanism by which estrogens stimulate cell proliferation.

Most importantly, estrogens act on several different cell types in breast tissue, both epithelial and stromal. ERα mediate most of the proliferation effects in female reproductive tissue. In contras ERβ appear to act mostly as an inhibitor of ERα action.

2.1.1. Estrogen Signals

About 80% of breast cancers are hormone-receptor-positive cancers. Treatment strategy is a suppression of estrogen production in the body. Estrogen exerts its biological effects by binding to ER. In breast cancer estrogen activates ER through genomic and non-genomic pathways and growth factors. Activation of ER promotes the proliferation of breast cancer. ER acts by the formation of homo or hetero-dimers of ERα and ERβ. ERα has been widely used as a predictive marker for endocrine therapy.

Growth factors which activate ER consist of EGF and IGF-1 via receptor phosphorylation [10].

The incidence of breast cancer is high even in postmenopausal women when the ovaries have ceased to produce estrogen. This is due to estrogen production within the breast cancer tissue, which is induced by the crosstalk between tumor and stromal cells in the tumor microenvironment (Figure 2) [10].

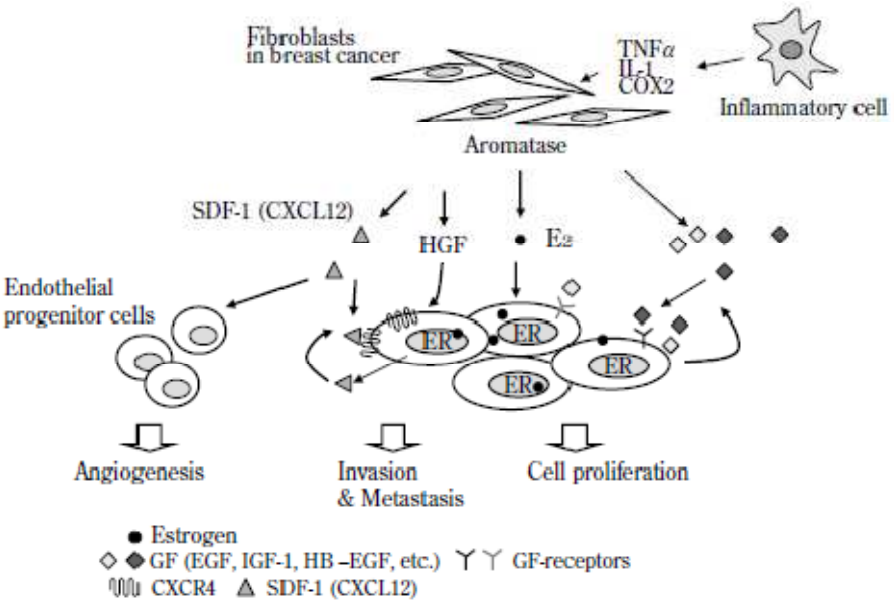

Figure 2.Tumor-stromal interactions in the microenvironment of breast cancer stimulate the secretion of estrogen, growth factors and chemokines, leading to tumor cell proliferation, invasion, metastasis and angiogenesis. Source: [10]

Aromatase express in the adipose stromal fibroblasts adjacent to the tumors, which produce estrogen from androgen. Aromatase expression levels in breast cancer tissues are significantly higher than those in benign breast lesions. Tumor-stromal crosstalk regulates aromatase gene expression via the production of various factors such as prostaglandin E2, COX2, tumor necrosis factor-**α**, IL-6 and IL-11 [10].

ER-activating ability is higher in postmenopausal than in premenopausal patients. In postmenopausal breast cancers, tumor cells activate stromal fibroblasts to express aromatase, resulting in intratumoral estrogen production. Therefore, aromatase inhibitors are used as a first-line and target endocrine therapy for ER-positive advanced breast cancers in these patients.

Among the stromal cells, fibroblasts are the most abundant cell type. Tumor cells actively recruit stromal cells, such as fibroblasts, inflammatory cells and endovascular cells, into the tumor, to create a supportive microenvironment for their own growth. In invasive human breast cancers, these fibroblasts are named CAFs (carcinoma associated fibroblasts) which exhibit biological characteristics distinct from normal fibroblasts [10, 11].

2.2. PROGESTRONE

Progesterone belongs to a class of hormones called progestogens and is the major naturally occurring human progestogen (figure 3). It is involved in the female menstrual cycle, pregnancy, and embryogenesis .

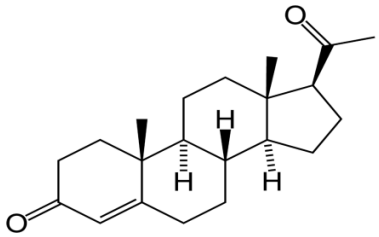

Figure 3.Structure of Progesterone

Progesterone is produced in the ovaries, in the corpus luteum after ovulation, and in the placenta during pregnancy. Progesterone reaches the highest levels during pregnancy. Progesterone exerts its action primarily through the intracellular

progesterone receptor. Progesterone’s physiological effects are amplified in the presence of estrogen. Estrogen up regulates the expression of progesterone receptors. Progesterone is sometimes called the "hormone of pregnancy", and it has many roles relating to the development of the fetus. It has effect on nervous system and other organs too.

Progesterone is used to support pregnancy and to control an ovulatory bleeding and to prepare uterine lining in infertility therapy and to support early pregnancy. Patients with recurrent pregnancy loss due to inadequate progesterone production may receive progesterone. It should be taken into account that hormonal contraception does not contain progesterone but a progestin.

Progesterone receptor (PR) is an intracellular steroid receptor that specifically binds progesterone. PR is encoded by a single gene PGR residing on chromosome 11q22. PRA and PRB, the two forms of progesterone differ in their molecular weight. PRA and PRB are translated from differently spliced mRNAs from the same gene. Expression of PR is induced by ERα. PRA (PRα) appears to act as a feedback inhibitor of ERα. A special transcription activation function (TAF), so called TAF-3, is present in the progesterone receptor-B, in a B-upstream segment at the amino acid terminal. This segment is not present in the receptor-A. Estrogen is necessary to induce progesterone receptors.

II. ETIOLOGY OF BREAST CANCER AND RISK FACTORS

Breast cancer becomes one of the major female cancers in female in the western industrialized countries. The life time risk of breast cancer for women in these countries is around 10%, and about 30% of them are lethal [1]. Most breast cancer become apparent after menopause. Western lifestyle favors the development of breast cancer.

In general, potential factors causing breast cancer in humans comprise prolonged exposure to estrogen, ionizing radiation, genetic predisposition (BRCA1, BRCA2, others), sedentary lifestyle, high-fat diet, alcohol, and tobacco smoking.

1. ESTROGEN EXPOSURE

Earlier menarche, later menopause, and fewer pregnancies associated with the Western lifestyle all lengthen the exposure to estrogen. With prolonged exposure to estrogen and a longer period of proliferation cycles, the number of cells that can contract mutation is increased and initiated tumor cell get more time to expand. Strong preventative effect is exerted by multiple pregnancies early in life with extended nursing periods.

Estrogens and their metabolites are phenolic compounds. Semiquinones, which are the result of oxidation of diphenolic estrogen metabolites, can react with macromolecules in the cell including DNA and induce mutation. Semiquinones can initiate quinone redox cycling that produce highly reactive oxygen species. So estrogen may act as chemical carcinogens. The risk of cancer in organs with high estrogen concentration, depend on individual ability to metabolize estrogen and to deal with quinone adducts to DNA and redox cycling [1]. There is an interaction between genes and environment because many genes are involved in estrogen biosynthesis and metabolism.

2. EXPOSURE TO IONIZING RADIATION

With high level of radiation cells experience a nonlethal DNA mutation that is passed on to subsequent cell divisions. This mutation may contribute to the formation of cancer. The relationship between exposure to radiation and catching the disease becomes specifically relevant, when we discuss prevention of breast cancer mortality by early detection. Certain carrier of mutation in the ATM gene which is caused by ionizing radiation may increase risk of breast cancer. Gene-environmental interactions are suspected here too.

3. HIGH FAT DIET

The breast cancer incidence is correlated to the content of fat, particularly of saturated animal fats in the diet [1]. The adipose tissue of postmenopausal women contains aromatase, a key biosynthetic enzyme for estrogens. Therefore, high-fat

diets may lead to expansion of adipose tissue leading to continued production of estrogens after menopause with inappropriate growth stimulation of breast epithelial cells.

4. GENETIC PREDISPOSITION

Genetic polymorphisms modulate cancer risk in interaction with environmental factors. Polymorphism in genes of steroid hormone metabolism, DNA repair, cell protection, and lipid metabolism may exert large effects on overall incidence of breast cancer [1]. In contrast, genetic factors predominate in smaller fraction of cases that are caused by inherited mutation in one of a limited number of high-risk genes. These high-risk genes will be discussed below.

III. SIGNS AND SYMPTOMS

Breast cancer can have no symptoms at the beginning. The first common symptom is a lump. Lump feels different from the surrounding breast tissue. In more than 80% of breast cancer cases, the woman discovers the lump herself [12]. A firm, distinctive thickening that appears in one breast and lumps found in lymph nodes located in the armpits or collarbone can indicate breast cancer. Other indications of breast cancer comprise changes in breast size or shape, skin dimpling, nipple inversion, or spontaneous single-nipple discharge. The lump may be painful, but pain is an unreliable sign. Pain without a lump is rarely due to breast cancer. In the early stages, the lump may move freely beneath the skin when it is pushed with the fingers. In more advanced stages, the lump usually adheres to the chest wall or the skin over it [12].

Inflammatory breast cancer (IBC) is manifested when breast cancer cells invade the small lymph vessels in the skin of the breast (dermal lymphatics). The nipple may turn inward (invert). It is a highly aggressive form of locally advanced breast cancer. Symptoms of inflammatory breast cancer include pain, swelling, warmth and redness throughout the breast, as well as an orange peel texture to the skin referred to as peau d’orange [12].

Paget's disease of the breast which is a syndrome manifested as eczematoid skin changes, redness and mild flaking of the nipple skin, could be another symptom of breast cancer. When Paget's disease becomes advanced, its symptoms comprise tingling, itching, increased sensitivity, burning, pain, and discharge from the nipple. Approximately half of women diagnosed with Paget's also have a lump in the breast [13].

Metastatic breast cancer (MBC) occurs when cancer spreads beyond the original organ, but it happens only rarely. The symptoms of MBC depend on the location where the cancer has spread. More common sites of metastasis include bone, liver, lung, and brain. The symptoms comprise unexplained weight loss, fevers, chills, bone or joint pains, jaundice, or neurological symptoms. These symptoms are "non-specific," meaning they can also be manifestations of many other illnesses [14].

Mastitis and fibroadenoma which belong to benign breast diseases have some symptoms which do not turn out to represent underlying breast cancer. The appearance of a new symptom should be taken seriously by both patients and their doctors, because of the possibility of an underlying breast cancer at almost any age [12].

IV. CLASSIFICATION OF BREAST CANCER

Breast cancers are described along four different classification schemes, or groups, each based on different criteria and serving a different purpose.

From the pathology point of view, each tumor is classified by its histological (microscopic anatomy) appearance and other criteria. Classical histological investigation distinguishes different subtype such as Paget carcinoma, intraductal carcinoma, lobular carcinoma, and benign tumor such as fibroadenoma [1].

According to protein and gene expression status breast cancers should be tested by immunohistochemistry for expression of the ER, PR and HER2/neu proteins. The profile of expression of a given tumor helps predict its prognosis and choose the most appropriate treatment. Molecular markers have improved this

classification. A diagnosis of ER+ or PR+ and ERBB2- , indicates a good prognosis and predicts a good response to anti-estrogenic drugs. Microarrays spotted with cDNA fragments or oligonucleotides corresponding to human genes are employed. RNA extracted from tumor or normal tissue are reverse transcribed and labeled to obtain cDNA mixture that remain representative of the relative abundancies of mRNA in the tissues. These are hybridized to the microarrays. The measured hybridization intensities at each spot give an estimate of the expression level of each mRNA represented on the array. These techniques allow the comparison of gene expression pattern between tumor and normal tissue or between individual tumors. More genes and/or proteins may be tested in the future.

According to the stage of tumor, the classification for breast cancer is called the TNM classification. The TNM classification of malignant tumors is a cancer staging system that describes the extent of cancer in a patient's body. T describes the size of the tumor and whether it has invaded nearby tissue, N describes regional lymph nodes that are involved, and M describes distant metastasis (spread of cancer from one body part to another). Table 2 describes the characterization of each stage.

Table 2. Stages of Breast Cancer. Source: [12]

Stage	Description
0	The tumor is confined to a milk duct or milk-producing gland and has not invaded surrounding breast tissue (in situ carcinoma)
I	The tumor is less than ¾ inch (2 cm) in diameter and has not spread beyond the breast.
II	The tumor is larger than ¾ inch but smaller than 2 inches (5 cm) in diameter and/or has spread to at least one lymph node in the armpit on the same side as the tumor.
III	The tumor is larger than 2 inches in diameter and/or has spread to lymph nodes that are stuck to one another or to surrounding tissues, or the tumor, regardless of size, has spread to the skin, the chest wall, or the lymph nodes that are beneath the breast inside the chest.
IV	The tumor, regardless of size, has spread to distant organs or tissues, such as the lungs or bones, or to lymph nodes distant from the breast.

According to the grade of tumor, tumors are classified as low, intermediate and high. A well-differentiated (low grade) tumor resembles normal tissue. A poorly differentiated (high grade) tumor is composed of disorganized cells and does not look like normal tissue. Moderately differentiated (intermediate grade) tumors are somewhere in between.

The ER+ cancers displayed many markers characteristic of luminal secretory cell phenotype, while other group of breast cancer showed expression profiles relating them to basal cells. This means that different subtypes of breast cancers may derive from different stage of the mammary epithelial lineage.

Breast cancer is usually primarily classified by its histological appearance. Rare variants are defined on the basis of physical exam findings. For example, IBC, a form of ductal carcinoma or malignant cancer in the ducts, is distinguished from other carcinomas by the inflamed appearance of the affected breast [15]. In the future, some pathologic classifications may be changed.

The most important goal of breast cancer classification is to provide a basis for choice of therapy in each individual patient. In fact, the more sophisticated understanding of cancer biology and its molecular basis emerging in breast cancer has initiated a kind of paradigm shift in its therapy that is spreading to other tumor entities [1]. In the past, the treatment of a cancer was adjusted according to its histological subtype, extension by stage, and apparent biological aggressiveness by grading.

V. GENETICS OF BREAST CANCER

Twelve genes which have been discovered responsible for breast cancer are categorized as high-risk and low-to-moderate-risk breast cancer susceptibility genes. The high-risk breast cancer susceptibility genes include BRCA1, BRCA2, PTEN, TP53, LKB1/STK11, and CDH1. The CHEK2, TGFβ1, CASP8, and ATM genes belong to the ‘low-to-moderate-risk’ breast cancer susceptibility genes. The high-risk genes are the main cause for strong familial aggregation of breast cancer.

1. HIGH-RISK GENES

1.1. BRCA1 and BRCA2

Both genes are reasonably large genes and ubiquitously expressed in humans with the highest levels in testis, ovaries and thymus. They are characterized by the presence of an extremely large exon 11. Both genes are generally considered to be “caretaker” genes (Caretaker genes act as sensors of DNA damage and participate in the repair process). Their inactivation allows other genetic defects to accumulate and leads to genetic instability. BRC1 and BRC2 are named for their role in breast cancer.

The prevalence of heterozygous carriers of high-risk mutations in the general Caucasian population has been estimated to be about one in 1000 for BRCA1, and one in 750 for BRCA2 [2]. Germline mutations in BRCA1 or BRCA2 confer strong lifetime risks of breast cancer. Within the setting of multiple-case families, the cumulative risk of breast cancer at age 70 years in BRCA1 and BRCA2 mutation carriers was 85% and 84%, respectively[2]. However, a more recent meta-analysis on 22 population-based and hospital based studies showed that the average cumulative risks in BRCA1-mutation carriers by age 70 years were 65% for breast cancer. The corresponding estimates for BRCA2 were 45% and 11% [2]. For both BRCA1 and BRCA2, cancer risks are influenced by the position of the mutation within the gene sequence.

The BRCA1 gene is located on chromosome 17q2 and it has 22 exons, spans approximately 100 kb of genomic DNA, and encodes an 1863 amino acid protein. Its roles include DNA-repair, protein ubiquitylation, chromatin remodeling and cell cycle checkpoint control. Homozygosity for BRCA1-inactivating mutations results in embryonic lethality. The relative risks of breast cancer declined significantly with age for BRCA1-mutation carriers [2]. Women with a mutation in the central region of the BRCA1 gene were shown to have a lower breast cancer risk than women with mutations outside this region.

The BRCA2 gene is located on chromosome 13q12. BRCA2 has 27 exons, spans around 70 kb, and encodes a protein of 3418 amino acids. BRCA2 is involved in double-strand break DNA repair through homologous recombination,

but little else is known about its function. A mutation in the central region of BRC2 is associated with a lower risk of breast cancer risk than mutations outside this region.

1.2. TP53 (Li-Fraumeni Syndrome)

The TP53 gene is located on chromosome 17p13.1, and encodes a protein involved in many overlapping cellular pathways that control cell proliferation and homeostasis, such as cell cycle, apoptosis and DNA-repair. The expression of the TP53 gene is activated in response to various stress signals, including DNA damage. Loss of TP53 function is thought to suppress a mechanism of protection against accumulation of genetic alterations (tumor suppressor). Germline mutations in TP53 are very rare. Mutations in the TP53 gene account for roughly 70% of families fulfilling the classical criteria for Li-Fraumeni syndrome. One of the most frequently occurring cancers in Li-Fraumeni families is breast cancer with an estimated penetrance in TP53 mutation carriers of 28–56% by the age of 45 years. The peak incidence for breast cancer is between 20 and 40 years [2].

1.3. PTEN (Cowden Syndrome)

Cowden syndrome (CS) is an uncommon autosomal dominant disorder. The prevalence of CS is estimated to be 1:300,000. Mutations in the PTEN gene are present in about 80% of CS families. Women carrying a PTEN-mutation have a 25–50% (2–4-fold) lifetime breast cancer risk. The majority of Cowden syndrome related breast cancers occur after the age of 30–35 years. Also, breast cancer at young age has been observed in male carriers of a germline PTEN mutation with the classical CS phenotype, suggesting an increased risk for males as well [2].

1.4. LKB1/STK11 (Peutz-Jegher Syndrome)

The LKB1/STK11-gene is located on chromosome 19p13.3 and encodes a transcript of ~1.3 kb, which acts as a tumor suppressor. Germline mutations in the serine/threonine kinase gene (LKB1/STK11) causes Peutz-Jeghers syndrome (PJS). Mutation in STK11, probably in conjunction with acquired genetic defects of the second allele in somatic cells, caused the manifestations of PJS. The risk of breast cancer by age 65 ranges between 29% and 54% [2]. It is suggested that

LKB1/STK11 can play the role of a tumor suppressor gene in sporadic breast cancer, and low expression of the LKB1/STK11 protein is significantly associated with a shorter survival [2].

1.5. CDH1/E-cadherin (HDGC-Syndrome)

The E-cadherin gene (CDH1) is located on chromosome 16q22.1. The mature protein product belongs to the family of cell–cell adhesion molecules and plays a fundamental role in the maintenance of cell differentiation and the normal architecture of epithelial tissues. The lifetime risk of developing breast cancer was estimated at 20–40% [2]. Somatic CDH1 mutations are frequently found in infiltrating lobular breast cancer and in-situ lobular breast cancer in contrast to breast cancers of other histopathological subtype. Today most breast tumors reported in HDGC families are of the lobular subtype.

2. LOW-TO-MODERATE-RISK GENES

2.1. ATM

The ATM gene is located on chromosome 11q22–23. The ATM protein plays a central role in sensing and signaling the presence of DNA double-strand breaks. In the unirradiated cell nucleus, ATM is held inactive, which is dissociated by rapid intermolecular autophosphorylation after irradiation [2]. Until today there has been much controversy about the exact role of germline ATM mutations in risk for breast cancer. The role of ATM gene in breast cancer susceptibility is plausible but the exact association remains unclear. Most probably it only plays a modest role in familial breast cancer susceptibility.

2.2. TGFβ1

The TGFβ1-gene is located on chromosome 19q13.1 and contains seven exons and very large introns. TGFB is a multifunctional peptide that controls proliferation, differentiation, and acts synergistically with TGFA in inducing transformation and as a negative autocrine growth factor. Dysregulation of TGFB activation and signaling may result in apoptosis. Many cells synthesize TGFB and almost all of them have specific receptors for this peptide.

TGFβ acts as a potent inhibitor of proliferation and migration and promotes apoptosis, properties associated with tumor suppression. TGFβ may induce cellular changes associated with malignant progression. TGFβ inhibits the development of early, benign lesions but promotes invasion and metastasis when the tumor suppressor activity is overridden by oncogenic mutations in other pathways[2].

2.3. CASP8

The CASP8 gene is located on chromosome 2q33–34, contains 13 exons and spans 51.2 kb. Caspases are important mediators of the apoptotic process thanks to this activity it was hypothesized that CASP8 and CASP10 might act as low-penetrance familial breast cancer susceptibility genes. In individuals carrying the protective alleles of both CASP10 (I410) and CASP8 (H302), the breast cancer risk was even more reduced [2].

2.4. CHEK2

The CHEK2 gene is located on chromosome 22q12.1 and several pseudogenes, encompassing exons 10–14 of the gene, are scattered throughout the genome. CHEK2 is a G2 checkpoint kinase that plays an important role in DNA repair and it is activated in response to ionising radiation through phosphorylation by ATM. Activated CHEK2 phosphorylates key cell cycle proteins BRCA1 and p53. CHEK2 conferred an increased risk of breast cancer of approximately two-fold in noncarriers of BRCA1/2 mutations. Patients carrying the CHEK2 1100delC mutation developed breast cancer earlier than non carriers and have an eight-fold risk of developing contralateral breast cancer when compared with matched controls[2]. Immunohistochemically, CHEK2 related breast tumors show in most cases an absent CHEK2 protein staining and are more often negative for luminal cytokeratin 19 staining compared to familial non-BRCA1/2 and BRCA1 related breast tumors [2].

VI. SCREENING

Screening for breast cancer is an approach which helps to find unsuspected cancers. It is carried out by self and clinical breast examination, x-ray mammography, breast Magnetic Resonance Imaging (MRI), and genetic testing.

Breast self-examination involves examining one's own breasts using a specific palpation technique in order to detect any lumps in the breast tissue, which may be cancerous. Clinical exams are similar; however, they are performed by a clinician or a doctor.

X-ray mammography uses x-rays in order to examine the breast for any uncharacteristic masses or lumps. Breast mammography is often recommended as a preventative measure, particularly for elder women and individuals at risk. Breast MRIs are another imaging technique which is used to spot potentially cancerous masses.

The most recent technology for breast cancer screening is ultrasound computed tomography. It uses sound waves creating a three-dimensional image and detecting breast cancer without the use of any dangerous radiation used in x-ray mammography.

Genetic testing for breast cancer is used for those who are at high risk for breast cancer and involves testing for mutations in the BRCA genes. In February 2007, the MammaPrint test became the first breast cancer predictor to win formal approval from the Food and Drug Administration. This is a new gene test to help predict whether women with early-stage breast cancer will relapse in 5 or 10 years, this could help influence how aggressively the initial tumor is to be treated.

VII. PROGNOSIS

Prognostic factors associated with breast cancer comprise staging, tumor size, location, grade, whether disease is systemic, recurrence of the disease, and age of a patient. Stage is the most important, as it takes into consideration the size, local involvement, lymph node status and whether metastatic disease is present. The higher the stage at diagnosis is, the worse the prognosis would be. Larger tumors, invasiveness of disease to lymph nodes, chest wall, skin, and aggressiveness of cancer cells raise the stage, while smaller tumors, cancer-free zones, and close to normal cell behavior lower the stage. Grading is based on how cultured biopsied cells behave. If cells are not well differentiated, they appear immature, divide more rapidly, and tend to spread. The presence of estrogen and progesterone receptors in a cancer cell is important in guiding treatment. Those whose tests are not positive

for these specific receptors will not respond to hormonal therapy. Patients whose cancer cells are positive for HER2/neu have more aggressive disease.

Younger women tend to have a poorer prognosis than post-menopausal women due to their breasts active cycles, nursing infants, and because they are often unaware of changes in their breasts. Therefore, younger women are usually at a more advanced stage when diagnosed.

VIII. DIAGNOSIS

Breast cancer is diagnosed by the examination of surgically removed breast tissue. A number of procedures such as fine-needle aspiration, nipple aspiration, ductal lavage, core needle biopsy, and local surgical excision can obtain tissue or cells prior to definitive treatment for histological or cytological examination. These diagnostic steps, when coupled with radiographic imaging, are usually accurate in diagnosing a breast lesion as cancer. Imaging tests are sometimes used to detect metastasis and include chest X-ray, bone scan, CT scan, MRI, and PET scanning. Only microscopic evaluation of a biopsy specimen can yield a cancer diagnosis. Carbohydrate antigen 15.3 (Ca 15.3, epithelial mucin) is a tumor marker determined in blood which can be used to follow disease activity over time after definitive treatment. Blood tumor marker testing is not routinely performed for the screening of breast cancer.

IX. TREATMENT

1. INTRODUCTION

Patients are divided to high risk and low risk cases according some clinical criteria like age, type of cancer, size, and metastasis. Different strategies would be use in different cases. Treatment possibilities include surgery, radiation therapy, chemotherapy, hormonal therapy, and immune therapy. When the tumor is localized, surgery with possible adjuvant hormone therapy, chemotherapy, and radiotherapy would be fundamental of therapy strategy.

Treatment strategies are derived from realized clinical trials which have proved efficient. Most of these treatment strategies improve the quality of life and

raise life expectancy of a patient. The treatment possibilities which have lately been used in the therapy setting of breast cancer and had beneficial effect for the patient will be considered. All information is based on reviewed articles which are done in each part. In the figure 4, breast cancer treatment overview is shown.

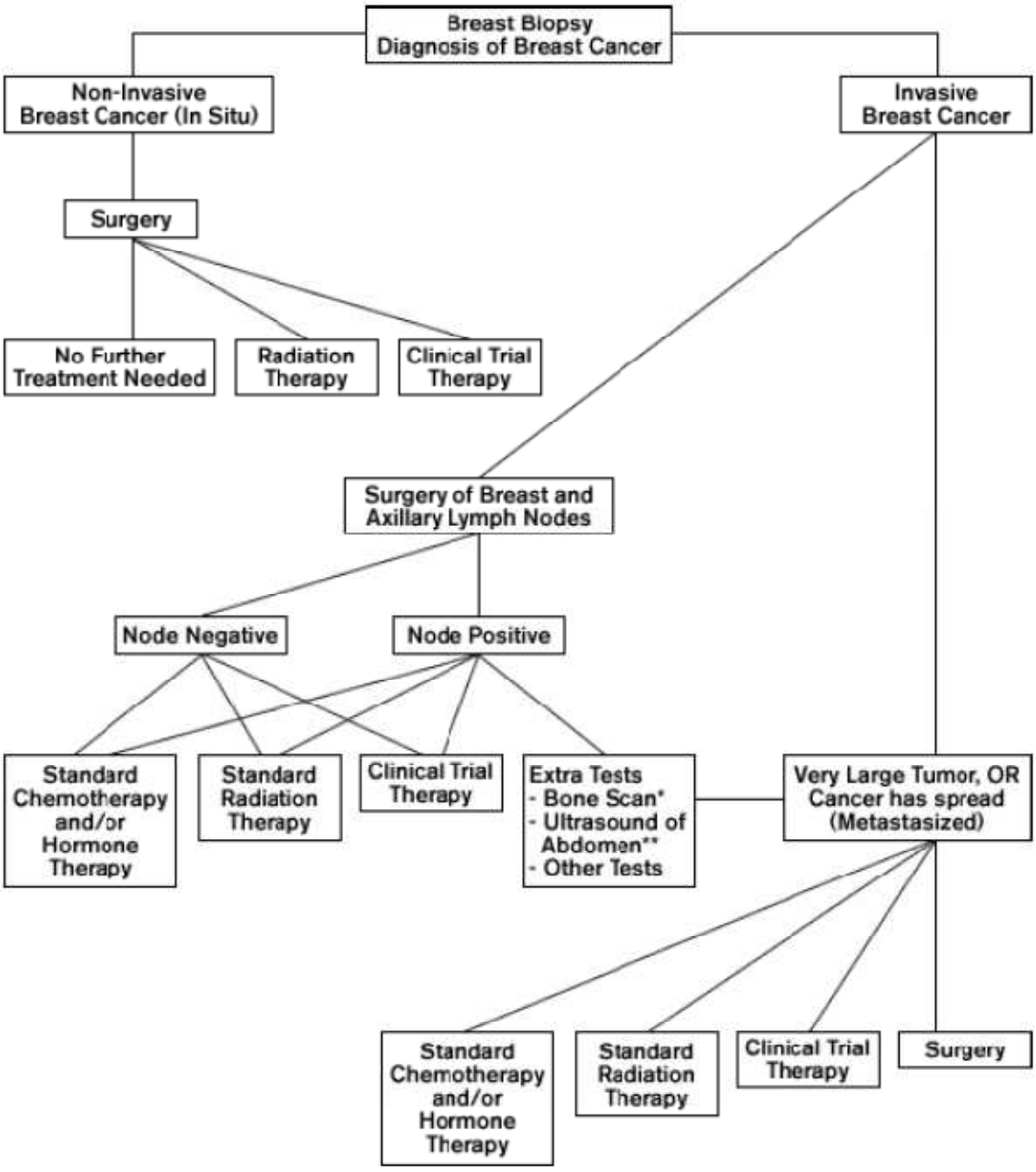

Figure 4. Breast cancer treatment overview. Source: [Copyright © 2000-2007 Alberta Breast Cancer Program (Last updated: Feb 11/2003)]

2. SURGERY

There two types of surgery used in breast cancer treatment. Depending on the stage and type of the tumor, lumpectomy (removal of the lump only or removal of larger amounts of breast tissue) or mastectomy (surgical removal of the entire breast) could be used.

Lumpectomy techniques have increasingly been used for breast-conservation. Mastectomy is done in patients who have two or more tumors in different areas of the breast (a "multifocal" cancer), and have previously received radiotherapy, the tumor size is relatively large compared to their breast size, patients with history of scleroderma or another disease of the connective tissue (it can complicate radiotherapy), patients living in areas with inaccessible radiotherapy treatment, or those who are apprehensive about the risk of recurrence after lumpectomy.

In standard surgery the tissue must be removed so that margins would be clear of cancer, if not, further operations removing more tissue are necessary. It may require removing part of the pectoralis major muscle or the lymph nodes in the axilla. In sentinel lymph node dissection, which has been used recently for a removal of the lymph node, the first node that drains the tumor is removed, that resulting in fewer side effects. In the past, 10 to 40 nodes were taken out that resulting in unfortunate side effects like lymphedema.

3. RADIATION THERAPY

Radiation therapy for breast cancer is usually performed after lumpectomy or mastectomy and also as an integral component of breast-conserving therapy. The purpose of radiation is to reduce the chance that the cancer will recur. Radiation therapy involves use of high-energy X-rays or gamma rays that target tumor or post surgery tumor site and kill cancer cells that may remain after surgery or recur where the tumor was removed. Linear Accelerator is used to deliver X-rays.

The dose of radiation must be strong enough to eliminate microscopic cancer cells that may remain near the area where the tumor was surgically removed. However, radiation affects normal cells too and may cause some damage to the normal tissue. Healthy tissue can repair itself, while cancer cells do not

repair themselves. The healthy tissue needs time to repair itself, therefore, radiation treatments are given over an extended period. Strategy treatments are performed five days a week over a period of five to seven weeks, each treatment lasts about 15 minutes. Radiation therapy can reduce the chance of breast cancer relapse.

3.1. Types of Radiotherapy

Radiotherapy can be delivered in many ways but is most commonly produced by a linear accelerator. After lumpectomy the whole breast and after mastectomy the whole chest would be radiated.

Intensity Modulated Radiation Therapy (IMRT) is a new technology which can change the shape and intensity of the radiation beam at different points across and inside the breast. This method helps to minimize dose to a healthy organ like the heart and lungs and maximize dose to breast tissue.

Mammosite is a new procedure which involves a type of brachytherapy. A radioactive source is temporarily placed inside the breast directly to the tumor bed (area where tumor was removed). However, this method is currently undergoing clinic trials.

Targeted Intraoperative Radiotherapy (TARGIT) is a method of delivering therapeutic radiation from within the breast using a portable X-ray generator called Intrabeam. It is undergoing clinical trials in several countries. The aim is to test whether it can replace the whole course of radiotherapy in selected patients. It may also be able to provide a much better boost dose to the tumor bed and appears to provide superior control [16].

Interstitial Laser Thermotherapy (ILT) is an innovative method of treating breast cancer in a minimally invasive manner and without the need for surgical removal, and with the absence of any adverse effect on the health and survival of the patient during intermediate follow up [17].

3.2. Indications for Radiation

Radiation therapy is not indicated in patients with advanced breast cancer (stage IV) unless there is a need to palliate symptoms like bone pain or fungating lesion.

In general it is indicated as a part of breast conserving therapy when the whole breast is not removed (in lumpectomy) and after mastectomy when patients have higher probability of cancer relapse for instance due to a large primary tumor or substantial involvement of the lymph nodes.

Tumor which is very close or involves the margins on pathology specimen, multiple areas of tumor (multicentric disease), microscopic invasion of lymphatic or vascular tissues, microcopic invasion of the skin, nipple, areola, or underlying pectoralis major muscle, patients with extension out of the substance of a lymph node (LN) and inadequate numbers of axillary LN sampled are factors which influence adding radiation therapy as adjuvant therapy.

3.3. Side Effects of Radiation Therapy

External beam radiation therapy is a non-invasive treatment with some short term and some longer term side effects. A patient undergoing radiation therapy experiences fatigue caused by the healthy tissue repairing itself, fibrosis of chest wall skin, suntan-like change in skin color in the area which is being treated, muscle stiffness, mild swelling and tenderness in the area. Darkening of the skin sometimes returns to normal in one or two months after the treatment and sometimes becomes permanent. On the other hand patients may experience no side effects at all.

Fibrosis of chest wall skin caused by radiation has significant potential effects if a patient has to undergo breast reconstruction surgery later on, because it

negatively affects skin elasticity and makes tissue expansion techniques difficult. For this reason, it is advised to make immediate breast reconstruction after surgery.

4. SYSTEMIC THERAPY

In this part there is a brief review of systemic therapy and new results of treatment strategies. Systemic treatments include chemotherapy, immune therapy, and hormonal therapy.

4.1. CHEMOTHERAPY

Breast cancer chemotherapy refers to the use of cytotoxic drugs (chemotherapy) in the treatment of breast cancer. The three major types of chemotherapy which are used in breast cancer treatment comprise neoadjuvant chemotherapy, adjuvant chemotherapy, and palliative chemotherapy.

Neoadjuvant chemotherapy refers to drug treatment given to people with cancer prior to surgery. The aim is to reduce the size of the cancer before surgery, thus making surgery easier and more likely to be successful. This chemotherapy is commonly used in cancers that are locally advanced where an operation is technically planned at a later stage. The use of such chemotherapy can effectively reduce the difficulty and morbidity of more extensive procedures.

Palliative chemotherapy is used to control (but not cure) the cancer in settings in which the cancer has spread beyond the breast and localized lymph nodes.

Granulocyte colony-stimulating factor (G-CSF) is used along with chemotherapy to reduce the rate of infection. This is due to effect of chemotherapy on the production of white blood cells which decrease their production. If there is a high incidence of bone marrow suppression and infection the adjuvant breast cancer chemotherapy regimens require growth factor support. These may include chemotherapy given in the dose dense fashion i.e. 2-weekly instead of 3-weekly or TAC chemotherapy.

Adjuvant chemotherapy is given after surgery to reduce the risk of recurrence. Multiple chemotherapeutic agents may be used in combination to treat patients with breast cancer [18]. The appropriate regimen depends on the character of the tumor, lymph node status, age, and health of a patient. Chemotherapy has

increasing side effects as the patient's age passes 65. The table 3 shows a list of commonly used adjuvant chemotherapy for breast cancer.

Table 3. List of commonly used adjuvant chemotherapy for breast cancer. Source: [By the helping from Wikipedia, Breast Cancer Chemotherapy]

Regimen	Treatment strategy
CMF: cyclophosphamide, methotrexate, and 5-fluorouracil	4-weekly for 6 cycles
FAC : 5-fluorouracil, doxorubicin, cyclophosphamide	3-weekly for 6 cycles
AC : Adriamycin (doxorubicin) and cyclophosphamide	3-weekly for 4 cycles
AC-Taxol: AC given 3-weekly for 4 cycles followed by paclitaxel	either 3-weekly for 4 cycles or weekly (at a smaller dose) for 12 weeks
TAC: Taxotere (docetaxel), Adriamycin (doxorubicin), and cyclophosphamide	3-weekly for 6 cycles
FEC: 5-fluorouracil, epirubicin and cyclophosphamide	3-weekly for 6 cycles
FECD: FEC given 3-weekly for 3 cycles followed by docetaxel	3-weekly for 3 cycles
TC: Taxotere (docetaxel) and cyclophosphamide	3-weekly for 4 cycles
Dose dense regimen: Some of the regimens above (e.g. AC followed by paclitaxel)	shorter period (i.e. every 2 weeks instead of every 3 weeks
In addition to chemotherapy, trastuzumab may also be added to the regimen depending on the tumor characteristics (i.e. HER2/neu status) and risk of relapse	either 3 weekly or weekly for a total duration of 1 year

Cytotoxic agents such as anthracycline or taxane had played a central role in the systemic treatment of breast cancer. Cytotoxic agents improve survival and they have become the standard for operable breast cancer. The use of multiple active drugs in combination, either concurrently or sequentially, may improve treatment efficacy. But, it is difficult to test multiple combined cytotoxic drugs due to their toxicities.

4.2. HORMONAL THERAPY

Hormonal therapy is administered in patients with estrogen receptor positive tumors when their chemotherapy is completed. Hormonal therapies comprise inhibitors of estrogen receptors, aromatase inhibitors and GnRH-analogues.

4.2.1 Selective Estrogen Receptor Modulators (SERMs)

Selective Estrogen Receptor Modulators (SERMs) act on the estrogen receptor. A characteristic that distinguishes these substances from pure receptor agonists and antagonists is that their action is different in various tissues, thereby granting the possibility to selectively inhibit or stimulate estrogen-like action in various tissues. Estrogenic compounds have a spectrum of activity ranging from full agonists such as the natural endogenous hormone estrogen, mixed agonists/antagonistics (agonistic in some tissues while antagonist in others) such as tamoxifen (TAM) and pure antagonists such as fulvestrant. The members of SERMs include TAM, raloxifene, clomifene, afimoxifene (4-hydroxytamoxifen), arzoxifene, bazedoxifene, lasofoxifene, ormeloxifene, toremifene, femarelle (DT56a). TAM and toremifene are used for breast cancer. Raloxifene (RAL) is used for osteoporosis and reducing risk of invasive breast cancer .

TAM is an orally active SERM that is typically given to premenopausal women to inhibit the estrogen receptors. TAM itself is a prodrug which is metabolized in the liver by the cytochrome P450 isoform CYP2D6 and CYP3A4 into active metabolites such as 4-hydroxytamoxifen and N-desmethyl-4-hydroxytamoxifen (endoxifen) [19] which have 30-100 times more affinity with the estrogen receptor than TAM itself. It is a nonsteroidal agent with potent antiestrogenic properties that compete with estrogen for binding sites in breast tissue. When it binds ER on the tumors, it makes a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. TAM causes cells to remain in the G0 and G1 phases of the cell cycle. It is cytostatic drugs because it prevents (pre)cancerous cells from dividing but does not cause cell death. It is also approved by the FDA for the prevention of breast cancer in women at high risk of developing the disease. Its side effect comprises endometrial cancer, rapid increase in triglyceride concentration in the blood, increased risk of thromboembolism, fatty liver, reduction of libido. A beneficial side effect of TAM is that it prevents bone loss by inhibiting osteoclasts by acting as an estrogen receptor agonist (i.e., mimicking the effects of estrogen) in this cell type, and therefore it prevents osteoporosis [20].

4.2.2. Aromatase inhibitors (AI)

Aromatase inhibitors (AI) are used in the treatment of breast cancer and ovarian cancer in postmenopausal women that block the aromatase enzyme. AIs are given to postmenopausal women to lower the amount of estrogen in their systems. AIs comprise of two types. Types I are irreversible steroidal inhibitors such as exemestane which form a permanent bond with the aromatase enzyme complex. Types II are reversible non-steroidal inhibitors such as anastrozole, letrozole which inhibit the enzyme by reversible competition.

Aromatase inhibitors work by inhibiting the action of the enzyme aromatase. Aromatase converts androgens into estrogens by a process called aromatization. Estrogens stimulate breast tissue; for this reason to decrease their production is a way of suppressing recurrence of the breast tumor tissue.

In postmenopausal women estrogen is produced mostly from conversion of androgen in the adrenal gland and the adipose tissue. Because some breast cancers respond to estrogen, lowering the estrogen level in postmenopausal women using AIs has been proven to be effective in breast cancer treatment [21]. AIs are generally not used to treat breast cancer in premenopausal women because most of the circulating estrogen is produced by the ovaries, not by conversion of androgens to estrogen.

TAM has been standard treatment as adjuvant hormonal therapy in postmenopausal women with breast cancer; however, ATAC trial has shown that clinical results are superior with an AI in postmenopausal women with localized breast cancer that is estrogen receptor positive. Further studies of various AIs are ongoing [21].

4.2.3. GnRH Agonists

Gonadotropin-releasing hormone agonists (GnRH agonists) are used in premenopausal women for ovarian ablation or suppression. They are synthetic peptide modeled after the hypothalamic neurohormone GnRH that interacts with

the gonadotropin-releasing hormone receptor to elicit its biologic response, the release of the pituitary hormones FSH (follicle-stimulating hormone) and LH (luteinizing hormone). They are pregnancy category X drugs.

The dissociation of agonist from the GnRH receptor takes some time and it is not quick. Consequence of this phenomenon is an initial increase in FSH and LH secretion, which is the so called flare effect. However, after about ten days a profound hypogonadal effect, which is decrease in FSH and LH, is achieved through receptor downregulation by internalization of receptors. Generally this induction and reversible hypogonadism is the therapeutic goal.

Generally GnRH agonists are used in treatment of breast and prostate cancer, treatment of delaying puberty in patients with precocious puberty, management of female disorders that are dependent on estrogen production, sex reassignment of male to female transsexuals, and in vitro fertilisation therapy. These agonists are leuprolide, buserelin, nafarelin, histrelin, goserelin, deslorein, and triptorelin. Leuprolide, buserelin, goserelin, and triptorelin can be used in the therapy of breast cancer, but further study is required to admit their effectiveness. They are decapeptide with specific amino acid substitution typically in position 6 and 10. They can be administered intranasally, by injection, or by implant.

The main side effect of GnRH agonists is hypoestrogenism, which is appearing in the form of hot flashes, headaches, and osteoporosis. In patients with long-term therapy estrogen they could be given to patients to prevent bone wastage.

Leuprorelin or leuprolide acetate is GnRH agonist, which act by constant stimulation of the pituitary GnRH receptors. It initially causes flare, but decreases secretion of LH and FSH later on. It is one of the GnRH agonists which could be used in therapy of breast cancer.

Buserelin is another GnRH agonist, which is used in therapy of breast cancer and is normally delivered via a nasal spray but is also available as an injection. Buserelin acetate is also marketed under the brand name Suprefact.

Goserelin Acetate is an injectable super GnRH agonist also known as a Luteinizing Hormone-Releasing Hormone (LHRH) agonist. Goserelin Acetate is

marketed with the brand name Zoladex. It stops production of testosterone and estrogen. One of its indications is that it is used in premenopausal women with breast cancer. It has one or three months long acting depot, but only one month depot has been approved for breast cancer.

Goserelin has almost complete bioavailability and a serum elimination half-life of two to four hours. It rapidly binds to the LHRH receptor cells in the pituitary gland, thus leading to an initial increase in production of LH and thus leading to an initial increase in the production of corresponding sex hormones. This initial flare may be treated by co-administration of anti-androgen Casodex (Bicalutamide) or similar medication. Eventually, after a period of about 14-21 days, production of LH is greatly reduced due to receptor downregulation, and sex hormones are generally reduced to castrate levels. Goserelin Acetate side effect consist of bone pain, hot flushes, headache, upset stomach, difficult urination , and weight gain.

Triptorelin (acetate or pamoate) is another GnRH agonist and like other GnRH agonists it may be used in the treatment of hormone-responsive cancers, such as breast cancer. It is marketed under the brand names Decapeptyl, Diphereline, and Gonapeptyl and in the United States, as Trelstar.

4.2.4. Endocrine Therapy

Breast cancer is a hormone-dependent cancer and estrogen plays an important role in the development and progression of breast cancer. Endocrine therapy is the treatment of choice for estrogen receptor- and/or progesterone receptor-positive breast cancer and has been used for several purposes, including chemoprevention, preoperative treatment, postoperative adjuvant treatment, and treatment for recurrent diseases. Endocrine agents are less toxic than chemotherapeutic agents. Endocrine therapies such as antiestrogen, AI, LHRH agonist and progestin have been used for almost 20 years.

SERMs or third-generation AIs significantly reduce the incidence of contralateral breast cancer in women with breast cancer [3]. SERMs or AIs, which target a growth pathway dependent on estrogen receptors, are also standard systemic treatments.

Endocrine agents prevent new breast cancers or suppress subclinical breast cancers in women at increased risk for breast cancer. The idea of breast cancer

prevention has been clarified in several clinical trials such a NSABP P-1, IBIS-I, Royal Marsden, Italian, MORE, CORE, NSABP P-2, and most of them have supported it [3, 22-24].

RAL is as effective as TAM in reducing the risk of invasive breast cancer and has a lower risk of thromboembolic events and cataracts but non-statistically significant higher risk of non-invasive breast cancer [3].

4.2.4.1. Preoperative Endocrine Therapy

The purpose to do clinical trials for preoperative cytotoxic chemotherapies consist of down-staging of primary tumors resulting in operability, down-staging of primary tumors to allow breast-conserving surgery, and achieving pathological complete response resulting in a survival benefit.

Recently, a positive interaction between HER2 signaling and aromatase activity has been reported. This study shows that overexpression of HER2 leads to elevated levels of cyclooxygenase-2 and increased prostaglandin E2 (PGE2) production in breast cancer cells. PGE2 stimulates the CYP19 gene encoding aromatase in stromal or tumor cells. Consequently, estrogen biosynthesis is enhanced, which leads to increased proliferation of ER-positive tumor cells. It is suggested that these findings may provide a rational explanation for the previous observation that ER-positive, HER1/2-overexpressing breast cancers showed an improved response rate to letrozole LET compared with TAM [3, 25].

TAM is ineffective because of its estrogen agonistic activity in ER positive breast cancers overexpressing HER1/2. TAM works as an estrogen agonist through the phosphorylated ER-$\boldsymbol{\alpha}$.

4.2.4.2 Postoperative Endocrine Therapy

Because MBC is hardly curable, prevention of metastatic diseases is important to reduce mortality from breast cancer. TAM has been the endocrine agent of choice for postoperative adjuvant therapy for breast cancer for over two decades. However, recent clinical trials have revealed that third-generation AIs are more potent than TAM in postmenopausal patients with hormone receptor positive breast cancer in the postoperative adjuvant setting.

There is a definitive effect of ovarian ablation or suppression both on recurrence and on breast cancer mortality. Ovarian ablation, such as surgical ovariectomy, radiation of ovaries or administration of LHRH agonists is useful for postoperative adjuvant therapy in premenopausal patients with ER-positive breast cancer. The overview of the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) has showed that ovarian ablation produced significant reductions in recurrence and death in these patients. In this study the usefulness of TAM in postoperative adjuvant setting for premenopausal patient has been shown [3, 26].

4.2.4.3 Endocrine Therapy for Metastatic Breast Cancer

Endocrine therapy is preferred to chemotherapy for MBC because of safety profile and the duration of response. The main purpose of therapy is to prolong survival while limiting toxicity and reduce malignancy of associated symptoms.

Clinical trials have shown that either TAM or an LHRH agonist alone is as effective as surgical ovarian ablation in premenopausal women with MBC [3].

The combination of the LHRH agonist buserelin with TAM prolongs progression-free survival and increases both the response rate and duration in premenopausal patients with hormone-responsive and MBC compared with the LHRH agonist alone [3].

The combination of an LHRH agonist and an AI as second-line endocrine therapy could be used in premenopausal women with advanced breast cancer, despite there are not available enough data about this combination.

Anastrozole (ANA) was compared with megestrol acetate (MA) in the Arimidex Study Group trials. It was reported that ANA was well tolerated and as effective as MA in the treatment of postmenopausal women and avoided the weight gain which is associated with MA treatment [3].

Fulvestrant (FUL) is a new type of ER antagonist that downregulates the ER and has no known agonistic effect. FUL is tolerated well and is as effective as ANA in the second-line treatment of patients with advanced breast cancer.

AI can be used in the third-line therapy in premenopausal women who have undergone ovarian ablation. AI can be also used in the first-line therapy of MBC in women who have received postoperative adjuvant TAM.

Endocrine agent works synergistically when combined with a chemotherapeutic agent. Combination of TAM and cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) is more active than CMF alone and induced a longer time-to-progression (TTP) and a better Overall Survival (OS), for this reason the sequential therapy is advisable for common clinical use. But adverse effect, like thromboembolism, is more frequent in patients treated with concurrent combination of chemotherapy and TAM. For this reason, the concurrent use of TAM with chemotherapy is inferior to sequential use.

Concurrent use of antiestrogen and the humanized anti-HER2 monoclonal antibody trastuzumab have synergistic antitumor activity in breast cancer cells expressing ER and HER1 and/or HER2 [3, 27].

Both prospective randomize clinical trials (RCTs) and basic research on the cellular and molecular biology of breast cancer have promoted remarkable progress in endocrine therapy for breast cancer during the past three decades [3].

The aim of systemic treatments for metastatic disease is a control of disease progression with relief of symptoms and prolongation of survival. Chemotherapy, endocrine therapy and herceptine are important parts of the management of MBC. If the tumor shows response to first-line endocrine therapy, the second-line endocrine therapy should be offered rather than cytotoxic therapy.

The selection of endocrine agents takes into account the menopausal status of the patient, the type of previous adjuvant endocrine treatment, the disease free interval and past medical history. In premenopausal women with metastatic disease, the combination of a LHRH agonist plus TAM is the first-line endocrine therapy. In postmenopausal women with metastatic disease, AI is the choice of therapy [28].

In premenopausal women with MBC if the tumors are resistant to TAM, because this is not cross resistant in endocrine therapy, an alternative endocrine therapy or systemic chemotherapy can be offered.

Treatment with medroxyprogesterone acetate (MPA) or surgical removal of the ovaries, followed by an AI should be considered as additional endocrine therapies for second-line and subsequent therapy in premenopausal women with MBC.

In postmenopausal women with advanced breast cancer AIs are preferred to TAM. MPA is the last choice of endocrine treatment. In the second-line setting selective AIs like anastrozole, letrozole, and exemestane have all been shown to offer efficacy and tolerability advantages over megestrol acetate, in TAM-resistant postmenopausal patients who are within one year of antiestrogen exposure with hormone dependent advanced breast cancer [28].

4.2.4.4 Endocrine Therapy in Early Breast Cancer

Adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer should include an AI [28, 29].

In 2005 the St.Gallen guidelines were updated as following:

- A fundamental change in the algorithm used for selecting adjuvant systemic therapy for early stage breast cancer

- Endocrine responsiveness (which should be better defined and validated) will dominate treatment selection for adjuvant therapy

- AIs have entered the adjuvant stage [28, 30].

Tumors are classified as endocrine-responsive, non-responsive or with uncertain endocrine responsiveness. These categories are then subdivided by menopausal status, and only then divided by risk. Risk categories were redefined as called low, intermediate and high [28].

Because most estrogen is produced by the ovaries in premenopausal patients, thus oophorectomy or prevention of estrogen through medical therapy is the choice of therapy.

TAM is a standard adjuvant treatment for premenopausal patients with endocrine responsive early breast cancer. Ovarian function suppression (OFS) or

ablation on combination with or without TAM for premenopausal women with endocrine responsive early breast cancer is recommended by National Institute of Health (NIH). The combination treatment of OFS plus TAM may be reasonable for very young patients of intermediate risk and high risk, and patients of high risk who have no chemotherapy induced OFS [28].

CMF followed by goserelin plus TAM improved outcomes for premenopausal node-positive, receptor-positive early breast cancer.

AIs are used as adjuvant endocrine therapy for postmenopausal women with early breast cancer. ANA improved disease-free survival with a lower incidence of thromboembolic events and endometrial cancers relative to TAM, while musculoskeletal complaints and bone fractures were more frequent with anastrozole.

Most of studies are based on comparison of TAM and AIs shows that switching to AIs after 2-3 years of therapy with TAM improved DFS with a lower incidence of thromboembolic events and gynecologic symptoms compared with continuation of TAM for a 5-year course of treatment. AIs can be improved by DFS, for this reason, women who have received 2 to 3 years of TAM should be considered candidates for switching to exemestane or anastrozole [28].

4.2.4.5 Neoadjuvant Use of Endocrine Therapy

Neoadjuvant endocrine therapy is a safe and effective alternative to chemotherapy. Neoadjuvant hormonal therapy is effective at down-staging tumors, particularly large tumors initially thought to be inoperable or requiring mastectomy. The neoadjuvant setting provides an opportunity to sample tumors during treatment and correlate biological changes with response [31].

AIs such as letrozole and anastrozole in the neoadjuvant strategies increase the number of women who are suitable for breast-conservation. They are agents of choice to be used as neoadjuvant in postmenopausal women with hormone sensitive breast cancer.

AIs are most effective in ER rich tumors, although letrozole is effective at even low ER, whereas TAM is not. The aromatase inhibitors letrozole and anastrozole are clinically and biologically effective in both HER2 positive and negative tumors, whereas HER2 positive tumors show a level of resistance to

TAM. Letrozole has been used safely in those who do show a response at 3–4 months for up to 12 months [31].

4.3. TARGETED THERAPY

Targets for treatment of breast cancer comprise ER/PR, HER2 receptor protein, HER1/EGFR, insulin-like growth factor1 (IGF-1) receptor protein, PI3K/AKT/mTOR cell survival pathway, VEGF receptor protein, P53, and other targets. There are many types of drug groups which could be used in target therapy. Monoclonal antibodies, kinase inhibitors, aromatase inhibitors, and vaccines are of these types.

Overexpression of human epidermal growth factor receptors (HER1/EGFR and HER2), and vascular endothelial growth factor in many solid tumors correlates with poor prognosis. These receptors are the best target for inhibition of the tumor proliferation.

Target therapy is one of new strategies used for a treatment of breast cancer. Trastuzumab (Herceptin) is used to block the activity of the HER2 protein in breast cancer cells, slowing their growth. In the advanced cancer setting, trastuzumab use in combination with chemotherapy can both delay cancer growth as well as improve the recipient's survival [32].

More recently, several clinical trials have also confirmed that in the adjuvant setting i.e. postoperative following breast cancer surgery, the use of trastuzumab for up to one year also delays the recurrence of breast cancer and improves survival [33-35].

4.3.1. ANTIANGIOGENIC THERAPY

Every solid tumor needs to generate blood vessels to keep it alive once it reaches a certain size. Usually, blood vessels are not built elsewhere in an adult body unless tissue repair is actively in process. An angiogenesis inhibitor is a

substance that inhibits angiogenesis (the growth of new blood vessels). The angiogenic factor could be used as a target for treatment of breast cancer.

4.3.1.1 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen and survival factor. VEGF also stimulates vascular permeability and recruits progenitor endothelial cells from bone marrow. VEGF has an anti-apoptotic, pro-survival effect on endothelial cells.

VEGF is essential pro-angiogenic growth factors expressed by most cancer-cell types and certain tumor stromal cells. Angiogenesis plays an essential role in development, invasion, and metastasis in breast cancer. Formations of blood vessel in tumors help cancer development. Inhibition of blood vessel formation by blocking the action of VEGF is one approach to treat breast cancer [36].

VEGF is produced by both malignant cells and non-malignant cells in response to hypoxia, inflammation, growth factors, cytokines, and by malignant cells and epidermal growth factor (EGF), transforming growth factor (TGF), keratinocyte growth factor (KGF), IGF, interleukin 1α (IL-1α), and interleukin 6 (IL-6) [37, 38]. It has been also shown that prostaglandin E2, thyroid stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH) can also increase the expression of VEGF mRNA [36].

Some cytokines such as interleukin 10 (IL-10) and interleukin 13 (IL-13) down-regulate VEGF expression.

Vascular endothelial growth factor receptors (VEGFR) consist of three cell-membrane type receptors that belong to the tyrosine-kinase receptor family (VEGFR-1/Flt-1, VEGFR-2/Flk-1 (KDR), VEGFR-3 (Flt-4)), and a soluble form of VEGFR-1 (sVEGFR-1), an intrinsic negative counterpart of the VEGF. VEGFR-1 and VEGFR-2 are tyrosine kinase receptors localized on vascular endothelial cells which signal VEGF. VEGFR-2 functions as the principal receptor for VEGF signaling, whereas VEGFR-1 functions as a decoy receptor to regulate the availability of VEGF [36].

VEGF status is correlated with neovascularization grade and prognosis in various types of solid tumors and can be predictive for the resistance to various treatments, including radiotherapy, chemotherapy, and hormone therapy.

4.3.1.1.1. Clinical Evidence of VEGF in Breast Cancer

In cancer patients compared to healthy individuals, there are higher levels of plasma platelets and these platelets contain high amounts of VEGF and because the VEGF storage system in these platelets is altered, it makes VEGF release more likely upon activation.

In preclinical evidence the increased level of hypoxia-inducible factor 1 (HIF-1α**)** expression is significantly correlated with an increased level of VEGF expression. VEGF expression level in the tumor tissue is a significant predictor of relapse-free survival and overall survival in node-negative breast cancer patients treated with loco-regional radiotherapy [36].

Fibrocystic lesions with the highest vascular density are associated with a greater risk of breast cancer. Histopathologically aggressive ductal carcinoma-*in-situ* lesions demonstrate higher microvessel density (MVD) and increased VEGF expression. Intratumoral VEGF expression is significantly correlated with MVD and poor prognosis in breast cancer [36].

4.3.1.1.2. VEGF-Targeting Treatment

Maximal anti-angiogenic activity would be achieved in a therapeutic system, the so called metronomic therapy. In metronomic therapy the combination of low, frequent dose chemotherapy plus an agent that specifically targets the endothelial cell compartment controlled tumor growth works more effectively than the cytotoxic agent alone. VEGF targeted therapy has arrived as frontline treatment for MBC in the adjuvant, neo-adjuvant, Her2-positive, and metronomic setting.

VEGF-targeting treatments include large molecules such as neutralizing antibodies against VEGF and VEGFRs and the soluble form of VEGFR-1, and small molecules such as signal transduction inhibitors.

Therapeutics strategies which could inhibit the function of VEGF are shown in the figure 5. Receptor Tyrosine Kinase Inhibitors (TKI) such as SU11248

(sunitinib malate), PTK787 (Vatalanib) Sorafenib (BAY 43-9006), and Natural Inhibitor of Angiogensis such as ribozyme, MMP and Neovastat (AE-941), which are agents targeting the VEGF pathways are currently under investigation [36].

Despite the mechanism of action of anti-VEGF therapy is not clear but according to the clinical evidence there some hypotheses which are as follows: (a) anti-VEGF agents can prune tumor vessels in patients and thus kill a fraction of cancer cells; (b) anti-VEGF agents can normalize tumor vasculature and microenvironment; and(c) anti-VEGF treatment can reduce the number of blood circulating endothelial cells and progenitor cells [36, 39].

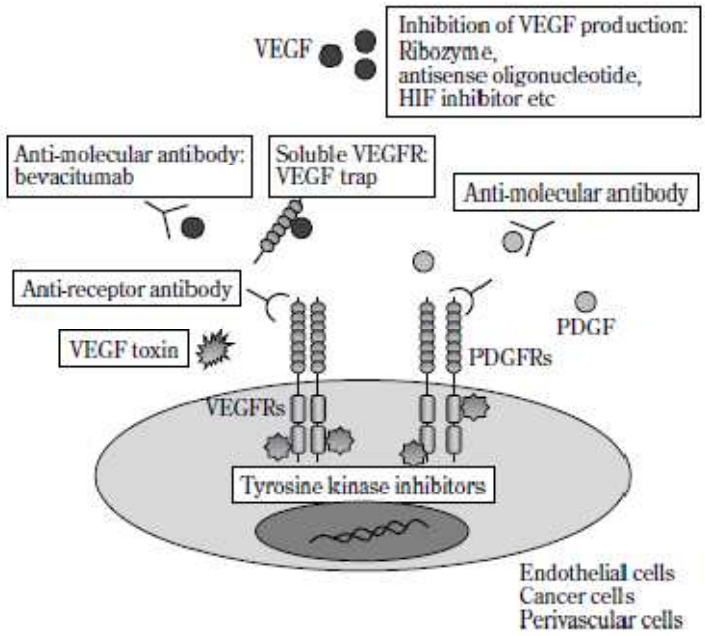

Figure 5. Schematic representation of the therapeutic targets and therapeutic agents which could inhibit the function of VEGF. Source: [36]

Most agents that target VEGF or its receptors have been well tolerated either as mono-therapy or in combination with standard chemotherapy. Their most important side effect is hypertension and heightened risk of bleeding. The other side effects include increased risk of congestive heart failure, thromboembolism, and impaired wound healing, and life-threatening or fatal hemorrhage. Fatigue is a common side effect for the multi targeted TKIs [36, 40, 41].Agents targeting the VEGF pathways are shown in figure 6.

The National Cancer Institute recently reported that the patients with inflammatory or locally advanced breast cancer who received bevacizumab alone for the first cycle followed by six cycles of bevacizumab with doxorubicin and docetaxel showed evidence of a decrease in vascular permeability on dynamic contrast enhanced magnetic resonance imaging after the first cycle of bevacizumab mono-therapy [36].

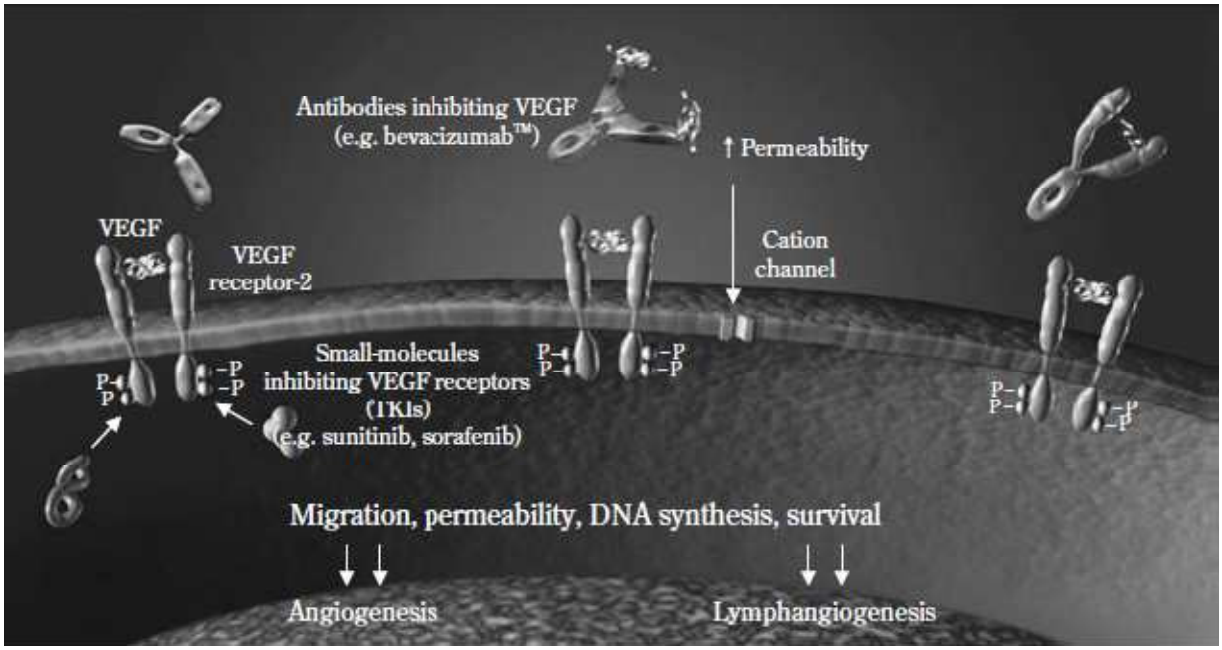

Figure 6. Agents targeting the VEGF pathways. Source: [42]

Bevacizumab (Avastin) is the most advanced therapeutic agent specifically designed to disrupt angiogenesis. It is a humanized monoclonal antibody directed against the VEGF and consists of 93% human and 7% murine components. It is well tolerated and has a half-life of 17-21 days. It normalizes the vascular architecture, thus allowing greater penetration of chemotherapy and resulting in further damage to the vasculature and tumor shrinkage. The combination of bevacizumab with chemotherapeutics such as the taxanes results in synergistic anti-endothelial cell activity [36, 43, 44].

Bevacizumab underwent testing in a randomized clinical trial whose preliminary results were announced by the National Cancer Institute in 2005. The preliminary data indicated that bevacizumab delays disease progression for up to five months over conventional chemotherapy, but survival was no better [45].

Sunitinib is a novel oral small-molecule multi-targeted receptor tyrosine kinase inhibitor that has demonstrated direct antitumor activity and anti-angiogenic action [46]. It targets VEGFR, platelet derived growth factor receptor (PDGFR), stem-cell factor receptor and Fms-like tyrosine kinase receptor 3 receptor tyrosine-kinases [42].

Sorafenib may stop the growth of tumor cells by blocking blood flow to the tumor and by blocking some of the enzymes needed for cell growth.

Combination therapies which contain sorafenib are under current study. Sorafenib and anastrozole may be useful for postmenopausal women with MBC. The combination of two molecular targeting drugs such as sorafenib and trastuzumab in phase Ⅱ studies will be a unique approach [42].

Sometimes hormonal therapy does not stop the growth of tumor cells which means that the tumor is resistant to hormonal therapy, for this reason sorafenib would add to therapeutic regime that may reduce drug resistance and allow the tumor cells to be killed.

HIF-1 is the central mediator of cellular responses to low oxygen and has recently become an important therapeutic target for solid tumor therapy. Inhibition of HIF-1 is expected to result in the attenuation of hypoxia inducible genes, which are vital to many aspects of tumor biology, including adaptive responses for survival under anaerobic conditions.

Other agent like AZD2171, Circulating Endothelial Cell (CEC), ILGF-IR Inhibitors BMS-554417, Src Inhibitor, Hsp90 Inhibitor, and Signal Transducers and Activators of Transcription (STAT) are currently under study and there is not so much evidence about them.

4.3.2. Epidermal Growth Factor Receptor

Epidermal growth factor receptor (EGFR) family is a family of four structurally related receptor tyrosine kinases. EGFR are also called ErbB protein family. The member of ErbB protein family consists of ErbB-1 (also named

epidermal growth factor receptor), ErbB-2, (also named HER2 in humans and neu in rodents), ErbB-3 (also named HER3) and ErbB-4 (also named HER4).

Human epidermal growth factor receptor type 2 (HER-2) is a protein giving higher aggressiveness in breast cancers. It is a cell membrane surface-bound receptor tyrosine kinase and is normally involved in the signal transduction pathways leading to cell growth and differentiation. HER-2 is over expressed in around 25% of human breast cancers, and is associated with poor outcome. However the incidence of HER-2 protein over-expression in IBC is higher than non-IBC. Action and site of molecular targeting drugs, especially in HER-2, and the signaling pathway are shown in figure 7.

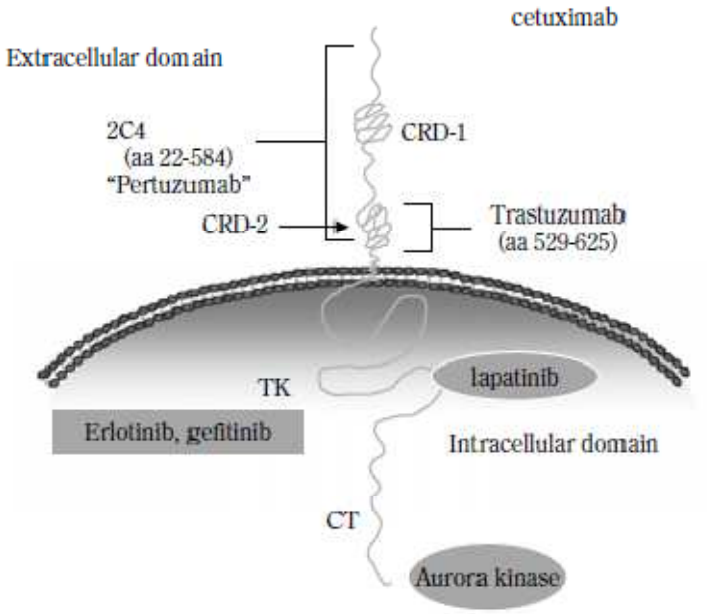

Figure7. Action and site of molecular targeting drugs, especially in HER-2, and the signaling pathway. Source: [42]

HER-2 is important as the target of the monoclonal antibodies trastuzumab (Herceptin) and Pertuzumab. Accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from HER-2-targeted therapy.

Trastuzumab (Herceptin), a humanized monoclonal antibody targeted to the extracellular domain of HER2, benefits patients with MBC, and improves DFS and OS after adjuvant chemotherapy, when administered weekly or every three weeks as monotherapy or in combination with chemotherapy. It is a chimeric monoclonal antibody against the Her-2 product. It binds to the domain IV of the extracellular segment of the HER2/neu receptor and inhibits Her-2 positive breast cancer cell growth. Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle so there is reduced proliferation.

Induction of the cyclin-dependent kinase (CDK) inhibitor p27Kip1 protein is one of the key mechanisms of action of HER2-targeting antibodies. Anti-HER2 antibodies inhibit HER2-mediated signaling in cancer cells, ultimately upregulating the levels and activity of p27Kip1 protein. Trastuzumab modulates some signaling targets and pathways by inhibiting CDK2 and decreasing Thr187 phosphorylation of p27Kip1, by inhibiting AKT and human kinase interacting stathmin (hKIS), by inhibiting Jun activation domain-binding protein 1 (Jab1), and by inhibiting cyclin D and c-Myc, by stimulating minibrain related kinase (MIRK). These targets and pathways which are affected by trastuzumab work in concert to maximize the expression and inhibitory effect of p27Kip1 on CDK2 and lead to cell cycle G1 arrest and growth inhibition.

Trastuzumab is active as a single agent in HER2 positive patients, well tolerated, and is associated with preservation of quality of life (QOL). Trastuzumab combined with chemotherapy increases response rates, time to disease progression, and survival. It has shown important activity when used with many chemotherapeutic agents such as platinum salts, gemcitabine, vinorelbine and capecitabine and liposomal anthracyclines [42].

Trastuzumab alone or trastuzumab in combination with chemotherapy regimens are standard treatment worldwide as first line therapy for MBC patients with HER2 overexpression/amplification that prolonged OS [47].

Adjuvant trastuzumab treatment improves DFS and OS for early breast cancer patients, based on data from four large trials such as National Surgical Adjuvant Breast and Bowel Project (NSABP) plus North Central Cancer Treatment Group (NCCTG) study, Herceptin adjuvant (HERA) trial, Breast Cancer International Research Group (BCIRG) 006, and Finland Herceptin (FinHer) trial [33-35, 47].

The National Comprehensive Cancer Network (NCCN) guidelines already recommend that adjuvant trastuzumab treatment should be used as a standard therapy for node negative high risk and node positive early breast cancer patients with HER2 overexpression [47].

Cardiac toxicity and decreased left ventricular ejection fraction (LVEF) are side effects. However, decrease of LVEF is fortunately reversible and LVEF is improved from 6 months after trastuzumab therapy is stopped [47].

Trastuzumab treatment has dramatically improved the prognosis of HER2-positive patients in adjuvant and metastatic settings. Trastuzumab treatment is already considered to be standard therapy for HER2-positive patients by many guidelines around the world.

Monoclonal antibodies such as trastuzumab can kill tumor cells and also be used as tumor-killing substances delivery to tumor location without harming normal cells.

EMD-72,000 is humanized antibody which is used to decrease the prevalence or severity of infusion reactions; therefore they will shorten the time of drug administration, and improve the patients' QOL.

The resistance mechanism to trastuzumab is not well understood. Action of trastuzumab may be blocked because the interaction between trastuzumab and its target receptor HER2, due to steric hindrance of HER2 by cell surface proteins such as mucin-4 (MUC4), would be decreased. Loss of function of the tumor suppressor PTEN gene, the negative regulator of Akt, results in heightened Akt signaling, which leads to decreased sensitivity to trastuzumab [42].

Recent studies of breast cancer cells have revealed a bi-directional connection between Her-2/neu and fatty acid synthase (FAS), a major lipogenic enzyme catalyzing the synthesis of long-chain saturated fatty acids. Her-2/neu overexpression stimulates the fatty acid synthase (FAS) promoter and ultimately mediates increased endogenous fatty synthesis, and this Her-2/neu-mediated induction of breast cancer-associated FAS can be inhibited by trastuzumab [42, 48]. Moreover, specific FAS blockade synergistically sensitizes breast cancer cells carrying Her-2/neu oncogene amplification and/or overexpression to trastuzumab-induced cell growth inhibition and apoptotic cell death [42].

4.3.3. Tyrosine Kinase Inhibitors

There are some agents targeting TKI. They consist of Erlotinib, Gefitinib, PI3K/Akt, and Lapatinib.

Erlotinib (Tarceva) is a potent HER1/EGFR tyrosine kinase inhibitor. Erlotinib may interfere with the growth of tumor cells and slow the growth of the tumor. Combining trastuzumab with erlotinib may kill more Her-2 overexpressing tumor cells [42, 49].

Erlotinib and pertuzumab are active against various human xenograft models, independently of HER1/EGFR or HER2 expression. A combination of these HERtargeted agents resulted in additive or greater than additive antitumor activity. (Pertuzumab (Omnitarg), a novel HER2-specific, recombinant, humanized monoclonal antibody, prevents heterodimerization of HER2 with other HERs) [42, 49].

Gefitinib, a small-molecule EGFR tyrosine kinase inhibitor, can be used in combination with docetaxel as first-line treatment for women with MBC [50].

The effects of gefitinib on the growth of three breast cancer cell lines showed high, intermediate, and low activity of the drug. The combination of gefitinib and taxane showed a strong synergistic effect.

The naturally occurring bioflavonoid Quercetin (Qu) is structurally homolog with the commercially available selective PI3K inhibitor, LY 294002 (LY). Both Qu and LY treatments for 24 h significantly decreased cell proliferation. Qu inhibits the PI3K-Akt/PKB pathway, in a manner similar to that of commercially-available LY [42].

Lapatinib is an oral receptor tyrosine kinase inhibitor, targeting both the ErbB-1 and ErbB-2 receptors [51, 52]. It can be used as monotherapy and also in combination therapy. Lapatinib is active against breast cancer. It is thought to penetrate the blood brain barrier for this reason it can be used in patients with brain metastase [42].

Antibodies and small molecule tyrosine kinase inhibitors targeting ErbB2 exhibit distinct, noncross resistant mechanisms of action.

Lapatinib combining with anti-ErbB2 antibodies like trastuzumab would enhance the apoptosis of ErbB2-overexpressing breast cancer cells and markedly downregulated survivin protein. The association between the inhibition of survivin and enhanced apoptosis may help identify therapeutic strategies which promote tumor cell apoptosis and might improve clinical response [36, 53].

4.3.4. Steroid Sulfatase Target

Enzyme, steroid sulfatase (STS) is the enzyme responsible for the hydrolysis of steroid sulfates to their unconjugated, biologically active forms. STS hydrolyzes steroid sulfates, such as estrone sulfate (ES) and dehydroepiandrosterone sulfate (DHEAS), to estrone and DHEA, which can be converted to steroids with potent estrogenic properties, that is, estradiol and androstenediol, respectively. The process which STS are involved in synthesis of estrogenic steroids and the site of action of its inhibitor is shown in figure 8 [54].

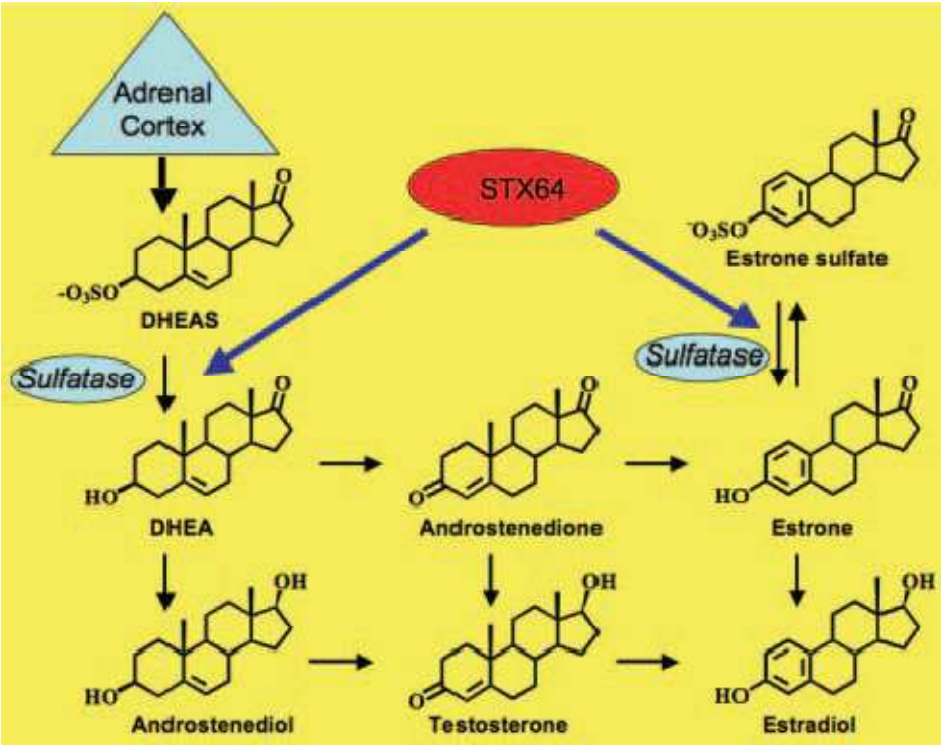

Figure 8. Pathways of steroidogenesis in postmenopausal women
And site of action of STX64 (BN83495). source: [54]

The halflife of ES in plasma (10–12 hours) is considerably longer than that for estrone and estradiol (20–30 minutes) that's why E1S would act as reservoir for the formation of active estrogens via the action of STS [54].

STS has a crucial role in regulating the synthesis of estrogenic steroids in breast cancer [55]. STS activity is much higher than aromatase activity in breast tumors and high levels of STS mRNA expression in tumors are associated with a poor prognosis.STS convert ES to estrone that is then reduced to the biologically active estrogen, estradiol, by 17β-HSD1, which is overexpressed in many breast tumors (Fig. 8).

The level of STS mRNA expression in breast tumors is much higher than aromatase mRNA expression and STS mRNA expression is higher in malignant than in normal breast tissue. The level of STS mRNA expression is important for prognosis [54].

Inhibitors of steroid sulfatase are being developed as a novel therapy for hormone-dependent breast cancer in postmenopausal women. The potent irreversible STS inhibitors include STX64, a tricyclic sulfamate ester and STX213 the second-generation inhibitor of STS with a steroid structure. They have sulfamate ester attach to aryl ring.

These STS inhibitors are orally active with a high level of bioavailability. This results from their binding to carbonic anhydrase Ⅱ in erythrocytes after absorption, which enables them to transit the liver without undergoing first-pass inactivation [54, 56].

STS inhibitors can offer therapeutic benefit in patients who have progressed on antiestrogen and/or aromatase inhibitor therapies [54].

STX64 is able to almost completely block STS activity in peripheral blood lymphocytes and tumor tissues. Inhibition of STS activity is associated with significant reductions in serum concentrations of androstenediol and estrogens.

In the strategy of drug administration the patients receiving an initial dose (cycle 0) followed by 3 × 2 weekly cycles (cycles 1–3), with each cycle consisting of daily dosing for 5 days followed by 9 days off treatment. If the dosage is 5 mg the inhibition of STS activity is more than 96%, however, with 20mg dosage complete inhibition of STS activity would be achieved. The concentration of

androstenedione also decreases by up to 86%. Because androstenedione is the substrate for aromatase in postmenopausal women, this means that androstenedione is derived mainly from the peripheral conversion of DHEAS in postmenopausal women [54].

STS inhibitors can offer therapeutic benefit in patients who have progressed on antiestrogen and/or aromatase inhibitor therapies. They have favorable risk/benefit ratio in the setting of locally advanced or metastatic disease.

4.4. Resistance to Chemotherapy in Breast Cancer

Cancer cells have the ability to become resistant to multiple different drugs. It has been established that membrane proteins, notably multidrug resistance (MDR), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP) of the ATP binding cassette (ABC) transporter family encoding efflux pumps, play important roles in the development of multidrug resistance [57] .

The breast cancer resistance protein, BCRP/ABCG2, is a half-molecule ATP-binding cassette transporter that facilitates the efflux of various anticancer agents from the cell. The expression of BCRP can thus confer a multidrug resistance phenotype in cancer cells, and its transporter activity is involved in the in vivo efficacy of chemotherapeutic agents[58].

The majority of tumors that initially respond to treatment with TAM develop resistance over time (acquired resistance), despite continued expression of ERα and approximately 30% of ERα-positive breast cancers do not respond to TAM treatment (*de novo* resistance) [59].

X. CANCER STEM CELLS

Recently there has been great interest in study on stem cells and their role in tumor formation. This chapter provides a brief introduction to the biology of stem cells and then their role in the malignant process. Unless we understand the biology of stem cells we will not be able to discover their role in tumor formation. Stem cells may be the target cell for malignant transformation, and tumor formation could be considered a disorder of stem cell self-renewal pathways.

As it is shown in the figure 9, two characteristics of a stem cell are self-renewal division and the production of daughter or ‘progenitor’ cells. In this

asymmetric division stem cells create exact copies of themselves and an early progenitor cell when they divide. The early progenitor cell then progresses to a late progenitor cell and then to the definitive cell line. They initially retain many of the characteristics of their parent stem cell but sequentially lose their self-renewing potential with each subsequent cell division as they differentiate to generate mature cells of at least one but often many cell types within an organ [60].

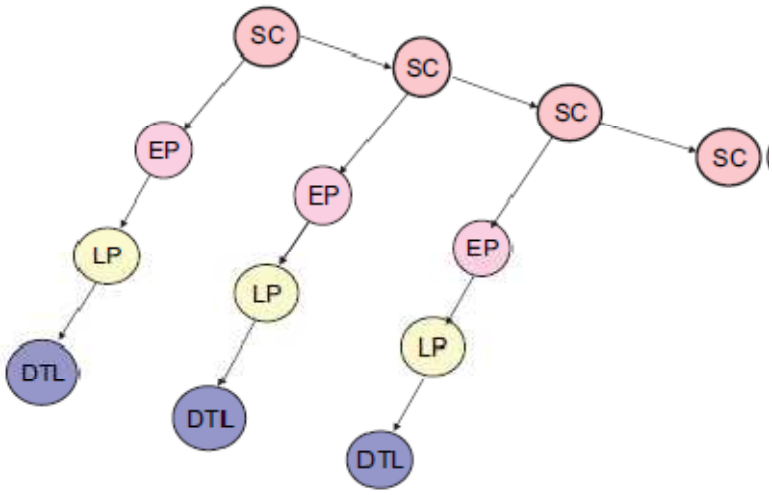

Figure 9. Stem cell self-renewal division and the production of daughter or 'progenitor' cells. DTL: definitive tissue line; EP: early progenitor; LP: late progenitor; SC: stem cell. Source: [60]

Table 4 compares the characteristics of somatic stem cells and cancer cells. Somatic stem cells generate normal tissues in a highly regulated process, whereas cancer stem cells produce tumors [61, 62].

Table 4. Comparison of somatic and cancer stem cells. Source: [60]

Somatic stem cell	**Cancer stem cell**
Self-renew, highly regulated	Self-renew, poorly regulated
Differentiate, produces mature tissues	Differentiate, produces tumor
Migrate to distance tissues	Metastasize to distant sites
Long lifespan	Long lifespan
Resistant to apoptosis	Resistant to apoptosis

The traditional model of tumor formation says that series of mutations affect a mature cell, causing it to become malignant. Any cell has the potential to form a tumor. In the new model of tumor formation mutation only at the stem cell or

progenitor cell level causes tumor cells. The cancer stem cell replicates forming an exact copy of itself as well as a continuous supply of heterogeneous tumor cells [60].

Tumors arise from a series of sequential mutations resulting from genetic instability and/or environmental factors affecting normal cells. Long-lived tissue stem cells undergo mutations that deregulate normal self-renewal pathways, leading to tumor formation. Cancer stem cells or their immediate progenitor cells may be sites for initial mutation. Tumor cells progress through a preneoplastic into a neoplastic phase and subsequently metastasize [60, 63].

1. BREAST CANCER STEM CELL

Cancer stem cells in breast cancer are the cells which expressing the CD44+CD24-lineage negative marker. This cell was discovered by injection into mice, and it has been shown that as few as 200 CD44+CD24-lineage negative cancer cells could consistently form breast cancers in mice [60, 62].

Tissue stem cells use multiple signaling pathways to control normal stem cell self-renewal. Deregulation of these pathways may lead to neoplastic proliferation with the development of a cancer stem cell. The Notch pathway and Wnt pathway are important in breast cancer[60-62, 64].

The mammary gland develops by differentiation from its mammary stem cell. A diverse range of breast cancers may, therefore, develop depending on where a mutation occurs in this pathway. According to this, three types of breast cancer for stem cell model for estrogen receptor expression has been proposed which comprise of ER-positive, ER-negative or heterogenous receptor and the mutation that could happened to in every case is shown in the figure 10 [60, 62, 64, 65].

Type 1 tumors develop from mutations in ER-negative stem/ progenitor cells, blocking differentiation and preventing the development of ER-positive progenitors. These tumors are poorly differentiated and appear to be more aggressive with a poorer prognosis. Less than 10% of these tumors are ER positive [60, 65].

Type 2 tumors are also derived from mutations in the ER-negative stem/progenitor cells. However, a variable percentage of the tumor will

differentiate into ER-positive cells. Antiestrogen therapy can lead to a decrease in tumor size but has no effect in ER-negative tumor [60, 65].

Type 3 tumors are well differentiated and result from mutations in ER-positive progenitor cells. Hormone replacement therapy use increases the risk of cancer formation. They respond best to antiestrogen therapy and have the best prognosis [60, 65].

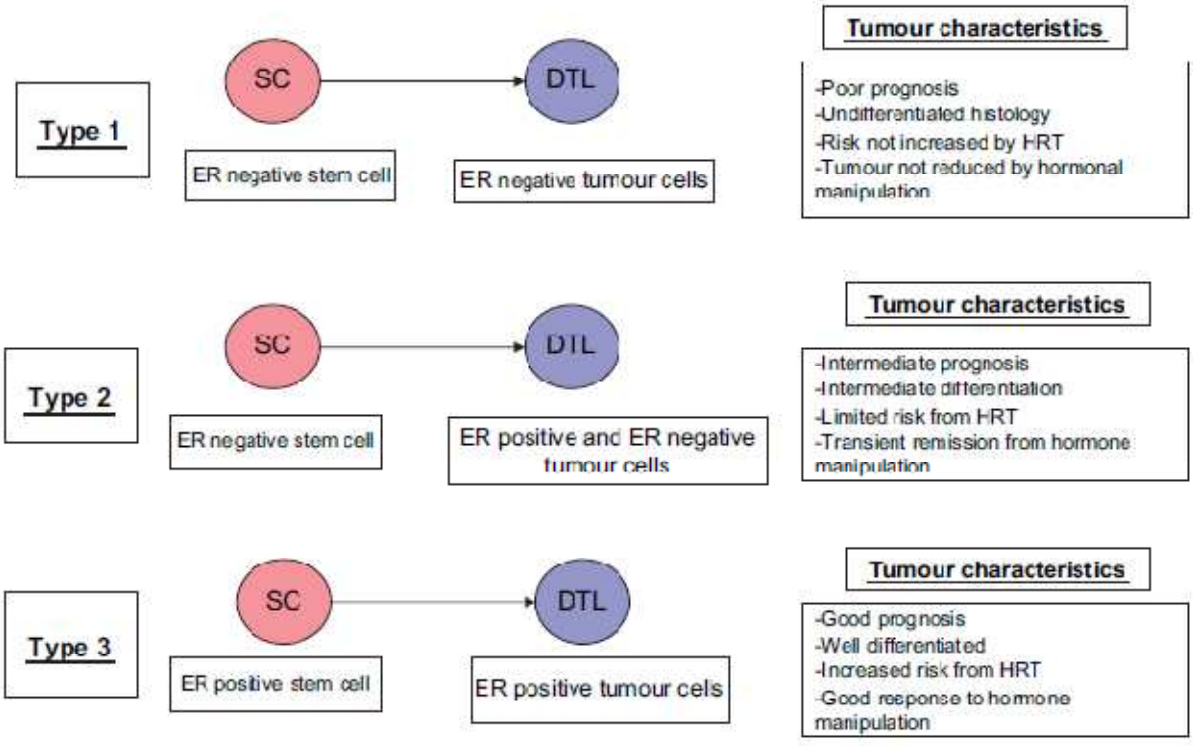

Figure 10. Three proposed models explaining how the point at which a mutation occurs will alter the estrogen receptor status of breast cancer. DTL: definitive tissue line; ER: estrogen receptor; SC: stem cell. Source: [60]

2. Therapeutic Aspect of Stem Cells

As stem cells have overexpression of anti-apoptotic agent such as Bcl-2 and express drug-resistance transporter proteins such as MDR1 and ABC transporters, they develop resistance to apoptosis and chemotherapy.

It is suggested that cancer stem cells also express these protein higher than that of the bulk population of tumor cells, which in turn may be resistant to

chemotherapeutic agents, permitting the reproliferation of tumors after chemotherapy [60].

Target therapies which are selectively toxic to cancer stem cells are more effective treatment for eradication of this crucial population of cells. To identify therapeutic targets that preferentially attack cancer stem cells it is necessary to compare gene expression profiles of cancer stem cells and normal stem cells.

XI. CONCLUSION

Breast cancer is the most common cancer among women and continues to be a major cause of death in European and American women. It occurs most frequently in postmenopausal women. For this reason it is one of the most important objects of study in the world. Significant progress has been made in the field of breast cancer treatment. However, there are still many questions which remain unanswered. They mainly concern the effectiveness and benefits of treatment and prevention guidelines. Treatment guidelines facilitate individual clinical decision making. Treatment guidelines are made according to the latest available evidence from randomized controlled trials and meta-analyses, therefore, the results of clinical trials are most important in the process of establishing these guidelines. The goal of all treatment strategies is maximal activity with minimal toxicity.

Generally, endocrine agents are less toxic than chemotherapeutic agents. They can be used in a long term therapy but the exact time of use in treatment setting is not obvious. On the other hand, the optimal sequence of endocrine therapy in metastatic breast cancer is also not clear and it needs further study.

Target therapy is going to become more valuable in the treatment of breast cancer but the key point is how to identify patients who would benefit from molecular targeted agents. The molecular mechanisms inducing the microenvironmental signals may be specific for individual breast cancers, and a therapy based on the individual microenvironment is needed. According to the molecular target, new agents can be developed. Molecular targeted agents can be safely used in combination setting of breast cancer treatment.

The recent discovery of breast cancer stem cells offers a new approach to understanding the biology of these conditions However, there is not enough evidence and clinical trials on this subject and further study is required in order to

find out about the development of normal and cancer stem cells and to verify whether cancer stem cells are present in other tumor types. From this point of view, new prognostic and predictive markers and new targeted therapeutic strategies may be developed.

Pharmacogenomics has a high potential to revolutionize cancer therapy. Lately in the UK, a breast cancer gene-free baby has been born. This is good news in the preventative setting of breast cancer in individuals who are in high risk condition. Identification of genetic profiles to subtype breast cancer and to identify the prognosis by microarray analysis has proven to be a promising step towards individualized cancer therapy.

The research and clinical trials are expanding and hopefully new strategies for breast cancer treatment will be established soon. Advanced strategies will guide patient selection and protocol design, which will contribute to patient survival and the quality of life.

References

1. Schulz, W.A., *Molecular Biology of Human Cancers: An Advanced Student's Textbook*. Vol. 1. 2005: Springer. 1-23 , 357-382.
2. Oldenburg, R.A., et al., *Genetic susceptibility for breast cancer: how many more genes to be found?* Crit Rev Oncol Hematol, 2007. **63**(2): p. 125-49.
3. Kurebayashi, J., *Current clinical trials of endocrine therapy for breast cancer.* Breast Cancer, 2007. **14**(2): p. 200-14.
4. Wajapeyee, N. and K. Somasundaram, *Pharmacogenomics in breast cancer: current trends and future directions.* Curr Opin Mol Ther, 2004. **6**(3): p. 296-301.
5. Shehadi, J.A., et al., *Surgical treatment strategies and outcome in patients with breast cancer metastatic to the spine: a review of 87 patients.* Eur Spine J, 2007. **16**(8): p. 1179-92.
6. (WHO), w.H.O., *breast cancer.* 2006.
7. Shetty, Y.C., et al., *Exemestane: a milestone against breast cancer.* J Postgrad Med, 2007. **53**(2): p. 135-8.
8. American Cancer Society, *What are the key statics about breast cancer in men?* 2007. (www.cancer.org/docroot/home/index.asp)
9. Yaghmaie, F., et al., *Caloric restriction reduces cell loss and maintains estrogen receptor-alpha immunoreactivity in the pre-optic hypothalamus of female B6D2F1 mice.* Neuro Endocrinol Lett, 2005. **26**(3): p. 197-203.
10. Yamaguchi, Y., *Microenvironmental regulation of estrogen signals in breast cancer.* Breast Cancer, 2007. **14**(2): p. 175-81.
11. Orimo, A., et al., *Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion.* Cell, 2005. **121**(3): p. 335-48.

12. Merck, Breast *cancer*. 2003. (www.merck.com/mmhe/sec22/ch251/ch251f.html#sec22-ch251-ch251f-525)
13. National Cancer Institute, *Paget's Disease of the Nipple: Questions and Answers.* 2005. (www.cancer.gov/cancertopics/factsheet/Site-Types/pagets-breast)
14. National Cancer Institute, *Metastatic Cancer: Questions and Answers.* 2004. (www.cancer.gov/cancertopics/factsheet/Site-Types/metastatic)
15. Giordano, S.H. and G.N. Hortobagyi, *Inflammatory breast cancer: clinical progress and the main problems that must be addressed.* Breast Cancer Res, 2003. **5**(6): p. 284-8.
16. Vaidya, J.S., et al., *Targeted intraoperative radiotherapy (TARGIT) yields very low recurrence rates when given as a boost.* Int J Radiat Oncol Biol Phys, 2006. **66**(5): p. 1335-8.
17. Dowlatshahi, K., J.J. Dieschbourg, and K.J. Bloom, *Laser therapy of breast cancer with 3-year follow-up.* Breast J, 2004. **10**(3): p. 240-3.
18. von Minckwitz, G., *Docetaxel/anthracycline combinations for breast cancer treatment.* Expert Opin Pharmacother, 2007. **8**(4): p. 485-95.
19. Desta, Z., et al., *Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6.* J Pharmacol Exp Ther, 2004. **310**(3): p. 1062-75.
20. Nakamura, T., et al., *Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts.* Cell, 2007. **130**(5): p. 811-23.
21. Howell, A., et al., *Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer.* Lancet, 2005. **365**(9453): p. 60-2.
22. Martino, S., et al., *Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene.* J Natl Cancer Inst, 2004. **96**(23): p. 1751-61.
23. Vogel, V.G., et al., *Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial.* Jama, 2006. **295**(23): p. 2727-41.
24. Veronesi, U., et al., *Italian randomized trial among women with hysterectomy: tamoxifen and hormone-dependent breast cancer in high-risk women.* J Natl Cancer Inst, 2003. **95**(2): p. 160-5.
25. Subbaramaiah, K., et al., *HER-2/neu status is a determinant of mammary aromatase activity in vivo: evidence for a cyclooxygenase-2-dependent mechanism.* Cancer Res, 2006. **66**(10): p. 5504-11.
26. *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.
27. Chu, I., et al., *The dual ErbB1/ErbB2 inhibitor, lapatinib (GW572016), cooperates with tamoxifen to inhibit both cell proliferation- and estrogen-dependent gene expression in antiestrogen-resistant breast cancer.* Cancer Res, 2005. **65**(1): p. 18-25.
28. Utsumi, T., N. Kobayashi, and H. Hanada, *Recent perspectives of endocrine therapy for breast cancer.* Breast Cancer, 2007. **14**(2): p. 194-9.
29. Winer, E.P., et al., *American Society of Clinical Oncology technology assessment on the use of aromatase inhibitors as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer: status report 2004.* J Clin Oncol, 2005. **23**(3): p. 619-29.
30. Goldhirsch, A., et al., *Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005.* Ann Oncol, 2005. **16**(10): p. 1569-83.
31. Macaskill, E.J. and J.M. Dixon, *Neoadjuvant use of endocrine therapy in breast cancer.* Breast J, 2007. **13**(3): p. 243-50.
32. Slamon, D.J., et al., *Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2.* N Engl J Med, 2001. **344**(11): p. 783-92.

33. Joensuu, H., et al., *Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer.* N Engl J Med, 2006. **354**(8): p. 809-20.
34. Piccart-Gebhart, M.J., et al., *Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1659-72.
35. Romond, E.H., et al., *Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer.* N Engl J Med, 2005. **353**(16): p. 1673-84.
36. Bando, H., *Vascular endothelial growth factor and bevacitumab in breast cancer.* Breast Cancer, 2007. **14**(2): p. 163-73.
37. Ferrara, N., H.P. Gerber, and J. LeCouter, *The biology of VEGF and its receptors.* Nat Med, 2003. **9**(6): p. 669-76.
38. Rak, J., et al., *Oncogenes and angiogenesis: signaling three-dimensional tumor growth.* J Investig Dermatol Symp Proc, 2000. **5**(1): p. 24-33.
39. Jain, R.K., et al., *Lessons from phase III clinical trials on anti-VEGF therapy for cancer.* Nat Clin Pract Oncol, 2006. **3**(1): p. 24-40.
40. Midgley, R. and D. Kerr, *Bevacizumab--current status and future directions.* Ann Oncol, 2005. **16**(7): p. 999-1004.
41. Miller, K.D., et al., *Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer.* J Clin Oncol, 2005. **23**(4): p. 792-9.
42. Hatake, K., N. Tokudome, and Y. Ito, *Next generation molecular targeted agents for breast cancer: focus on EGFR and VEGFR pathways.* Breast Cancer, 2007. **14**(2): p. 132-49.
43. Ferrara, N., et al., *Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer.* Nat Rev Drug Discov, 2004. **3**(5): p. 391-400.
44. Jain, R.K., *Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy.* Science, 2005. **307**(5706): p. 58-62.
45. Institute, N.C., *Bevacizumab Combined With Chemotherapy Improves Progression-Free Survival for Patients With Advanced Breast Cancer.* 2005.
46. Cabebe, E. and H. Wakelee, *Sunitinib: a newly approved small-molecule inhibitor of angiogenesis.* Drugs Today (Barc), 2006. **42**(6): p. 387-98.
47. Iwata, H., *Perspective of trastuzumab treatment.* Breast Cancer, 2007. **14**(2): p. 150-5.
48. Menendez, J.A., L. Vellon, and R. Lupu, *Targeting fatty acid synthase-driven lipid rafts: a novel strategy to overcome trastuzumab resistance in breast cancer cells.* Med Hypotheses, 2005. **64**(5): p. 997-1001.
49. Nakamura, H., et al., *Cooperative cell-growth inhibition by combination treatment with ZD1839 (Iressa) and trastuzumab (Herceptin) in non-small-cell lung cancer.* Cancer Lett, 2005. **230**(1): p. 33-46.
50. Zhuang, Y., et al., *Topotecan central nervous system penetration is altered by a tyrosine kinase inhibitor.* Cancer Res, 2006. **66**(23): p. 11305-13.
51. Nelson, M.H. and C.R. Dolder, *Lapatinib: a novel dual tyrosine kinase inhibitor with activity in solid tumors.* Ann Pharmacother, 2006. **40**(2): p. 261-9.
52. Moy, B. and P.E. Goss, *Lapatinib: current status and future directions in breast cancer.* Oncologist, 2006. **11**(10): p. 1047-57.
53. Konecny, G.E., et al., *Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells.* Cancer Res, 2006. **66**(3): p. 1630-9.
54. Stanway, S.J., et al., *Steroid sulfatase: a new target for the endocrine therapy of breast cancer.* Oncologist, 2007. **12**(4): p. 370-4.
55. Reed, M.J., et al., *Steroid sulfatase: molecular biology, regulation, and inhibition.* Endocr Rev, 2005. **26**(2): p. 171-202.

56. Ho, Y.T., et al., *Inhibition of carbonic anhydrase II by steroidal and non-steroidal sulphamates.* Biochem Biophys Res Commun, 2003. **305**(4): p. 909-14.
57. Kuo, M.T., *Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities.* Antioxid Redox Signal, 2009. **11**(1): p. 99-133.
58. Noguchi, K., et al., *Functions of the breast cancer resistance protein (BCRP/ABCG2) in chemotherapy.* Adv Drug Deliv Rev, 2009. **61**(1): p. 26-33.
59. Riggins, R.B., et al., *Pathways to tamoxifen resistance.* Cancer Lett, 2007. **256**(1): p. 1-24.
60. Spillane, J.B. and M.A. Henderson, *Cancer stem cells: a review.* ANZ J Surg, 2007. **77**(6): p. 464-8.
61. Pardal, R., M.F. Clarke, and S.J. Morrison, *Applying the principles of stem-cell biology to cancer.* Nat Rev Cancer, 2003. **3**(12): p. 895-902.
62. Dontu, G., et al., *Stem cells in normal breast development and breast cancer.* Cell Prolif, 2003. **36 Suppl 1**: p. 59-72.
63. Al-Hajj, M., et al., *Prospective identification of tumorigenic breast cancer cells.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 3983-8.
64. Shackleton, M., et al., *Generation of a functional mammary gland from a single stem cell.* Nature, 2006. **439**(7072): p. 84-8.
65. Dontu, G., D. El-Ashry, and M.S. Wicha, *Breast cancer, stem/progenitor cells and the estrogen receptor.* Trends Endocrinol Metab, 2004. **15**(5): p. 193-7.