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FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DIPLOMA THESIS

2010 Pavlina Menelaou

CHARLES UNIVERSITY IN PRAGUE
FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

Department of Pharmacology and Toxicology

Pharmacotherapy of breast cancer:
Novel approaches, overcoming of multidrug resistance.

Diploma thesis

Research Advisor: PharmDr. Martina Čečková, Ph.D.

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DECLARATION

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

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ABBREVIATIONS

ABCG2	BCRP , ATP-binding cassette transporter subfamily G member 2
ADCC	antibody dependent cellular toxicity
AIs	Aromatase Inhibitors
ALT	Alanine Transaminase
Anti-Mtor	intracell signaling pathway
Anti-ECFR	Epidermal Growth Factor Receptor Inhibitor
AST	Aspartate AminoTrasferase
BC	Breast Cancer
BCRP	ABCG2 , Breast Cancer resistance protein
BMD	Bone Mineral Density
BMI	Body Mass Index
BRC-230	SkBr3 Breast Cancer cells lines
BSE	Breast Self-examination
CBE	Clinical Breast Examination
CD	Cowden Disease
CDT	Complex Decongestive Therapy
CP	Pneumatic Compression
DC	dendritic cells
DCIS	Ductal Carcinoma In Situ
DLT	Dose Limiting Toxicity
EGFR	Epidermal Growth Factor Receptor
ERbB	protein family or epidermal growth factor receptor EGFR family
ER(+/-)BC	Estrogen Receptor positive/negative Breast Cancer
FDA	Food and Drug Administration
HER2	Human Epidermal Growth Factor Receptor 2 protein
HRT	Hormonal Replacement Therapy
HSP90	Heat Shock Protein 90
HT	Hormonal Therapy
HVES	High Voltage Electrical Stimulation
Ig-G1	immunoglobulin G
IL	interleukin
INFs	interferons
LCIS	Lobular Carcinoma In Situ
LFS	Li-Fraumeni syndrome
LFLS	Li-Fraumeni like syndrome
LT	Laser Therapy
mAbs	monoclonal antibodies
MCF-7	(breast cancer cell line) Michigan Cancer Foundation
MDR	Multidrug resistance
MRI	Magnetic Resonance Imaging
MRP	Multidrug resistance associated protein
MUC-1	multiple clinical trials

MXR	Mitoxantrone resistance protein
NSABP	study National Surgical Adjuvant Breast And Bowel Project
OS	Overall Survival
PARP	poly (ADP-ribose) polymerase
P-gp	P-glycoprotein
PLD	pegylated liposomal doxorubicin
P53	protein 53
QOL	Quality Of Life
RT-PCR	Reverse transcription polymerase chain reaction genetic diagnosis
RUTH	Raloxifene Use for the Heart Trial
SCLC	small lung carcinoma
SERMs	Selective Estrogen Receptor Modulators
STAR	Study of Tamoxifen And Raloxifene Trial
SNRs	single nucleotide polymorphism
TAM	Tamoxifen
TKIs	tyrosine kinase inhibitors
TOP2a	Topoisomerase 2a
TP53	tumor protein 53
VEGF	Vascular endothelial growth factor
WHI	Woman's Health Initiative

INTRODUCTION TO BREAST CANCER

Humans have had cancer throughout recorded history. The oldest description of cancer (although the word cancer was not used) was discovered in Egypt and dates back to about 1600 B.C. It is called the Edwin Smith Papyrus, and is a copy of part of an ancient Egyptian textbook on trauma surgery. The origin of the word cancer is credited to the Greek physician Hippocrates (460–370 B.C.), considered the "Father of Medicine." Hippocrates used the terms *carcinos* and *carcinoma* to describe non-ulcer forming and ulcer-forming tumors.

During the Renaissance, famous Scottish surgeon John Hunter (1728–1793) said that some cancers cannot be cured by surgery and suggested also how the surgeon might decide which cancers to operate on. If the tumor had not invaded nearby tissue and was "moveable", he said, "There is no impropriety in removing it''. In 19^{th} century there was the use of scientific oncology with modern microscope in studying diseased tissues [1].

Cancer begins when cells in a part of the body start to grow out of control. There are many kinds of cancer, but they all start because of out-of-control growth of abnormal cells [1].

Despite years of intensive study and substantial progress in understanding susceptibility to breast, this disease remains important cause of death in women. However, many recent critical advances — sequencing of the human genome and the development of high-throughput techniques for identifying DNA-sequence variants, changes in copy numbers, and global expression profiles — have given an acceleration at preventing and curing this disease [2].

Disease occurs almost only in women, but men can get it, too. Breast Cancer (BC) is a malignant tumor that starts from cells of the breast. A malignant tumor is a group of cancer cells that may grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body [1]. Most often the tumor involves glandular breast cells in the ducts or lobules [3]. Cancer starts when a cell in a part of a body starts to grow up out of control. Cancer cell growth is different from other normal cell growth because cancer cells do not die but they form new ones, abnormal ones. Another difference from normal cells is that the cancer cells can grow in other tissues and that is what makes cancer. Cell becomes cancer cell because of the damage of DNA without repairmen like in normal cells [1]. Most patients present with an asymptomatic lump discovered during examination or screening mammography. Diagnosis is confirmed by biopsy. Primary therapy usually includes surgical excision, often in combination with radiation therapy. Physicians can also give adjuvant therapy to kill any cancer cells that may have spread, even if they cannot be detected by imaging or laboratory tests. Studies have shown that adjuvant therapy for BC may increase the chance of long-term survival by preventing a recurrence [3].

Breast Cancer today is considered a leading cause of morbidity around the world, with the statistics showing increasing incidence rates to the levels of an epidemic [4,5]. The treatment given for BC is highly variable and dependent on a

number of factors including the type, location and amount of disease and the health status of the patient. The treatments are designed to either directly kill/remove the cancer cells or to lead to their eventual death by depriving them of signals needed for cell division. Other treatments work by stimulating the body's own defenses. However, despite the available treatment choices available, up to this moment the fact remains that there is not a “cure” for BC or cancer in general. Therefore, medical scientists around the world are striving to come up with novel treatments that could enhance the “battle” against cancer.

ABSTRACT

Breast cancer arises when there is an uncontrolled growth of abnormal cells in the breast. Most often the tumor involves glandular breast cells in the ducts or lobules. The most common sign of breast cancer is a new lump or mass. Other possible signs can be redness, swelling, nipple discharge and more. Some risk factors of breast cancer can be alcohol, obesity, oral hormonal contraceptives, radiation, gender, age, hereditary/genetic aspects, estrogen and progesterone receptors.

Breast cancer can be treated by surgery including mastectomy, lymphedema surgery and lumpectomy, by radiation therapy or chemotherapy. The novel methods include hormonal therapy using selective estrogen receptor modulators like tamoxifen, raloxifene, aromatase inhibitors like anastrozole and letrozole. One of the promising therapies is immune therapy with the use of interferons and other cytokines, dendritic cells and vaccines. Another novel approach is represented by the targeted therapy that is using monoclonal antibodies, tyrosine kinase inhibitors and more promising ways. The gene therapy that is made to correct specific molecular defects that contributes to the cause or progressions of breast cancer belong also into one of the perspective methods for the treatment of breast cancer. Last but not least is the stem cells therapy that is working with the replacement of the diseased cells.

Chemotherapy of breast cancer can be complicated by multidrug resistance (MDR), which is caused by various mechanisms including increased drug efflux from the cell by ATP dependent transporters, decreased drug uptake into the cell, activation of detoxifying enzymes and defective apoptotic pathways. The methods to overcome the resistance include control of expression of multi drug resistance proteins and suppression of the activity of multi drug resistance membrane transporter proteins.

AIMS OF THE STUDY

The aim of this work is to overview current knowledge on breast cancer in relation to the diagnosis, screening and methods used to treat the disease, with emphasize put on the novel approaches. The main causes of multidrug resistance of the cancer and the approaches to overcome this phenomenon are also summarized.

METHODOLOGY

All the information in this diploma thesis was obtained from original articles, scientific reviews and guidelines retrieved from various search databases and webpages. These included mainly PubMed, Merck manual online, cancernetwork.com, UBMMedica, BreastCancer.org and pages of American Cancer Society (cancer.org). The key words that have been used in order to retrieve the relevant literature included: breast cancer, novel treatment, chemotherapy, stem cells, gene therapy and appropriate Mesh terms.

I. BACKGROUND

1. Definition and classification of Breast Cancer

Breast Cancer is the most common type of cancer among women worldwide and its rate is increasing in both developed and developing countries [4]. The load is not evenly distributed and there are large variations in the incidence rates of BC between different countries. BC is an uncontrolled growth of breast cells.

Cancer occurs as a result of mutations, or abnormal changes, in the genes responsible for regulating the growth of cells and keeping them healthy. Normally, the cells in our bodies replace themselves through an orderly process of cell growth: healthy new cells take over as old ones die out. But over time, mutations can "turn on" certain genes and "turn off" others in a cell. That changed cell gains the ability to keep dividing without control or order, producing more cells just like it and forming a tumor. A tumor can be benign (not dangerous to health) or malignant (has the potential to be dangerous). Benign tumors are not considered cancerous: their cells are close to normal in appearance, they grow slowly, and they do not invade nearby tissues or spread to other parts of the body. Malignant tumors are cancerous. The term "breast cancer" refers to a malignant tumor that has developed from cells in the breast. Usually BC either begins in the cells of the lobules, which are the milk-producing glands, or the ducts, the passages that drain milk from the lobules to the nipple. Over time, cancer cells can invade nearby healthy breast tissue and make their way into the underarm lymph nodes, small organs that filter out foreign substances in the body (Table 1). If cancer cells get into the lymph nodes, they then have a pathway into other parts of the body. BC is always caused by a genetic abnormality (a "*mistake*" in the genetic material). However, only 5-10% of cancers are due to an abnormality inherited from your mother or father. About 90% of breast cancers are due to genetic abnormalities that happen as a result of the aging process and the "wear and tear" of life in general [5]. Most breast lumps which are due to fibrocystic changes and other breast complaints are due to benign conditions. Breast pain can be produced also by mastitis, pendulous breasts, or hidradenitis suppurativa. Moreover they constitute the greatest percentage of symptoms leading to a diagnosis of cancer. Nipple discharge is usually benign, especially if non bloody, bilateral, and not spontaneous and can be due to medications, hormonal factors, prior nursing or pregnancy, or, less commonly, cancer [6]. Many genetic, epidemiologic, and laboratory studies support that a series of genetic changes contribute to the dynamic process which can cause BC is known as carcinogenesis [7]. Accumulation of these genetic changes corresponds to the phenotypic changes associated with the evolution of malignancy at the end. The carcinogenesis sequence is starting with tissue of normal appearance followed by changes leading to hyperplasia and dysplasia, the most severe forms of which are difficult to distinguish from carcinoma *in situ* [8]. The concept that BC may be preventable is supported by the wide international variation in BC rates, which is an indicator that there are potentially modifiable environmental and lifestyle determinants of breast cancer. Migration studies reinforce this premise;

for example, it has been observed that Japanese immigrants to the United States increase their BC risk from Japanese to American levels within two generations [9, 10, 11].

Table 1 . Stages of breast cancer. Adopted from BreastCancer.org [1]

STAGE	DEFINITION
0	Cancer cells remain inside the breast duct, without invasion into normal adjacent breast tissue.
I	Cancer is 2 centimeters or less and is confined to the breast (lymph nodes are clear).
IIA	No tumor can be found in the breast, but cancer cells are found in the auxiliary lymph nodes (the lymph nodes under the arm) OR the tumor measures 2 centimeters or smaller and has spread to the auxiliary lymph nodes OR the tumor is larger than 2 but no larger than 5 centimeters and has not spread to the auxiliary lymph nodes.
IIB	The tumor is larger than 2 but no larger than 5 centimeters and has spread to the auxiliary lymph nodes OR the tumor is larger than 5 centimeters but has not spread to the auxiliary lymph nodes.
IIIA	No tumor is found in the breast. Cancer is found in auxiliary lymph nodes that are sticking together or to other structures, or cancer may be found in lymph nodes near the breastbone OR the tumor is any size. Cancer has spread to the auxiliary lymph nodes, which are sticking together or to other structures, or cancer may be found in lymph nodes near the breastbone.
IIIB	The tumor may be any size and has spread to the chest wall and/or skin of the breast AND may have spread to auxiliary lymph nodes that are clumped together or sticking to other structures, or cancer may have spread to lymph nodes near the breastbone.
IIIC	There may either be no sign of cancer in the breast or a tumor may be any size and may have spread to the chest wall and/or the skin of the breast AND the cancer has spread to lymph nodes either above or below the collarbone AND the cancer may have spread to auxiliary lymph nodes or to lymph nodes near the breastbone.
IV	The cancer has spread — or metastasized — to other parts of the body.

2. Statistics of Breast Cancer

Around 429,900 new cases of BC occur each year in Europe [12]. The lowest European rates are in eastern and southern Europe and the highest are in Northern and Western Europe (**Figure 1**).

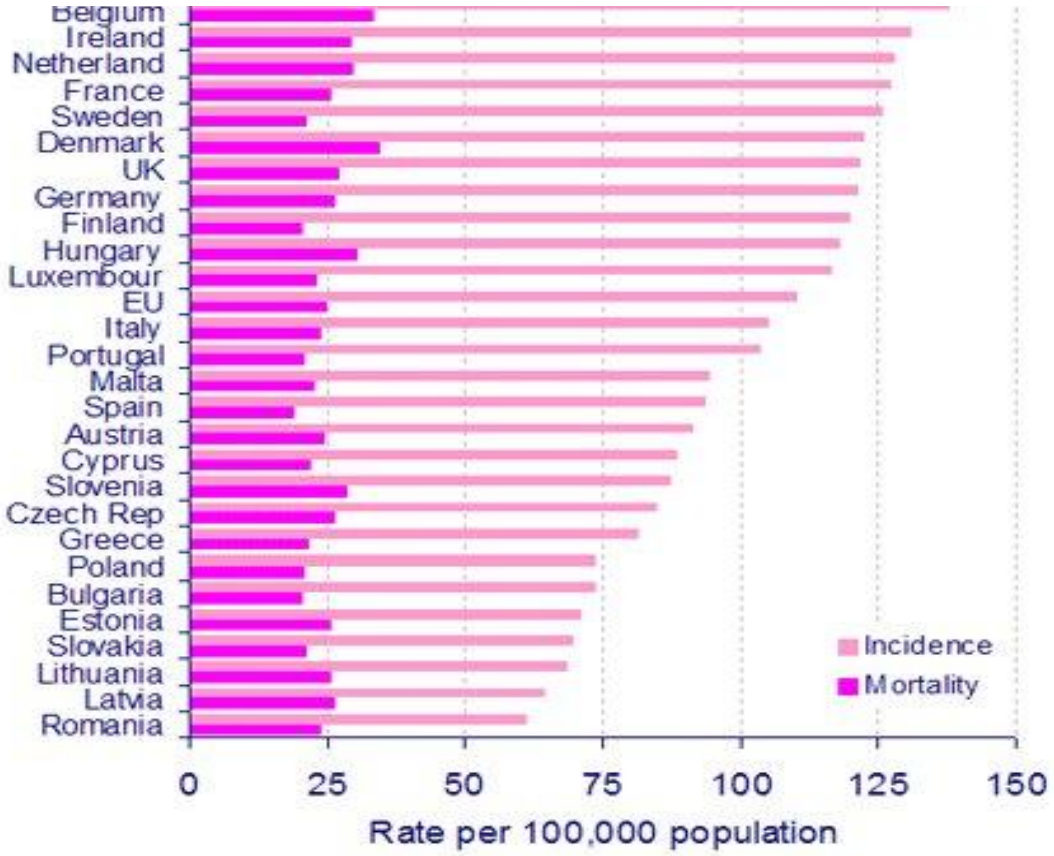

Figure1. Estimates of the cancer incidence and mortality in Europe in 2006. Adapted from Parkin et al [4]

3. Signs of Breast Cancer

The most common sign of BC is a new lump or mass. A mass that is painless, hard, and has irregular edges is more likely to be cancerous, but breast cancers can be tender, soft, or rounded. For this reason, it is important that any new mass, lump, or breast change is checked by a health care professional with experience in diagnosing breast diseases.

Other possible signs of BC include:

- swelling of all or part of a breast (even if no distinct lump is felt)
- skin irritation or dimpling
- breast or nipple pain
- nipple retraction (turning inward)
- redness, scaliness, or thickening of the nipple or breast skin
- a nipple discharge other than breast milk [1].

4. Diagnosis and screening

The American Cancer Society released the following recommendations for the diagnosis of Breast Cancer:

- Women with age 40 and older should have a mammogram every year continuously until they are in good health [1].
- Women in their 20s and 30s should have a clinical breast exam (CBE) as part of a periodic (regular) health exam by a health professional preferably every 3 years. Starting at age 40, women should have a CBE by a health professional every year [1] .
- Breast self-examination (BSE) should be started for women in their 20s. Any report for breast changes should be told to their health professional right away [1,2] .
- Women at high risk (greater than 20% lifetime risk) should get an MRI (Magnetic Resonance Imaging) and a mammogram every year. Women at moderately increased risk (15% to 20% lifetime risk) should talk with their doctors about the benefits and limitations of adding MRI screening to their yearly mammogram. Yearly MRI screening is not recommended for women whose lifetime risk of Breast Cancer is less than 15% [1] .

5. Risk Factors

A “risk factor” is anything that has the potential to increase the risk of developing cancer. Many of the most important risk factors for BC are beyond one’s control, such as age, family history, gender, estrogen exposure (early menstruation, late menopause), estrogen receptors (ER) and progesterone receptors (PR) and radiation therapy to chest [6]. However, there are some risk factors that can be controlled, such as weight, physical activity, oral contraceptives and alcohol consumption and others that cannot be controlled such as gender and radiation [5].

5.1 Obesity

Obesity is an increased BC risk, especially for postmenopausal women that did not use Hormonal Therapy (HT). The Woman’s Health Initiative (WHI) observational study observed 85,917 women aged 50 to 79 years and collected information on their weight history [13]. Height, weight, and waist and hip circumferences were measured. With a median follow-up of 34.8 months, 1,030 of the women developed invasive breast cancer. Among the women who never used HT, increased BC risk was associated with weight at entry, body mass index (BMI) at entry, BMI at age 50 years, maximum BMI, adult and postmenopausal weight change, and waist and hip circumferences. Weight was the strongest predictor, with a Relative Risk (RR) of 2.85 (95% CI, 1.81–4.49) for women weighing more than 82.2kg, compared with those weighing less than 58.7 kg [13].

5.2. Alcohol

Many epidemiologic studies have shown that alcohol consumption is also an increased risk of BC. Individual data from 53 case-control and cohort studies were included in a British meta-analysis [14]. Compared with women who reported no alcohol consumption, the RR of BC was 1.32 (95% CI, 1.19–1.45; $P < .001$) for women consuming 35 g to 44 g of alcohol per day and 1.46 (95% CI, 1.33–1.61; $P < .001$) for those consuming at least 45 g of alcohol per day. The RR of BC increases by about 7% (95% CI, 5.5%–8.7%; $P < .001$) for each 10 g of alcohol (i.e., one drink) consumed per day. The same result was obtained, even after additional stratification for race, education, family history, age at menarche, height, weight, BMI, breast-feeding, oral contraceptive use, menopausal hormone use and type, and age at menopause. Alcohol can limit liver’s ability to control blood levels of the hormone estrogen, which in turn can increase risk [5, 6].

5.3. Exercise

Active exercise may reduce BC risk, particularly in young women [15, 17]. Many observational studies have examined the relationship between physical activity and BC risk [16]. Most of these studies have shown that increased physical activity is contrast to BC incidence. The average RR reduction is reportedly 30% to 40%. However, it is not known to what degree, if at all, the observed association is to the result of confounding variables, such as diet or a genetic predisposition to breast cancer. A prospective study of more than 25,000 women in Norway suggests that doing heavy manual labor or exercising 4 or more hours per week is associated with a decrease in BC risk. This result is more for premenopausal women and in women of normal or lower-than-normal body weight [15]. In a case-control study of African American women, strenuous recreational physical activity (>7 hours per week) was associated with decreased BC incidence [15]. The American Cancer Society recommends engaging in 45-60 minutes of physical exercise 5 or more days a week [17].

5.4. Oral Contraceptives-Hormonal risk factor

Using oral contraceptives (OC), also known as birth control pills (estrogen and progesterone), appears to slightly increase a woman's risk for breast cancer, but only for a limited period of time. Results from 10 studies and one pooled analysis of 54 studies suggest that the use of OC does not significantly modify the risk of BC among women with a familial history of BC [18]; however, evidence from four studies shows that some women may be at a greater risk, particularly women who took OCs prior to 1975. Current evidence shows that women with a family history of BC do not increase their disease risk by using OCs [91]. During the period of June 1991-February 1994 in Italy, other researchers conducted a case control study to examine the association between oral contraceptive use and BC risk [20]. Cases comprised 1991 women aged less than 65 with confirmed BC. Controls included 1899 same-age patients at the same network of hospitals as the cases with an acute, non-neoplastic, non-hormone-related condition. 18% of cases and 14% of controls had ever used OCs. Overall, there was no association between OC use and BC. Very short term OC use (1 year) had an odds ratio (OR) of 1.3 (borderline significance only). However, women who had recently stopped using OCs faced an elevated risk of BC (OR = 1.6 for 1-4 years since last OC and 1.7 for 5-9 years). Among women who had last used OCs 10 years ago, the risk of BC was directly related to duration of use (OR = 1.3 for 5 years and 1.7 for =or 5 years; p = 0.02 [for the trend]). The OR for women who had last used an OC longer than 10 years ago reached unity. Among these women, those that used OCs for at least five years had a lower risk of developing BC (OR = 0.66). The increased OR for women who had recently ceased using OCs and the apparent protection against BC among women who ceased using OCs for at least 10 years match the pattern of BC risk noted after a full-term pregnancy. These findings should reassure the public that OCs apparently does not induce breast carcinogenesis [20].

5.5. Radiation

Having radiation therapy to the chest area as a child or young adult as treatment for another cancer significantly increases BC risk. The increase in risk seems to be highest if the radiation was given while the breasts were still developing (during the teen years) [5]. Radiation as a carcinogen can interact with DNA to produce a range of mutations. Radiation causes double-strand breaks [21]. Exposure to ionizing radiation has clearly been established as one of the risk factors for the development of breast cancer. Much data on the relationship between radiation exposures and subsequent BC are derived from atomic bomb survivors and women who received medical exposures either for diagnostic or therapeutic purposes. Although therapeutic radiation is rarely used to treat benign conditions, it remains an important and effective treatment modality for a wide range of cancers [22].

5.6. Gender

Being a woman is the most significant risk factor for developing BC. Although men can get breast cancer, too, women's breast cells are constantly changing and growing, mainly due to the activity of the female hormones estrogen and progesterone. This activity puts them at much greater risk for Breast Cancer [1, 2].

5.7. Age

Simply growing older is the second biggest risk factor for breast cancer. From the age of 30 to 39, the risk is 1 in 233, or .43%. The risk increases to 1 in 27, or almost 4%, by the time that the woman will be in her 60s [5].

5.8. Hereditary/genetic aspects

People who have a first degree relative (mother, father, sister) that had BC are in high risk of getting BC as well. Twenty percent of BC is familial (family history of breast cancer). Approximately 5% to 10% of BC is hereditary; a gene mutation has been inherited, which puts the patient at an increased risk of cancer (Figure 2). Two-thirds of these hereditary cancers occur in individuals with BRCA1 or BRCA2 mutations, which are germline mutations. The remaining 10% to 15% is due to some other factor involving the family, such as an environmental factor, chance, or an undiscovered gene mutation [23].

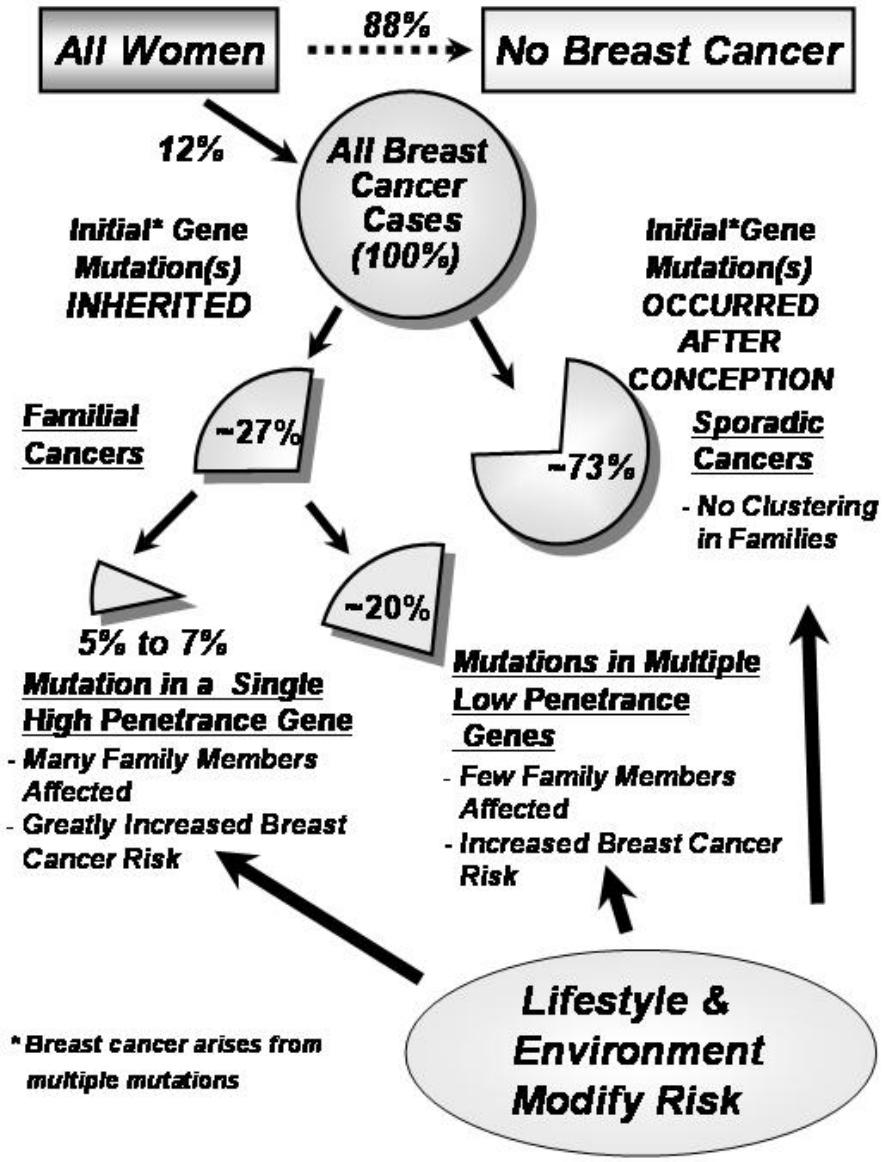

Figure2. The percentages of all Breast Cancer Cases. Adapted from Kelloff et al [9]

5.9. Estrogen and Progesterone receptors

Estrogen and progesterone receptors are reported as positive or negative. Laboratories establish a cutoff point related to the percentage of cells which stain with the antibody [5]. If the number of cells which stain is greater than that predetermined cutoff point, the result is called positive. If the number is below that number, the result is called negative. If hormone receptor status is positive, that predicts two things, first, a better overall outcome, and second, a good response to hormonal therapy. The meaning of the test in terms of choosing treatment options is much more important. The higher the percentage of positive estrogen receptors, the greater the chance of responding well to hormonal therapy. The lower the hormone receptor positivity, the better response will have to chemotherapy. Pathology report may say that the BC cells tested negative for estrogen receptors (ER-), progesterone receptors (PR-), and HER2, also called human epidermal growth factor receptor 2 (HER2-). Testing negative for all three receptors means "triple-negative breast cancer." Without these receptors, the cancer's growth is not likely to be fueled by estrogen or progesterone, or by growth signals coming from the HER2 protein. Therefore, triple-negative BC does not respond to hormonal therapy (such as tamoxifen or aromatase inhibitors) or therapies that target HER2 receptors, such as Herceptin (trastuzumab). About 10-20% of breast cancers are found to be triple-negative [5].

II. EXISTING AND NEW APPROACHES TO TREAT BREAST CANCER

1. Introduction to therapy

Cancer treatment has traditionally been dominated by intravenous drug therapy [17] There has, however, been a constant increase in the production of different oral anticancer drugs offering obvious benefits in terms of convenience and ease of administration, as well as addressing patients' preference for oral therapy [17 , 18 , 24, 25]. It is proved that one-quarter of all anticancer agents under development are oral agents [26] Several agents that are already approved in a range of tumor types (e.g. capecitabine, erlotinib, gefitinib, imatinib, lapatinib, lenalidomide, thalidomide, sunitinib, sorafenib) and many others in development (e.g. vatalanib, satraplatin) are or will be available only as oral formulations. Novel approaches to drug delivery, such as the development of hydrophilic polymer carriers to deliver drugs to the gut [27], are likely to further increase the number of oral drugs available promising even better results in the deal of Breast Cancer. Remarkable and additional to this is to say that therapy is also based on the fact that cells have receptors on their surface and in their cytoplasm and nucleus. Chemical messengers such as hormones bind to receptors, and this causes changes in the cell. BC cells may or may not have three important receptors: estrogen receptor, progesterone receptor (PR), and HER2/neu. Cells with these receptors are called ER positive (ER+), ER negative (ER-), PR positive (PR+), PR negative (PR-), HER2 positive (HER2+), and HER2 negative (HER2-). Cells with none of these receptors are called basal-like or triple negative. ER+ cancer cells depend on estrogen for their growth, so they can be treated with drugs to reduce estrogen (e.g. tamoxifen), and generally have a better prognosis. Generally, HER2+ had a worse prognosis, however HER2+ cancer cells respond to drugs such as the monoclonal antibody, trastuzumab, (in combination with conventional chemotherapy) and this has improved the prognosis significantly. That is why scientists have done the estrogen receptor assay that is a laboratory test done on a sample of the cancer in order to see whether estrogen receptors are present. The growth of normal breast cells and some breast cancers is stimulated by estrogen. BC cells without these receptors (called estrogen-receptor negative or ER negative) are unlikely to respond to hormonal therapy. Estrogen-receptor positive cancers are more likely to respond to hormonal therapy. Since estrogen nourishes some types of breast cancer, scientists question whether estrogen replacement therapy increases the Breast Cancer risk.

2. Surgery

2.1 Mastectomy

For the first 80 years of the last century, mastectomy was considered to be the choice of most of the women with newly diagnosed, early stage of BC for their treatment. For more than 3 decades breast-conserving surgery in the treatment of BC has been proved by randomized controlled trials and meta-analysis trials to be safe. These have all shown equivalent long-term survival in women treated with breast-conserving surgery or mastectomy [28, 29 , 30 , 31 , 32].

Mastectomy can be used when breast conservation therapy is not possible (due to a large or multicentric tumor) or would result in poor cosmetic outcome, or when the patient specifically chooses a mastectomy. Simple Mastectomy involves removal of breast only without removal of lymph nodes. Skin sparing mastectomy performed when patient undergoes immediate breast reconstruction. The goal is to remove all breast tissue, along with the nipple-areola complex. The procedures Nipple-areola–sparing mastectomy attempt to preserve either the whole complex, termed nipple areola–sparing mastectomy or just the areola, with removal of the nipple [32, 33].

2.2. Lymphedema surgery

Lymphedema is defined as the excessive and persistent accumulation of fluid and extravascular and extracellular proteins in tissue spaces, due to the inefficiency of the lymphatic system [34]. Surgery and Radiotherapy of BC can be accompanied by auxiliary lymph node drainage, which may cause upper limb lymphedema. [34, 35, 36, 37] . It is extremely important to reduce and control lympedema due to the severity of complications resulting from post-surgical lymphedema. It can be significantly reduced during the first week of treatment and less significantly after the third week [38] reducing in this way the incidence of probable infections. Physiotherapy resources for lymphedema treatment are for example complex decongestive therapy (CDT), pneumatic compression (CP), high voltage electrical stimulation (HVES) and laser therapy (LT).

2.3. Lympectomy

It is a common surgery that involves the removing of the lump of the Breast Cancer and the surrounding tissue without removing the entire breast. It is a less radical procedure than mastectomy and is usually followed by radiotherapy (RT). Comparing to mastectomy, as the tissue is removed the procedure is generally quite limited and non-invasive. A lumpectomy is considered a viable means of "breast conservation" or "breast preservation" surgery with all the attendant physical and emotional advantages of such an approach. Lumpectomy may be performed for ductal carcinoma in situ (DCIS), for invasive ductal carcinoma, or for other conditions.

3. Radiaton Therapy

Radiation therapy is an adjuvant treatment for most women and is following lumpectomy and mastectomy surgery. Management of Breast Cancer requires a multimodality approach and an integration of the services of surgery, radiotherapy (RT), and chemotherapy. Recent reports have shown not only the established two-thirds reduction in local recurrence when RT is used after mastectomy or breast conserving surgery (BCS), but also an overall survival benefit of around 5-10% [28, 39, 40] raising in that way the use of Radiation therapy after the undergo of mastectomy and/or lumpectomy surgery.

Radiotherapy can be delivered in many ways but the most common way is by a linear accelerator. Currently it is set that for stage T1, T2 breast cancer, patients with at least four positive auxiliary nodes should receive postoperative radiotherapy of the chest wall and lymphatic drainage area, and patients with negative auxiliary nodes need no radiotherapy after mastectomy [41, 42]. However, whether patents with one to three positive auxiliary nodes need postoperative radiotherapy after mastectomy has been controversial [43]. Radiation as in other therapeutic approaches to treat cancer has various side effects which may include muscle stiffness, mild swelling and tenderness in the area.

4. Chemotherapy

Chemotherapy (drug treatment for cancer cells throughout the body) may be used before surgery, after surgery, or instead of surgery for those cases in which surgery is considered unsuitable. Multiple chemotherapeutic agents may be used in combination to treat patients with breast cancer [44]. Determining the appropriate regimen to use depends on many factors; such as, the character of the tumor, lymph node status, and the age and health of the patient. In general, chemotherapy has increasing side effects as the patient's age passes the age of 65 [45, 46].

There are three major types of chemotherapy.

- Neoadjuvant chemotherapy

Given before surgery to minimize the size of a tumor

- Adjuvant chemotherapy

Given after surgery to decrease the risk of recurrence of cancer cells. Some of the drugs used for adjuvant therapy include the single use or the combinations of the following: doxorubicin, docetaxel, paclitaxel, cyclophosphamide, fluorouracil, methotrexate, epirubicin.

- Palliative chemotherapy

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

Taxanes

Taxanes are between the most active chemotherapeutic agents used to treat metastatic breast cancer. They have a novel mechanism of action and non-cross resistance (see chapter on Multi Drug Resistance – MDR) with anthracyclines provides a sound rationale for testing their activity in the adjuvant setting. The addition taxanes appears to improve disease-free and overall survival (OS) in women with early-stage breast cancer, independently of receptor expression and lymph node involvement [41]. Today, there are too many different choices for the therapy of early stage BC than for any other type of cancer in humans. Despite this fact, that there is a broad armamentarium of therapy, it is still stays considerable uncertainty regarding the optimal therapeutic strategy for each individual patient. Although the precise role of taxanes is somewhat uncertain, based upon the data from first-generation taxane trials it is reasonable to consider taxane therapy in women that have an elevated risk of relapse where endocrine sensitivity is absent or incomplete. In the future, it may be possible to limit anthracyclines to particular subgroups of patients with specific molecular alterations, such as co-amplification of HER-2 and TOP2A. Amplification of the HER-2/NEU oncogene (17q12-q21), which encodes a transmembranous tyrosine kinase, has been described in numerous solid tumors, including breast, colon, endometrium, lung, ovary, stomach, thyroid and bladder cancers, and has been associated with short survival in patients suffering from breast, lung and ovary tumors [47-49]. The TOP2A is an essential nuclear enzyme involved in DNA replication by regulating transient breakage and rejoining of double-stranded DNA. Subsequent studies revealed that TOP2A is located adjacent to the HER-2 gene and suggested that coamplification or deletion of TOP2A may be responsible for the altered chemosensitivity of some HER-2-amplified tumors [49-52]. However, further prospective data are required before anthracyclines can be routinely omitted in patients who do not harbor such biomarkers. For patients with HER-2-positive disease, adjuvant trastuzumab therapy has a clearly established benefit, in spite of the recent negative findings from the PACS 04 trial [53, 54].

Doxorubicin

In the efforts to treat Breast cancer the conventional anthracyclines appear to be the most widely used agents both as an adjuvant treatment, as well as in metastatic disease. In the literature there is evidence studies that doxorubicin presents many advantages such as its response rate, time to disease progression, and overall survival of the patients' receiving this kind of treatment.

Despite the fact that doxorubicin has excellent antitumor activity, it has also a relatively low therapeutic index, and its use is limited by myelosuppression, alopecia, acute nausea and vomiting, stomatitis, and above all cumulative cardiotoxicity [48].

Early studies have shown promise with liposomal doxorubicin and pegylated liposomal doxorubicin (PLD) as neo-adjuvant therapy in patients with locally advanced Breast Cancer [50] or in combination with trastuzumab [52]. In fact, combination of trastuzumab with liposomal anthracyclines currently under investigation have shown better cardiac safety than that observed when trastuzumab was combined with conventional doxorubicin [54, 55].

5. Hormonal Therapy

Tamoxifen - selective estrogen receptor modulators (SERMs)

Some new drugs called selective estrogen receptor modulators (SERMs) are being studied. They seem to have many of the helpful effects of estrogen replacement without increasing Breast Cancer risk; in fact, recent studies suggest that some SERMs may actually reduce BC risk [1] Also they have done the progesterone receptor assay which is not as important clinically as ER. It is a laboratory test done on a sample of the Breast Cancer that shows whether the cancer depends on progesterone for growth [1]. It can provide useful information and should also be performed on all samples of invasive BC or DCIS [56]. Progesterone and estrogen receptor tests provide more complete information to help decide the best cancer treatment for the patient [1].

Tamoxifen is one of the important discoveries in the use of synthetic selective estrogen modulators (SERMs) in the treatment strategy for estrogen receptor (ER)-positive breast cancer. Hundreds of thousands of lives have been saved because of this advanced discovery. Tamoxifen it is used as we said in the treatment of Breast Cancer but also it is used for prevention in high-risk premenopausal women [57] Tamoxifen, is the first targeted agent for the treatment of Breast Cancer [57] that according to its results it is saving thousands of lives. Its use has significantly contributed to a reduction in BC mortality, because Breast Cancer patients that have been treated with TAM for 5 years exhibit a 30-50% reduction in both the rate of disease recurrence after 10 years of patient follow-up and occurrence of contralateral breast cancer. However, in patients treated with TAM there is substantial interindividual variability in the development of resistance to TAM therapy, and in the incidence of TAM-induced adverse events, including deep vein thrombosis, hot flashes, and the development of endometrial cancer. [58] The laboratory strategy of tamoxifen is targeting OER positive tumors [59] with long-term adjuvant therapy [48, 49] ultimately resulted in the improved survivorship of hundreds of thousands of women around the world. Indeed, the fact that tamoxifen is cheap and accessible to under-funded healthcare systems worldwide means that this form of targeted therapy continues to save lives till today. However, unlike the targeted therapies of today that

usually have a single anticancer application, tamoxifen became the gold standard for the targeted therapy of all stages of Breast Cancer (including male breast cancer), the treatment of ductal carcinoma in situ [59] a pioneering agent for the chemoprevention of BC in high risk women [58, 59] and the lead compound for the new drug group, the SERMs [57, 59]. The extensive laboratory studies of tamoxifen and the related nonsteroidal antioestrogen LY156,758 (keoxifene) undertaken as a prelude to initiating major trials in BC prevention, described the pharmacology of SERMs that switch on and switch off target sites throughout the body. As an example of the immediate translation of the discovery of SERM action, tamoxifen was noted to block Breast Cancer growth but enhances the growth of endometrial cancer growth under laboratory conditions [59] .This laboratory concept translated to improved clinical care through awareness that tamoxifen increased the incidence of endometrial cancer in postmenopausal women treated for breast cancer.

Clinical Implications of CYP2D6 in Tamoxifen Treatment for Breast Cancer

Recently the Food and Drug Administration (FDA) recommended an update in the label of Tamoxifen and o make it to show the increased risk of recurrence in BC patients that have cytochrome P450 (CYP2D6) poor metabolized. Six studies were analysed, and three of them were found consistent with FDA recommendations. The CYP2D6 genotype might be one of the first predictors of therapeutic response in cancer care based on germline DNA creating the possibility to analyze blood instead of tumor [86, 173].

The study design and main results of the six studies are summarized in Tables 4 and 5. The results by Goetz [174] Schroth [175] and Gonzalez-Santiago [174] all show lower recurrence-free survival in poor metabolizers compared with extensive metabolizers. The results presented by Nowell [177] and by Wegman in her 2005 publication [178] fail to show any association, whereas in 2007 Wegman [179] shows an even better recurrence-free survival in poor metabolizers. All studies combined the heterozygous genotypes (e.g. *1/*4 or *1/*41) either with the poor metabolizers (e.g. *4/*4) or with the homozygous wild-type genotype (*1/*1). A total of 471 tamoxifen-using patients were genotyped and included in the Goetz, Schroth, and Gonzalez-Santiago studies, whereas 915 patients were studied in the Nowel and Wegman publications.

Table 4. ‘‘Positive’’ studies on mainly Caucasian Breast Cancer patients using adjuvant tamoxifen: higher recurrence in Poor Metabolizers. Adapted from Vincent O. Dezentje, et al. [180]

Author	Study design	N	Results
Goetz et al. 2005 [174]	*4/*4 vs. 1/*1 + *1/*4	190	RFS HR, 1.86; P = 0.08
Goetz et al. 2007 [174]		197	RFS HR, 1.74; P=0.02(+CYP2D6inhibitors)
Schroth et al. [175]	Rest (*4,*5, *10, *41)* vs. *1/*1	197	EFS HR, 1.89; P = 0.02
Gonzalez-Santiago et al. [176]	*4/*4 + *1/*4 vs. *1/*1	84	RFS HR, 2.82; P = 0.05
Abbreviations: N, number of patients; RFS, recurrence-free survival; EFS, event-free survival; HR, adjusted hazard ratios. * Rest group includes all heterozygous and homozygous variant genotypes. C Abstract at 2007 ASCO Annual Meeting.			

Table 5. ‘‘Negative’’ studies on mainly Caucasian BC patients using adjuvant tamoxifen: lower recurrence in Poor Metabolizers. Adapted from Vincent O. Dezentje, et al. [180]

Author (population)	Study design	N	Results
Wegman et al. 2005 [179]	*4/*4 + *1/*4 vs. 1/*1	76	DRFS HR, <1; nonsignificant
Wegman et al. 2007 [178]	*4/*4 vs. *1/*4 or 1/*1	677	RFS HR, <1; P = 0.055
Nowell et al. [177]	*4/*4 + *1/*4 vs. 1/*1	162	PFS HR, 0.67; P = 0.19
Abbreviations: DRFS, distant recurrence-free survival; PFS, progression-free survival			

Raloxifene

Raloxifene is a second-generation selective estrogen receptor modulator (SERM) that acts as an estrogen antagonist on breast and uterine tissues, and an estrogen agonist on bone. Raloxifene reduces the risk of invasive Breast Cancer in postmenopausal women at high risk of invasive Breast Cancer and in postmenopausal women with osteoporosis. There in the risk of invasive Breast Cancer achieved in postmenopausal women at high risk of such cancer. Raloxifene was associated with an increased, albeit rare, risk of venous thromboembolism from 1.5- to 3-fold [60], across several placebo-controlled trials and an increased risk of fatal stroke in one placebo-controlled trial in postmenopausal woman at increased risk for major coronary events. However, raloxifene was associated with a lower risk of venous thromboembolic events and cataracts than tamoxifen in a head-to-head trial [61]. Among these adverse effects, the most noteworthy is a 7% increase in hot flashes in the initial postmenopausal period [60] . Another adverse reaction that has been observed is leg cramps. Cardiovascular risks were assessed in RUTH (Raloxifene Use for the Heart Trial) study [60] this was a randomized, double-blind, placebo-controlled, international study which was conducted in postmenopausal women at risk for major coronary events. A total of 10,101 postmenopausal women with established coronary heart disease or at increased risk for coronary heart disease were randomly assigned to either placebo (N = 5057) or raloxifene 60 mg/day (N = 5044). Raloxifene treatment neither increased nor decreased the risk of coronary events. Insofar as genitourinary safety is concerned, raloxifene did not cause endometrial hypertrophy nor increased the risk vaginal bleeding [60] In fact, in the clinical trials in which raloxifene (n = 317) was compared to continuous combined hormone replacement therapy (n = 110) or with cyclic hormone replacement therapy (n = 205), the incidence of breast symptoms and uterine bleeding in the women treated with raloxifene was significantly lower than in the women treated with either of the two hormone replacement therapy (HRT) regimens. Raloxifene should not be administered to pregnant women or to men, since its innocuousness has not been demonstrated in these patients. One example of the application of SERMs, a failed drug for Breast Cancer, keoxifene, was reinvented [57,59] as raloxifene, the first SERM to be successfully used to treat osteoporosis with the beneficial side effect of preventing BC indirectly [60, 59] It should be stressed, however, that raloxifene cannot be used to reduce Breast Cancer risk in premenopausal women. Raloxifene is as effective as tamoxifen in reducing the risk of invasive Breast Cancer [62].

Comparison of tamoxifen and raloxifen

The NSABP Study of Tamoxifen and Raloxifene (STAR), was designed in order to compare the tamoxifen with raloxifene in a population of healthy postmenopausal women at increased risk for Breast Cancer [63].

The study was undertaken between July 1999 and November 2004, and in total 19,747 women was randomized to receive either tamoxifen (20 mg, plus placebo) or raloxifene (60 mg, plus placebo) daily for a 5-year period. The mean predicted 5-year risk of developing Breast Cancer among the study population was 4.03% (SD, 2.17%) with a lifetime predicted risk of 16%. The mean time of follow-up was 3.9 years (SD, 1.6 years).

In this report of the STAR trial, raloxifene and tamoxifen were equivalent in efficacy for lowering the risk of invasive breast cancer [61, 62]. The cumulative incidence rates were 25.1 per 1000 Women (raloxifene) vs 24.8 per 1000 (tamoxifen) (P=.83).

There were fewer cases of noninvasive BC (LCIS and ductal carcinoma in situ [DCIS]) in the tamoxifen group (57 cases) than in the raloxifene group (80 cases), although the difference is not yet statistically significant (incidence 1.51 vs 2.11 per 1,000; RR, 1.40; 95% CI, 0.98-2.00). There were 36 cases of uterine cancer with tamoxifen and 23 cases with raloxifene (RR, 0.63; 95% CI, 0.35-1.08). [59] Both tamoxifen and raloxifene are known to increase a woman's risk of blood clots (see table 2).

Consistent with preclinical findings and with results from other large-scale studies showing that, compared with placebo, raloxifene does not increase endometrial cancer risk,[64, 65] the rate of endometrial cancer in the STAR trial, although not statistically significant, was 38% lower in the raloxifene group than in the tamoxifen group [61]. In contrast to tamoxifen, raloxifene does not reduce the risk of noninvasive breast cancer. Raloxifene also was associated with significantly less risk of thromboembolic events and cataracts (see table 2) [66]. Combined, these results demonstrate that raloxifene is an alternative for lowering risk of invasive breast cancer in postmenopausal women with higher Gail risk scores and in those with LCIS for whom the Gail model does not apply.

Table 2: Safety outcomes from the STAR trial2. Adopted from Evista [71]

SAFETY OUTCOMES FROM THE STAR TRIAL 2		
	TAMOXIFEN	**EVISTA**
Uterine Cancer	36 cases of uterine cancer	23 cases of uterine cancer
Uterine hyperplasia ‡	84 cases of uterine hyperplasia	14 cases of uterine hyperplasia
Hysterectomy ‡	244 hysterectomies	111 hysterectomies
Ishemic Heart Disease	114 cases	126 cases
Stroke and TIA	53 strokes; 41 TIAs	51 strokes; 50 TIAs
Thromboembolic events ‡	141 cases of deep vein thrombosis and pulmonary embolism	100 cases of deep vein thrombosis and pulmonary embolism
Fractures	104 cases of 1 type of fracture; 26 hip fracture	96 cases of 1 type of fracture; 23 hip fractures
Cataracts ‡	394 cases of cataracts; 260 cataracts surgeries	313 cases of cataracts; 215 cataracts surgeries
Deaths	101 deaths	96 deaths
(‡)The highlighted rows depict areas where the difference between treatment groups was statistically significant.		

Aromatase Inhibitors

Many breast tumors are "estrogen sensitive," meaning the hormone estrogen helps them to grow. Aromatase inhibitors (AIs) can help block the growth of these tumors by lowering the amount of estrogen in the body. AIs are categorized into two types [65]:

- Irreversible steroidal inhibitors such as exemestane form a permanent bond with the aromatase enzyme complex.
- Non-steroidal inhibitors (such as anastrozole, letrozole) inhibit the enzyme by reversible competition.

Aromatase inhibitors are effective and well tolerated drugs in the endocrine therapy of estrogen-receptor positive Breast Cancer in postmenopausal women. The long-term side effects and the safety of aromatase inhibitors still remain to be seen, and studies have been designed towards this direction. The most commonly used aromatase inhibitors include Aminoglutethimide, Testolactone, Anastrozole, Letrozole, Vorozole, Formestane and Fadrozole

The results of ongoing studies may indicate the role of aromatase inhibitors in the prevention of breast cancer. Many tests on chemoprevention of mammary carcinogenesis in female rats have been shown desirable effects of aromatase inhibitors--anastrozole and letrozole in premenopausal women affected by breast cancer [67] . The third-generation aromatase inhibitors (AIs) anastrozole, exemestane and letrozole have largely replaced tamoxifen as the preferred treatment for hormone receptor - positive BC in postmenopausal women [64] due to their superiority shown in several recent head-to-head trials [65].

Statistics show that approximately 185,000 new cases of invasive BC and are recorded each year and from these cases at least half are eligible for adjuvant therapy with AIs [67]. In addition, AIs are currently being tested as primary prevention therapy in large randomized trials involving tens of thousands of women at increased risk for breast cancer. Given the volume of use, internists will increasingly see postmenopausal women who are taking or considering treatment with AIs. Physicians need to be able to: (a) briefly discuss the advantages and the disadvantages of using a selective estrogen receptor modulator such as tamoxifen or raloxifene instead of an AI for risk reduction and (b) recognize and manage AI-associated adverse events [64].

Side Effects of Aromatase Inhibitors

Systemic adjuvant therapy has proven to be significantly effective at reducing recurrences and deaths in patients who have received primary therapy for early breast cancer. However, as with all treatments, adjuvant therapy can cause some unwanted side effects. With the presence of side-effects the importance of their effective management and control becomes apparent. Only this way one can achieve the compliance of patients with the treatment. Adjuvant endocrine therapy can be taken for many years because is not associated with the more severe, acute toxicities of chemotherapy. As it is known, the standard duration of postoperative adjuvant endocrine therapy is approximately 5 years. Prevention and treatment of adverse events associated with long-term endocrine therapy is particularly important in the adjuvant setting, where patients are clinically cancer free [68]. Anastrozole, exemestane, and letrozole - are generally well tolerated.

Most side-effects are mild to moderate and common to menopause [73]. The most commonly reported adverse events associated with adjuvant AI therapy include hot flushes and musculoskeletal complaints/arthralgia [73, 70]. In contrast to tamoxifen, they do not increase the risk of serious life-threatening thromboembolic or cerebrovascular events or endometrial cancer. However, there is a small but significant increase in the risk of osteoporosis and fractures with AI therapy [74]. An adequate monitoring of bone mineral density (BMD) and lipid profile could be recommended for post-menopausal women candidate to AIs. Sexual dysfunction is one of the side-effects too. The AI-associated reduction in estrogen levels can increase vaginal/vulvar symptoms and adversely influence sexual function. Although non-hormone-containing local agents with demonstrated efficacy are available, optimal therapy for estrogen deprivation-associated vaginal/vulvar symptoms might require local or systemic estrogen use. However, the use of systemic estrogen in the breast cancer, especially for women on AIs, has been challenged by recent randomized clinical trial evidence. In addition, maintenance of estrogen levels in the postmenopausal range cannot be assured with local estrogen use. Thus, for postmenopausal women with limiting vaginal/vulvar symptoms on adjuvant AIs that are not manageable with non-hormone-containing agents, a switch to tamoxifen might be preferred rather than adding local or systemic estrogens to the AI regimen [75].

6. Immune Therapy

Immune system is the way to effectively protect our body from scavenging germs every day. Without it we would be all time effected by different bacteria, viruses, protozoa, parasites and fungi. Also cancer is very easy to be developed too. We now understand that there is a firm link between the immune system and cancer, and that by properly stimulating the immune system we can impact many cancers. Through the years immune system has proved to be a standard treatment for variety of cancers. Monoclonal antibodies, immune adjuvants, and vaccines against oncogenic

viruses are now well-established cancer therapies. Immune modulation is a principal element of supportive care for many high-dose chemotherapy regimens. In addition, immune activation is now appreciated as central to the therapeutic mechanism of bone marrow transplantation for hematologic malignancies [181].

Interferons and other cytokines

In recent decades many advances have occurred in the understanding of the role of cytokines in breast cancer [76]. New signaling pathways of interleukin (IL)-1 family, IL-6, IL-11, IL-18, interferons (IFNs) and interferon regulatory factors 1 (IRF-1) and 2 (IRF-2) have been found within tumor microenvironments and in metastatic sites. Some cytokines (IL-1, IL-6, IL-11, TGFbeta) stimulate while others (IL-12, IL-18, IFNs) inhibit BC proliferation and/or invasion. Similarly, high circulating levels of some cytokines seem to be favorable (soluble IL-2R) while others are unfavorable (IL-1beta, IL-6, IL-8, IL-10, IL-18, gp130) prognostic indicators. So far IL-2, IFNalpha, IFNbeta and occasionally IFNgamma, IL-6, IL-12 have been the cytokines used for anti tumor treatment of advanced BC either to induce or increase hormone sensitivity and/or to stimulate cellular immunity [77]. Disappointing results occurred in most trials; however, two long-term pilot studies suggest that IL-2 and IFNbeta, when used appropriately can have a positive effect on clinical benefit and overall survival of patients with minimal residual disease after chemotherapy or with disseminated disease controlled by conventional endocrine therapy [77]. Cytokines play a major role in the immune response to tumors. Single nucleotide polymorphisms (SNPs) in the regulatory or coding regions of many cytokine genes lead to functional alterations in the transcriptional regulation of these genes or the proteins they encode [78].

Distant metastases from Breast Cancer are incurable. In endocrine-responsive patients antiestrogens are commonly administered as first and second line therapy. Regrettably, tumor growth becomes resistant to this relatively innocuous therapy. Beta-interferon was unsuccessfully added to tamoxifen to induce estrogen receptor enhancement. In mice, interleukin-2 added to tamoxifen increased their mutual anti-tumor activities. Nevertheless, no effective clinical application has been developed. It started an exploratory clinical trial based on the association of these immunostimulating cytokines with antiestrogens for first line salvage therapy of hormone dependent BC with distant metastases. Twenty-six consecutive BC patients with distant metastases, 23 of which had metastases at multiple sites, were studied for responsiveness to treatment with first line salvage antiestrogen therapy, combined with beta-interferon and interleukin-2 immuno-therapy. Clinical response and survival were compared with that of 30 consecutive historical control patients treated with antiestrogen therapy alone. Controls showed, as expected, a median duration of response, a median survival time after treatment, and after diagnosis of distant metastases, of 16, 31 and 34 months, respectively. After a mean follow-up of 62+/-36 months (range 17-155), the interval times in the non-control patients were 61

(P<0.001), 101 (P<0.000001) and 106 (P<0.000001) months. Two long-term survivors appeared to be cured after 155 and 94 months from the time of diagnosis with multiple bone metastases. Nineteen of the patients treated with beta-interferon and interleukin-2 have survived. Hormone immuno-therapy was given in an outpatient setting and was very well tolerated [77, 79].

Vaccines for Breast Cancer

A true cancer vaccine contains cancer cells, parts of cells, or pure antigens. The vaccine increases the immune response against cancer cells that are already present in the body. It may be combined with other substances or cells called adjuvant that help boost the immune response even further [1, 2, 6] .

Tumor cell Vaccines

Tumor cell vaccines are made up of actual cancer cells that have been removed during surgery. The cells are treated in the lab, usually with radiation, so they cannot form more tumors. In most cases, doctors also change the cells in certain ways, often by adding chemicals or new genes, to make them more likely to be seen as foreign by the immune system. The cells are then injected into the patient. The immune system recognizes antigens on these cells, then seeks out and attacks any other cells with these antigens that are still in the body.

MUC-1

Multiple clinical trials have also tested the safety and bioactivity of vaccines that target MUC-1-derived peptide or carbohydrate epitopes.[82] One group of trials tested a MUC-1 tandem repeat peptide-KLH conjugate given with the immunologic adjuvant QS-21 (a saponin) to patients with stable metastatic breast cancer. This vaccination strategy induced antigen-specific IgM and IgG antibody titers associated with NK-directed antibody- dependent cellular cytotoxicity (ADCC) as measured in vitro. Although concomitant KLH-specific T cell immunity was also induced, no evidence of MUC- 1-specific T cell immunity was found [82].

Dedritic Cells

Dendritic cells (DC) are recognized as the most potent antigen-presenting cells with the ability to stimulate naive resting T cells and to initiate primary immune responses [83,84]. Encouraging results in vaccination studies in animal models and the development of protocols to generate sufficient numbers of human DC for clinical application have led to attempts to verify the feasibility and efficacy of this approach in patients in the context of Phase I/II vaccination trials [83]. There has been a surge of interest in the use of dendritic cell (DC) vaccination as cellular immunotherapy for numerous cancers [84]. Results from early clinical trials pointed to a need for additional improvement of DC-based vaccines before they could be considered as practical alternatives to the existing cancer treatment strategies. In this regard,

subsequent studies have shown that DCs that express transgenes encoding tumor antigens are more potent primers of antitumor immunity both in vitro and in vivo than DCs simply pulsed with tumor peptides. Furthermore, DCs that have been engineered to express certain cytokines or chemokines can display a substantially improved maturation status, capacity to migrate to secondary lymphoid organs in vivo, and abilities to stimulate tumor-specific T cell responses and induce tumor [85].

7. Targeted Therapy of Breast Cancer

A monoclonal antibody known as Trastuzumab (Herceptin) is used to block the action of HER2 protein in Breast Cancer cells and slow down their growth in patients whose cancer expresses an over-abundance of the HER2 protein. Trastuzumab can be used in combination with chemotherapy in advanced therapy and so can both delay cancer growth as well as improve the recipient's survival [72]. More recently, several clinical trials have also confirmed that in the adjuvant setting i.e. postoperative following BC surgery, the use of trastuzumab for up to one year also delays the recurrence of BC and improves survival [59] making the patient less worried for re-occurrence.

Other types of antibodies that are being researched to fight cancer include:

- Angiogenesis inhibitors. These antibodies prevent the growth of new blood vessels, cutting off the supply of oxygen and nutrients to cancer cells [86,87].
- Signal transduction inhibitors. These antibodies block signals inside the cancer cell that helps the cells divide, stopping the cancer from growing [88, 89].

Trastuzumab

Trastuzumab (Herceptin) is a humanized IgG(1) kappa monoclonal antibody, specifically targeted against the extracellular domain of the human epidermal growth factor receptor 2 (HER2), and is indicated for the treatment of HER2-positive early or metastatic breast cancer [73]. Trastuzumab, when administered concurrently with chemotherapy regimens, consistently prolonged disease-free survival (primary endpoint) and overall survival (secondary endpoint) in patients with HER2-positive early breast cancer. Trastuzumab was generally well tolerated when added to, or administered following, a chemotherapy regimen in clinical trials. While cardiac adverse events, such as a decreased left ventricular ejection fraction and congestive heart failure, are a concern, these effects are treatable and appear to be mostly reversible [73]. Therefore, major guidelines recommend using trastuzumab as a key drug in the management of HER2-positive metastatic breast cancer. Despite the encouraging results obtained with this humanized monoclonal antibody directed against the HER2-receptor, used alone or in association with chemotherapy in metastatic patients, progression under trastuzumab are usually observed and resistances to this treatment are described [74]. Thus, many other monoclonal

antibodies and tyrosine-kinase inhibitors emerged. (antiangiogenic therapy)These therapeutics, used alone or in association with chemotherapy or trastuzumab have variable properties: anti-HER2 and anti-EGFR such as lapatinib, pertuzumab and neratinib; anti-EGFR such as gefitinib; antiangiogenesis (bevacizumab); anti-mTOR pathway (temsirolimus, everolimus) or inhibitor of HSP90 (tanespimycine) [74].

HSP90 (tanespimycine)

Heat shock protein 90 (HSP90) is the core of a multi-chaperone complex critical for the folding, trafficking, and stabilization of many client proteins that are involved in tumor cell proliferation, survival, and angiogenesis [90,91] .Targeting HSP90 results in degradation of these client proteins. Tanespimycin can be given safely at biologically active doses with mild toxicity such as nausea, vomiting, diarrhea, and fatigue. Further development of HSP90-targeted strategies includes testing of novel chemical structures having better solubility and stability and the potential for oral administration. Targeting of HSP90 in combination with other heat shock proteins, such as HSP70 or HSP27, may be an alternative strategy that warrants further exploration [88]. Phase I study examined whether a heat shock protein (Hsp) 90 inhibitor tanespimycin (17-AAG; KOS-953) could be administered safely in combination with trastuzumab at a dose that inhibits Hsp90 function in vivo in lymphocytes. Patients with an advanced solid tumor progressing during standard therapy were eligible. Patients were treated with weekly trastuzumab followed by intravenous tanespimycin, assessed in escalating dose levels. Twenty-five patients were enrolled onto four tanespimycin dose levels: 225 (n = 4), 300 (n = 3), 375 (n = 8), and 450 mg/m2 (n = 10). Dose-limiting toxicity (DLT) was observed at the third and fourth cohort (1 patient each): more than 2-week delay for grade 4 fatigue/grade 2 nausea and anorexia (375 mg/m2); more than 2-week delay for thrombocytopenia (450 mg/m2). Drug-related grade 3 toxicity included emesis, increased ALT, hypersensitivity reactions (two patients each), and drug-induced thrombocytopenia (n = 1). Common mild to moderate toxicities included fatigue, nausea, diarrhea, emesis, headache, rash/pruritus, increased AST/ALT, and anorexia.

Pharmacokinetic analysis demonstrated no difference in tanespimycin kinetics with or without trastuzumab. Pharmacodynamic testing showed reactive induction of Hsp70 (a marker of Hsp90 inhibition) in lymphocytes at all dose levels. Antitumor activity was noted (partial response, n = 1; minor response, n = 4; stable disease > or = 4 months, n = 4). Tumor regressions were seen only in patients with human epidermal growth factor receptor 2 (HER-2)-positive metastatic breast cancer. Tanespimycin plus trastuzumab is well tolerated and has antitumor activity in patients with HER-2+ BC whose tumors have progressed during treatment with trastuzumab [91].

Aromatase Inhibitor

Letrozole

Letrozole is a third-generation, nonsteroidal aromatase inhibitor. Adjuvant therapy with letrozole is more effective than tamoxifen in postmenopausal women with hormone-responsive early breast cancer, and extended adjuvant therapy with letrozole after the completion of adjuvant tamoxifen therapy is more effective than placebo in this patient population [75]. Letrozole is generally well tolerated too. Letrozole should be considered a valuable option in the treatment of postmenopausal women with hormone-responsive early breast cancer, both as adjuvant and extended adjuvant therapy [75] .One large randomized trial demonstrated that administration of letrozole to high-risk (node-positive) postmenopausal patients who have completed 5 years' adjuvant tamoxifen further prevents late recurrences and contralateral breast cancer, contrary to the lack of obvious benefit of extending tamoxifen treatment to 10 years found in another large randomized study [91].

The PI3K/AKT/mTOR-pathway

The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin-pathway (PI3K/AKT/mTOR-pathway) plays a role in the regulation of cell proliferation, cell survival, angiogenesis and resistance to anti-tumor treatments [92]. In many tumor types the PI3K/AKT/mTOR-pathway is found activated through several different underlying mechanisms. Since this pathway is believed to largely drive the malignant behavior of several of these tumors, mTOR-inhibition is considered an attractive means to apply as anti-tumor treatment. Currently, four mTOR-inhibitors are explored for clinical use: rapamycin, temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573). As monotherapy, mTOR-inhibitors yield interesting anti-tumor activity against various tumor types at the expense of relatively mild toxicities [92]. This recently resulted in the registration of two mTOR-inhibitors for patients with metastatic renal cell carcinoma (RCC) while randomized studies in other tumors are currently in progress. Furthermore, mTOR-inhibitors are well-suited drugs to combine with other anti-tumor drugs as in preclinical models mTOR-inhibition overcomes chemo resistance. Consequently, mTOR-inhibitor-containing multidrug regimens are subject to clinical studies. As holds true for all anti-tumor therapies, identification of patients who are likely to respond to mTOR-inhibitor-containing therapies is of utmost importance to avoid over- or under treatment. Preliminary results suggest that several factors reflecting activation of mTOR in tumors may be used for this purpose [92].

Exemestane

Exemestane is an orally active steroidal irreversible inactivator of the aromatase enzyme [93]. It is for an adjuvant treatment in postmenopausal women

with estrogen receptor-positive early-stage BC that it is following 2-3 years of adjuvant treatment with tamoxifen, and for the treatment of advanced BC in postmenopausal women whose their disease occurred by using tamoxifen or other antiestrogen therapy. In early-stage disease, switching to exemestane for 2-3 years after 2-3 years of adjuvant tamoxifen treatment was more effective in prolonging disease-free survival than continuing tamoxifen therapy, although it was not associated with an overall survival benefit, except in those with estrogen receptor-positive or unknown receptor status disease when nodal status, hormone replacement therapy (HRT) and chemotherapy use were adjusted for [93]. Moreover, preliminary data suggest that the efficacy of exemestane is the same with tamoxifen in the primary adjuvant treatment of early-stage breast cancer, but exemestane may be better in prolonging the time to distant recurrence. In advanced disease, exemestane showed equivalent efficacy to megestrol in patients with disease refractory to tamoxifen and an efficacy not significantly different from that of fulvestrant in those refractory to a nonsteroidal aromatase inhibitor. Exemestane is also effective in the first-line hormonal treatment of advanced BC in postmenopausal women. Exemestane is generally well tolerated, although there is bone fracture risk. It is an emerging treatment option for postmenopausal women with advanced BC resistance to one or more antiestrogen therapies [92].

Fulvestrant

Fulvestrant an estrogen receptor antagonist that has different and distinct mode of action and no agonist effects [94] may offer an effective treatment option in the post-AI setting [95]. Four Phase III clinical trials in postmenopausal women with advanced BC have found fulvestrant at the approved dose of 250 mg/month to be at least as effective and well tolerated as anastrozole or [94] exemestane [96] when following disease progression or recurrence when using tamoxifen. In addition, fulvestrant has also demonstrated activity in patients with visceral and HER2+ disease, who are generally regarded as being less responsive to endocrine therapy. Data from a recent neoadjuvant study [96] shows that higher dose of fulvestrant may possess greater activity. This systematic review was undertaken to examine and support if fulvestrant can be use as systemic therapy for metastatic or topically improved BC in postmenopausal women. Based on the findings, Fulvestrant can be considered as alternative therapy to anastrozole or exemestane in postmenopausal women with topically increased or metastatic BC [96].

Comparison of anastrozole, letrozole and exemestane in the management of early breast cancer

The hormonal therapy of patients with endocrine-sensitive early BC has mainly consisted, for several decades, of the gold standard tamoxifen [97]. The efficacy and favorable toxicity profiles of third-generation aromatase inhibitors (AIs), anastrozole, letrozole and exemestane, in advanced disease led to their development

in early breast cancer. Recent results consistently show the superiority of these agents over tamoxifen. Adjuvant trials by Nabholtz et al [97] evaluated AIs using four different therapeutic approaches: (1) Upfront strategy: randomization of newly diagnosed patients: tamoxifen for 5 years versus AI for 5 years, (2) Sequential strategy: randomization of newly diagnosed patients: tamoxifen (2 - 3 years) followed by AI or the inverse for a total of 5 years versus upfront AI for 5 years, (3) Switch strategy: delayed randomization (or analysis) after 2 - 3 years of tamoxifen (patients free of disease): 2 - 3 years of tamoxifen versus 2 - 3 years of AI (total treatment 5 years) and (4) Extended strategy: delayed randomization after 5 years of tamoxifen (patients free of disease): 2 - 5 years of AI versus placebo.

Overall, AIs showed evidence of superiority over tamoxifen in the adjuvant setting with proven improved efficacy and better toxicity profile. Despite some common characteristics, a body of evidence on AIs indicates some specific differences between the three agents in mechanism of action, pharmacokinetics, efficacy as well as toxicity profiles [97]. Consequently, these hormonal agents may not be considered interchangeable in clinical practice. This review explores available results from AI trials and tries to define their present role in the adjuvant management of postmenopausal patients with BC [97].

Monoclonal Antibodies

Bevacizumab

Bevacizumab is a humanized monoclonal antibody to VEGF, and the incorporation of bevacizumab to chemotherapy is one of the rapidly evolving areas in the treatment of breast cancer. Preclinical studies have shown that Bevacizumab (Avastin) in combination with chemotherapy versus chemotherapy alone improves progression-free survival and increases the response rate in first-line therapy for locally recurrent or metastatic breast cancer. This approach has been and is still being evaluated for early BC in neoadjuvant and adjuvant settings. Bevacizumab is well tolerated and has an established tolerability profile. The biomarkers of benefit will ultimately help identify the subgroups of patients who specifically benefit from anti-VEGF therapy with bevacizumab [68]. Vascular endothelial growth factor (VEGF) has emerged as a key target for the treatment of cancer. As the ligand to the VEGF receptor, it plays a central role in promoting tumor angiogenesis. Over expression of VEGF leads to poor outcomes in patients with BC and other tumors. As a single agent or added to vinorelbine, bevacizumab has produced encouraging results in phase II clinical trials in patients with refractory metastatic breast cancer. When added to capecitabine chemotherapy in a phase III trial, bevacizumab produced a greater response rate, but did not prolong progression-free survival. This may reflect the late disease stage and poor prognostic factors in the patient population. A large, ongoing, phase III, cooperative group trial is evaluating the effect of bevacizumab in combination with paclitaxel as first-line therapy for metastatic disease. The adverse

effect profile of bevacizumab differs from that of cytotoxic chemotherapy and includes hypertension, proteinuria, thrombosis, and epistaxis [80].

Cetuximab and Panitumunab

Numerous cellular pathways have a significant impact in the growth and metastatic potential of tumors [81]. Essential element of such pathways is the epidermal growth factor receptor (EGFR), a member of the HER family of receptor tyrosine kinases. One of the most important issues in cancer, which attracted the attention of clinical oncologists, is the potential use of targeted therapies. EGFR signaling pathway is implicated in the control of cell survival, proliferation, metastasis and angiogenesis. EGFR is, therefore, an appealing target for molecular-targeted cancer therapy as it is expressed in a variety of solid tumors (colorectal, breast, head and neck, etc.). Receptor antagonists that target EGFR have already been of high interest for a number of years [81]. Multiple therapeutic strategies have been developed to target EGFR, including monoclonal antibodies (mAbs), tyrosine kinase inhibitors (TKIs), ligand-toxin conjugates, and antisense oligonucleotides. In particular, mAbs block ligand from binding to the extracellular domain of the receptor. Two mAbs that block EGFR (erbB1), cetuximab and panitumumab, have been approved by FDA. Cetuximab is a chimeric IgG1 anti-EGFR monoclonal antibody, whereas panitumumab is a fully human IgG2 anti-EGFR monoclonal antibody [81].

Tyrosine kinase inhibitors

Tyrosine kinases (TKs) are attractive targets for cancer therapy, as quite often their abnormal signaling has been linked with tumor development and growth [99]. Constitutive activated TKs stimulate multiple signaling pathways responsible for DNA repair, apoptosis, and cell proliferation. In the last few years, thorough analysis of the mechanism underlying tyrosine kinase's activity led to novel cancer therapy using TKs blockers [99,100]. These drugs are remarkably effective in the treatment of various human tumors including head and neck, gastric, prostate and Breast Cancer and leukemia. The most successful example of kinase blockers is Imatinib (Imatinib mesylate, Gleevec, STI571), the inhibitor of Bcr/Abl oncoprotein, which has become a first-line therapy for chronic myelogenous leukemia. The introduction of STI571 for the treatment of leukemia in clinical oncology has had a dramatic impact on how this disease is currently managed. Others kinase inhibitors used recently in cancer therapy include Dasatinib (BMS-354825) specific for ABL non-receptor cytoplasmic kinase, Gefitinib (Iressa), Erlotinib (OSI-774, Tarceva) and Sunitinib (SU 11248, Sutent) specific for VEGF receptor kinase, AMN107 (Nilotinib) and INNO-406 (NS-187) specific for c-KIT kinase. The following TK blockers for treatment of various human tumors are in clinical development: Lapatinib (Lapatinib ditosylate, Tykerb, GW-572016), Canertinib (CI-1033), Zactima (ZD6474), Vatalanib (PTK787/ZK 222584), Sorafenib (Bay 43-9006, Nexavar), and Leflunomide (SU101, Arava) [99].

Alterations in tyrosine kinase expression or functionality have been linked to tumor growth, and detailed analysis of tyrosine kinase pathways has led to the development of novel anticancer drugs based on their inhibition.[98] The aim was to examine the cytotoxicity and cellular alterations correlated with multidrug resistance mechanisms induced by three tyrosine kinase inhibitors, lapatinib, sorafenib and gefitinib. The study was performed on three Breast Cancer cell lines (BRC-230, MCF-7 and SkBr3). Lapatinib and gefitinib induced a cytotoxic effect and mitochondrial membrane depolarization in BRC-230 and SkBr3 cells, while sorafenib induced apoptosis and a high and rapid dissipation of mitochondrial potential in all cell lines.[98] Moreover, all three drugs produced a rapid cytosolic calcium mobilization from endoplasmic reticulum stores in the investigated cell lines and a strong decrease in multidrug transporter activity in BRC-230 and MCF-7 cells. Mitochondrial membrane depolarization and inhibition of multidrug transporter activity induced by tyrosine kinase inhibitors were independent of cytosolic calcium mobilization. These data suggest that the investigated drugs possess mechanisms of action that are independent of drug target expression, opening up further possibilities for the development of new therapeutic strategies [98].

Lapatinib

Lapatinib is an oral, small-molecule, that inhibits epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER2) tyrosine kinases [101] by binding to the ATP-binding site of the receptor's intracellular domain. This results in inhibition of tumor cell growth. The drug is relatively well tolerated in patients, with few and mostly low-grade adverse effects. In particular and unlike to trastuzumab, it has very little (if any) adverse effects on cardiac function [102] Some data show that lapatinib could cross the blood-brain barrier, with some evidence of activity in treating or preventing brain metastases [102] Recently, lapatinib gained approval for use in the USA and Europe, for the use in combination with capecitabine in the treatment of advanced BC overexpressing HER2 (HER2+) and for the treatment of advanced HER2-positive breast cancer respectively [103, 101] .

Sunitinib

Sunitinib is an orally available small-molecule multikinase inhibitor [104]. This agent potently inhibits the vascular endothelial growth factor receptor, platelet-derived growth factor receptor, and c-Kit in addition to other kinases in biochemical and cell-based assays [105]. In several relevant preclinical cancer models, sunitinib exerts significant antiangiogenesis and antitumor effects.

In phase I studies, using intermittent dosing schedules, oral administration of doses up to 50 mg/day were reasonably well tolerated and resulted in plasma

concentrations in the range of targeted levels needed for sustained kinase inhibition [100].

Biomarker and functional imaging studies showed modulation of circulating markers of angiogenesis as well as a reduction in tumor metabolism. Sunitinib showed clinical activity in patients with renal cell cancer and in patients with imatinib-resistant gastrointestinal stromal tumors [100]. Definitive randomized clinical trials showed significant clinical activity in these two indications leading to regulatory approval [100]. In addition, this drug has showed activity in a variety of other tumor types such as breast, colon, and lung cancer and is being explored in combination with standard drugs in these diseases. The observation that biological and functional imaging effects are reduced during drug-free intervals has prompted the evaluation of protracted dosing schedules. A better understanding of mechanisms involved in resistance to sunitinib provides the rationale for combination strategies that hopefully will result in better clinical effect [100].

TP53

TP53 encodes the tumor suppressor protein p53, which inhibits cell cycle progression in the presence of radiation-induced DNA breaks [107]. TP53 mutations are associated with a syndrome named Li-Fraumeni syndrome (LFS) and Li-Fraumeni-like syndrome (LFLS) (see also Table 3) [106]. In families with LFS, TP53 is frequently mutated. Studies have shown that although mutations in TP53 are extremely rare in the general population, those with the mutation will develop cancer at some point [106]. In a study of 100 women who had breast cancer, 4 women below 31 years of age had a mutation in TP53, independent of BRCA-gene mutation status; 2/37 familial BC cases had features of LFS or LFLS and 2/63 non-familial cases had mutations in TP53]. In Walsh's study] of 300 women with a strong family history of BC who had neither mutations nor genomic rearrangements within the BRCA1 and BRCA2 genes, three families had LFS and 7 families had LFLS. Two of the 3 families with LFS and 1 in family history of Breast Cancer without LFS or LFLS, none carried mutations in TP53. In this selected population, about 1% of families with hereditary Breast Cancer may carry mutations in TP53 [106].

Table 3 the criteria for LFS and LFLS syndromes that are associated with the TP53 mutations adapted from Rosman et al [106]

Li-Fraumeni Syndrome (LFS)
Sarcoma <45 years old with 1st degree relative < 45years old with cancer and 1st or 2nd degree relative < 45years old with any cancer
Li-Fraumeni-like syndrome (LFLS)
Sarcoma, brain tumor or adrenocortical carcinoma < 45years old or childhood leukemia and 1st or 2nd degree relative with LFS tumor and 1st or 2nd degree relative < 60years old with cancer

NeuVax

Apthera is developing NeuVax which is a vaccine consisting of an immunogenic peptide derived from the HER2/neu protein which is over-expressed in many Breast Cancer patients [108,109]. It is in Phase II/III testing to treat early-stage, HER2-positive breast cancer and in Phase I/II testing to treat HER2-positive prostate cancer [109]. According to Apthera, patients demonstrated in vivo immunologic responses as well as in vivo DTH responses post-treatment. Clinical recurrence rate was 5.6% (5/90) for treated patients versus 14.8% (12/81) for the observation patients (p=0.04)[108].

NeuVax can be administered safely and with minimal local or systemic toxicity. Both in vitro functional assays and in vivo DTH responses indicate a good immunologic response to the treatment. NeuVax appears to reduce rate of recurrence in disease-free conventionally treated BC patients. It may represent a useful adjunct to current standard therapy in reducing the rate of BC recurrence [109].

8. Gene Therapy

Gene therapy is a therapeutic approach that is designed to correct specific molecular defects that contribute to the cause or progression of cancer [111]. Genes that are mutated or deleted in cancers include the cancer susceptibility genes p53 and BRCA1. Because mutational inactivation of gene function is specific to tumor cells in these settings, cancer gene correction strategies may provide an opportunity for selective targeting without significant toxicity for normal non-tumor cells [111]. Both p53 and BRCA1 appear to inhibit cancer cells that lack mutations in these genes, suggesting that the so-called gene correction strategies may have broader potential than initially believed. Increasing knowledge of cancer genetics has identified these and other genes as potential targets for gene replacement therapy. Initial patient trials of p53 and BRCA1 gene therapy have provided some indications of potential efficacy, but have also identified areas of basic and clinical research that is needed before these approaches may be widely used in patient care [110].

The BRCA1/2 mutations are the most commonly identified germ line gene mutations in patients with hereditary breast cancer [112]. These proteins have many critical cellular functions, including repair of DNA double-strand breaks. The role of defective BRCA1/2 as a predictor of response to DNA-damaging agents has been studied extensively in preclinical models, but prospective clinical validation is lacking [113, 114]. Poly [ADP-ribose] polymerase (PARP) inhibitors illustrate the concept of synthetic lethality in cells with defective BRCA1/2 and numerous PARP inhibitors are being evaluated in patients with BRCA1/2-associated tumors [115]. BRCA1/2 mutation or functional loss will likely serve as a useful predictive biomarker of response to treatment with PARP inhibitor [117, 116].

Despite Gene therapy for cancer treatment representing a promising approach that has shown selectivity and efficacy in experimental systems as well as clinical trials, some major problems remain to be solved before this strategy becomes routinely adopted in the clinic. One of the main challenges being the improvement of gene delivery [118]. Namely, the development of DNA vectors characterized by maximum efficiency and minimal toxicity will define the success of gene therapy and its chances of being accepted by public and clinicians. A number of issues need to be considered. The "magic" vector should be targeted, protected from degradation and immune attack, and safe for the recipient and the environment. Moreover, it should express the therapeutic gene for as long as required, in an appropriately regulated fashion. Vehicles such as retroviruses, adenoviruses and liposomes have been adopted in clinical studies, with varying results. New therapeutic modalities are also being explored in order to overcome the limitation of poor gene transfer and patient toxicity, including bacteria, adeno-associated and herpes simplex viruses, lentiviruses, cationic polymer-DNA complexes and electroporation [118].

Once the mutation is identified in the gene, patients and their relatives have the option of preimplantation genetic diagnosis (PGD) in order to select embryos without familial cancer-predisposing mutations [119]. This procedure has already been performed in several syndromes, including the common syndromes of genetic predisposition to colon and breast cancer. Despite the numerous ethical objections and legal arguments, PGD for adult-onset cancers is today a reality and couples with an inherited predisposing mutation deserve the same respect, support and right to choose if their child will be born having an extremely high risk for cancer development as in the case of other life-threatening diseases for which prenatal screening has become a standard [97] Mutations within the BRCA1 and BRCA2 genes are common among women with a strong family history of breast cancer, they account for at most 3–8% of all BC cases. Mutations in the TP53 and PTEN genes, which cause Li- Fraumeni syndrome and Cowden syndrome respectively (as seen above), are exceedingly rare, and probably account for less than 0.1% of breast cancers. Other candidate genes that may cause BC have been identified in the past few years. Cancer susceptibility genes that are associated with an increased risk of BC include TGFBR1*6A, CHEK2*1100delC, and BRIP1 [120, 121].

A recent analysis of 22 studies involving 8,139 index case patients unselected for family history shows that carrying a deleterious BRCA1 mutation confers an estimated lifetime risk for developing BC of 65% (95% CI 44–78%) [122]. By the age of 40, carrying a deleterious BRCA1 mutation confers a 20% chance of developing breast cancer, and the risk increases with age, with the lifetime risk being 82% by the age of 80. In a combined analysis of 22 studies, BRCA2 mutation carriers were found to carry a cumulative BC risk by age 70 of 45% (95% CI = 31% – 56%), and for ovarian cancer of 11% (95% CI = 2.4%–19%) [123].

ERBB3-protein family or epidermal growth factor receptor (EGFR) family

ERBB3, a member of the epidermal growth factor receptor (EGFR) family, is unique in that its tyrosine kinase domain is functionally defective [123]. It is activated by neuregulins, by other ERBB and non ERBB receptors as well as by other kinases, and by novel mechanisms [131].

There are likely important but poorly understood roles for nuclear localization and for secreted isoforms. Studies of ERBB3 expression in primary cancers and of its mechanistic contributions in cultured cells have implicated it, with varying degrees of certainty, with causation or sustenance of cancers of the breast, ovary, prostate, certain brain cells, retina, melanocytes, colon, pancreas, stomach, oral cavity and lung [120]. Recent results link high ERBB3 activity with escape from therapy targeting other ERBBs in lung and breast cancers [124].

MicroRNAs

MicroRNAs (miRNAs) are small 20-22 nucleotide-long members of the non-protein-coding RNA family and cause an inhibition of translation and some degree of degradation of the target messenger RNAs (mRNAs) through binding to partially complementary sites, usually in the 3' untranslated regions of the target mRNAs [189]. Therefore, miRNAs play pivotal roles as negative regulators of gene expression in a wide array of physiological processes.

Recent observations reveal that many miRNAs have been implicated in various human cancers. Both losses and gains of miRNA function have been shown to contribute to cancer development through a variety of mechanisms [182].

In oncology, detection and monitoring of tumors are now becoming possible by the evaluation of tumor-derived secretory miRNAs. However, the secretory mechanism and biological function of extra cellular miRNAs remain unclear.

The fact that several miRNA genes are dysregulated in multiple types of cancer indicates that significant pathways involved in tumorigenesis may have miRNAs as downstream targets. Thus, in tumors where miRNA genes are lost or amplified, miRNA mimetics or antagomirs, respectively, are considered as promising drugs to induce apoptosis and/or cell cycle arrest in cancer cells that depend on

miRNA dysregulation for growth and survival. There is growing evidence that miRNA therapy could be a potent means to curtail tumor growth [191].

MYC proteins

MYC proteins (c-MYC, MYCN, and MYCL) regulate processes involved in many if not all aspects of cell fate. Therefore, it is not surprising that the MYC genes are deregulated in several human neoplasias as a result from genetic and epigenetic alterations [127]. MYC encodes a transcription factor that, as part of a heterodimeric complex with MAX, regulates the expression of a multitude of genes involved in regulating cellular proliferation and growth [129, 128].

The near "omnipotency" together with the many levels of regulation makes MYC an attractive target for tumor intervention therapy [127]. Overexpression of MYC is commonly associated with tumorigenesis. MYC exerts its neoplastic function by inducing autonomous cellular proliferation and cellular growth, blocking differentiation, and inducing genomic destabilization [128].

T cell receptor

T cell receptor (TCR) gene therapy provides patients with autologous T cells that are genetically engineered with TCR alphabeta chains and constitutes a promising approach for the treatment of tumors and virus infections.[129] Among the current challenges of TCR gene therapy is the optimization of TCR alpha and beta transgene pairing to enhance the functional avidity of therapeutic T cells. Recently, various genetically modified TCRs have been developed that enhance TCR pairing and minimize mispairing, i.e. pairing between transgenic and endogenous TCR chains. [130].

p53 gene therapy

The p53 is the most commonly mutated tumor suppressor gene in solid tumors and is also mutated in the germline of patients with the rare hereditary Li-Fraumeni syndrome [105]. The p53 gene is specifically relevant to the development and progression of breast cancer, because p53 is frequently mutated in BC specimens and Li-Fraumeni syndrome patients develop BC as part of their multiple cancer syndrome. Thus, p53 genetic correction is a rational approach for breast cancer, particularly in those rare patients with BC as part of Li-Fraumeni syndrome [107].

The function of wild-type p53 is suppression of cell proliferation through a multiprotein regulatory pathway that is focused around the retinoblastoma gene and control of apoptosis [116].Because p53 may naturally function as an inhibitor of cell proliferation, it inhibits cell growth in most normal and malignant cells, with few exceptions [119,120, 121]. For this reason it may effectively inhibit tumor growth even in cancers that do not have p53 mutations .Preclinical animal studies of

adenovirus-based p53 gene therapy for cancer in both cell culture and animal models have demonstrated tumor suppression [107].

BRCA1 gene therapy

Although the molecular function of BRCA1 is controversial and may include DNA repair or transcriptional functions, overexpression of BRCA1 into sporadic breast or ovarian cancer cells, which usually show low BRCA1 expression, results in growth inhibition and tumor suppression [122,123].

The mechanism of growth inhibition by BRCA1 is unknown and may involve interactions with WAF1/CIP1, p53, Rb or induction of apoptosis. Although BRCA1 is only mutated in a small percentage of breast or ovarian cancers, the majority of sporadic breast and ovarian cancers appear to express low levels of BRCA1 messenger RNA and protein. This appears to be a consequence of loss of heterozygosity and promoter methylation of the remaining BRCA1 allele [124, 125].

PTEN

The PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that inhibits cell growth during the G1 phase of cell cycle by activating the cyclin-dependant kinase inhibitor p27 (KIP1) .Mutations in PTEN are rare, but are associated with a high penetrance syndrome termed Cowden disease (CD). Individuals with Cowden syndrome have a high risk for developing BC as well as hamartomas and benign tumors in the skin, thyroid, breast, endometrium, and brain. At least three different mutations in PTEN have been found in families with CD and early onset of Breast Cancer [80].

9. Stem Cells Therapy

Stem cells are the natural sources of embryogenesis and continuous regeneration throughout adult life [132]. Stem cells provide for life-long cell replacement in tissues and organs, and have inherent homing abilities that are critical in therapeutic applications. Stem cells are also the driving force of cancer where genetic/epigenetic alterations culminate in tumorigenesis either in tissue stem cells or in some of their derivatives.[132] As a rare subset of the tumor, cancer stem cells are the only drive of tumor initiation/propagation. Autologous and cancer stem cells are thus the key targets of a) long-term and transient-regenerative/epigenetic gene therapy and b) of recurrence-free anticancer therapy, respectively [132, 133].

Stem cells can be distinguished from other cell types based on two unique characteristics. First, they are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue-

or organ-specific cells with special functions. In some organs, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions [133].

Scientists around the world, primarily work with two types of stem cells from animals and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem cells [134]. In 1998, scientists developed a method to derive stem cells from human embryos and grow the cells in vitro. These cells are called human embryonic stem cells [135]. The embryos used in these studies were created for reproductive purposes through in vitro fertilization procedures. In 2006, researchers identified conditions that would allow some specialized adult cells to be "reprogrammed" genetically to assume a stem cell-like state. This new type of stem cell is called induced pluripotent stem cells (iPSCs) [135].

Stem cell therapy is the replacement of diseased, dysfunctional or injured cells with either adult or embryonic stem cells [134]. It's somewhat similar to the organ transplant process but uses cells instead of organs. Stem cell therapy is sometimes called regenerative medicine. The stem cells are manipulated to specialize them into specific types of cells, such as heart muscle cells, blood cells or nerve cells. This manipulation may involve changing the material in which the stem cells are grown or even injecting genes into the cells. The specialized cells could then be implanted into a person [135].

Recently, a model promised to find the way to explain the different biological features of breast cancer cell populations and their different response to therapeutic agents. This model links the emergence of breast cancer cells to stem cells and progenitors, an observation originally made in other cancer entities. It is supporting that tumors are coming from a small population of undifferentiated cells. These cells can undergo self-renewal and are able to generate a large number of partially differentiated cells, which constitute the mass of the tumor. If breast cancer is a stem and progenitor cell disease, this will have important implications for the understanding of the emergence of cancer cells. A combination between stem cells, early or late progenitors and the particular oncogenic mutations acquired can give a new classification to the different types of breast cancer. So these parameters might determine the mechanisms of cancer progression and the responsiveness of patients to drug treatment [183].

Whereas human breast epithelial stem cells may exist within the basal layer, the luminal compartment or its reprogrammed equivalent can provide precursor cells for breast cancer [184].

Understanding the origin of breast cancer stem cells, their relationship to breast cancer development, and the differences between normal and cancer stem cells

may lead to novel approaches to breast cancer diagnosis, prevention, and treatment [185].

Multidrug Resistance – MDR

1. Definition

Multidrug resistance (MDR) in tumor cells is a significant obstacle to the success of chemotherapy in many cancers. Multidrug resistance is a phenomenon whereby tumor cells in vitro that have been exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds [136].

MDR is termed 'intrinsic' when the disease is refractory to chemotherapy from the outset, or 'acquired' when the disease becomes insensitive to treatment upon relapse [137]. Multidrug resistance can affect patients with a variety of blood cancers and solid tumors, including breast cancer. Tumors usually consist of mixed populations of malignant cells, some of which are drug-sensitive while others are drug-resistant. Chemotherapy has the ability to destroy only the drug-sensitive cells, leaving however the drug-resistant cells unharmed. As a result the tumor begins to grow again, and chemotherapy fails because the remaining tumor cells are now resistant [137].

2. Etiology of multidrug resistance

A number of different mechanisms can mediate the development of MDR, including increased drug efflux from the cell by adenosine triphosphate (ATP)-dependent transporters, decreased drug uptake into the cell, activation of detoxifying enzymes, and defective apoptotic pathways. The presence of at least two molecular “pumps” in tumor-cell membranes that actively expel chemotherapy drugs from the interior are correlated to resistance to therapy [138]. This allows tumor cells to avoid the toxic effects of the drug or molecular processes within the nucleus or the cytoplasm. The two pumps commonly found to confer chemoresistance in cancer are P-glycoprotein (also referred to as MDR1 and ABCB1 according to the gene classification) and the multidrug resistance associated protein (also referred to as MRP). Because of their function and importance, they are the targets of several anticancer efforts.

Active drug transporters such as MRPs (MDR associated proteins) and MDR1 have been described in both prokaryotic and eucaryotic cells. According to Seral et al [129] these were originally described as conferring resistance to anticancer agents in cancer cells, antibiotics in bacteria, or antifungal agents in fungi, these proteins appear today to be part of a very general mechanism that cells have developed to protect themselves from invasion by diffusible, foreign molecules. The P-glycoprotein and MRP transporters belong to the family of ATP binding cassette transporters and use

ATP hydrolysis as an energy source [139]. They play a key role in drug disposition by modulating drug transport through epithelia and other biological barriers to an extent that was completely unsuspected before [140].

P-glycoprotein (P-gp) is a cell membrane-associated protein that transports a variety of drug substrates [141]. Although P-gp has been studied extensively as a mediator of multidrug resistance in cancer. However, P-gp is expressed in normal tissues as well and acts as a determinant of drug pharmacokinetics. P-glycoprotein is present in organ systems that influence drug absorption (intestine), distribution to site of action (central nervous system and leukocytes), and elimination (liver and kidney), as well as several other tissues. Many marketed drugs inhibit P-gp function, and several compounds are under development as P-gp inhibitors. Similarly, numerous drugs can induce P-gp expression. While P-gp induction does not have a therapeutic role, P-gp inhibition is an attractive therapeutic approach to reverse multidrug resistance. Clinicians recognize that P-gp induction or inhibition may have a substantial effect on the pharmacokinetics and pharmacodynamics of concomitantly administered drugs that are substrates for this transporter [141].

P-gp belongs to the ATP-binding cassette (ABC) family of transporters, currently numbering 48 members that share sequence and structural homology [142]. It is believed that, while this class of transporters has a large number of members, only 10 or so are reported to confer the drug-resistant phenotype [143]. These transporters use the energy that is released when they hydrolyze ATP to drive the transport of various molecules across the cell membrane [1402]. In addition to their physiologic expression in normal tissues, many are expressed and, importantly, over-expressed, in human tumors. Their role in the development of MDR and in normal tissues has been reviewed elsewhere [143].

In cancerous tissue, the expression of P-gp is usually highest in tumors that are derived from tissues that normally express P-gp, such as epithelial cells of the colon, kidney, adrenal, pancreas, and liver, resulting in the potential for resistance to some cytotoxic agents before chemotherapy is initiated. In other tumors, the expression of P-gp may be low at the time of diagnosis but increases after exposure to chemotherapy agents, thereby resulting in the development of MDR in those cells [144]. There is a growing body of literature that links the failure of certain chemotherapeutic agents to the expression of P-gp. Indeed, the induction of MDR1 RNA can be rapid following exposure of tumor cells to chemotherapy [145].

As mentioned earlier, increased Pgp is not the only cause of MDR. Several cell lines selected for resistance do not contain increased amounts of Pgp but nevertheless are resistant to a broad range of natural-product drugs [146-149]. In one of these non-Pgp MDR lines, the H69AR small-cell lung carcinoma (SCLC) line, Cole et al. [150] found amplification and increased expression of MRP genes. There are currently 9 isoformic members of MRP proteins (MRP1-6 = ABCC1-6 and MRP

7-9 = ABCC10-12). Overexpression of MRP has since been observed in several other [151-153], but not all [151], non-Pgp MDR cell lines. Transfection of HeLa cells with an expression vector containing the MRP cDNA results in the acquisition of resistance to doxorubicin, vincristine, and VP-16, but not cisplatin [154]. Here it is shown that transfection of the MRP cDNA into human lung carcinoma cells also results in MDR. MRP belongs to the ABC superfamily of transporter proteins [150,155], and therefore one could claim that it could simply act like Pgp, as a plasma membrane pump extruding drugs. This claim is partially supported by the literature [154] which demonstrates decreased drug accumulation for several non-Pgp MDR cell lines that were later found to overexpress MRP [151-153]. However, there appears to be an exception to this rule. The exception is the MDR H69AR cell line in which the MRP gene was discovered [150]. Drug accumulation was reported to be the same as in the parental cell line and this led Cole et al. [141, 157, 158] to consider other mechanisms than decreased drug accumulation for MRP action. Moreover, the subcellular location of MRP did not seem to be similar to that of a plasma membrane transporter such as Pgp. A 190-kDa protein detected in non-Pgp MDR cells and thought to be MRP was found mainly in the endoplasmic reticulum, rather than in the plasma membrane [153].

A study by Zaman et al al [155] set out to investigate the exact mechanism by which MRP acts. They concluded that MRP is remarkably similar to the drug-transporting Pgps in its mode of action:

(a) Like Pgp, MRP can cause resistance to a range of hydrophobic drugs.
(b) MRP is predominantly located in the plasma membrane.
(c) MRP can decrease drug accumulation in the cell and this decrease is abolished by permeabilization of the plasma membrane.
(d) MRP can increase the efflux of drugs from cells. MRP is believed to act as a drug pump, like Pgp, extruding hydrophobic compounds from cells against a concentration gradient. Presumably the two ATP-binding motifs in MRP allow the protein, just like Pgp, to use ATP hydrolysis for active transport [155].

The BCRP is 95-kDa phosphoglycoprotein drug transporter of a somewhat different structure [159]. BRCP differs from Pgp and MRP because it contains only one transmembrane and one ATP binding domain, however its mode of action remains quite similar to those of Pgp. It is classified as the ABCG2 transporter , the ATP-dependent pump belonging to the G subfamily of ABC transporters [159].

In a study by Litman et al, there were developed four polyclonal antibodies that were against peptides corresponding to four different epitopes on the mitoxantrone resistance- associated protein, ABCG2. Three epitopes on the cytoplasmic region of ABCG2 gave rise to high-affinity antibodies, which were demonstrated to be specific for ABCG2. The Western blot analysis of cells with high levels of ABCG2 showed a single major band of the expected 72-kDa molecular size

of ABCG2 under denaturing conditions. The Immunoblot analysis was performed under non-reducing conditions and after treatment with cross-linking reagents there was a shift in the molecular weight from 72 kDa to several bands of 180kDa. Evidence of N-linked glycosylation was also obtained using tunicamycin and N-glycosidase F. Fluorescence and electron microscopic immunohistochemical staining, showed cytoplasmic and predominantly plasma membrane localization of ABCG2 in cell lines with high levels of expression. Also there was an observation about staining of plasma membrane on the surface of the chorionic villi in placenta. The conclusion was that ABCG2 is an ABC half-transporter of the G subfamily that can forms dimers in the plasma membrane and functions as an ATP-dependent outward pump for substrate transport [187].

In another study of Litman et al about ABCG2, was shown that ABC half-transporter is a potent, new mechanism for conferring multiple drug resistance. They used four multidrug-resistant human colon (SI) and breast (MCF-7) cancer cell lines. Despite the different selection history of the colon and breast cancer sublines a similar phenotype was displayed. The cross-resistance patterns seem to be almost identical for the two sublines. Both had high level of resistance to mitoxantrone, the anthracyclines and topotecan. Then as visualized by microscopy, sublines had similar intracellular drug distribution profiles with reduced mitoxantrone, daunorubicin, bisantrene, topotecan, BODIPY-prazosin and rhodamine 123. The restoration of mitoxantrone accumulation to the level in sensitive, parental cells after preincubation with azide and deoxy-glycose shown that the mitoxantrone resistance was correlated with that of an energy-dependent accumulation defect in both sublines. The efflux of mitoxantrone after restoration of energy was higher from the resistant cells that from the parental cell lines. According to a previous finding that transfection of the BCRP gene conferred mitoxantrone resistance to parental cancer cells is further confirming BCRP/MXR as the mechanism of resistance in these cells, Litman et al demonstrated that these cells exhibit decreased mitoxantrone accumulation as compared to control cells transfected with the vector alone. They further demonstrated that MXR-expressing cells are capable of glucuronidating cytotoxic drugs.[189]

In the recent years another type of drug transporter has been recognized which is not associated with the cytoplasmic membrane, but operates by controlling the drug transport from the nucleus to the cytoplasm via vaults. This is a 110-kDa vault protein, the lung resistance protein (LRP) [159]. Its drug substrate spectrum is similar to that of Pgp.

A study was designed to determine whether BCRP is expressed at a mitochondrial level and to see its function in various MDR and parental drug-sensitive cell lines. Experiments like Western blot analysis, immunofluorescence confocal and electron microscopy, flow cytometry analysis, and the BCRP (ABCG-2) small interfering RNA, showed that BCRP is expressed in the mitochondrial cristae and is functionally active there. In comparison to parental drug-sensitive cells

Mitoxantrone accumulation was significantly reduced in mitochondria and in cells that overexpress BCRP. In comparison with basal conditions in both whole cells and in mitochondria of BCRP-overexpressong cell lines, the specific inhibitor of BCRP, fumitremorgin c, increased the accumulation of mitoxantrone . So the conclusion of this study is that BCRP is overexpressed and functionally active in the mitochondria of MDR-positive cancer cell lines. Also BCRP can be involved in the physiology of cancer cells because its presence in the mitochondria of parental drug-sensitive cells [188] .

3. Strategies to overcome multidrug resistance

Scientists around the world have been struggling to develop nontoxic agents that would overcome the MDR of tumors. The way to achieve this incorporates various strategies, based on diverse mechanisms and effects. According to Borowski et al [159] these strategies use two general approaches: (a) rational design of agents that retain their cytostatic activity towards MDR tumor cells, and (b) development of augmenting compounds able to restore the cytotoxicity of available antitumor drugs against resistant cells. The latter approach is aimed at interfering with either the expression of the transporter proteins or their functioning.

Control of expression of MDR protein

Since its amplification is not a prerequisite for Pgp-related resistance in human tumor cells [160].
An MDR1 specific polypeptide transcriptional repressor has been screened out. Modification of MDR1 promoter region was achieved by 5-azacytidine [161].In consequence, the transcriptional activity was inhibited, and resistant K562 cells were transformed to a non-MDR type. Interesting results were obtained with ecteinascidin 743, an isoquinoline derivative of marine origin [159]. This cytostatic agent of high activity selectively inhibits the activation of induced MDR1 gene blocking its transcription, without affecting constitutive MDR1 expression [162].

Overcoming the activity of MDR membrane transporter proteins

There are several general strategies to circumvent the drug efflux action of expressed MDR transporter proteins. These strategies may include the: (a) development of compounds that are not substrates of the efflux pump(s), (b) use of agents that inactivate (inhibit) MDR proteins, (c) design of cytostatics characterized by fast cellular uptake, surpassing their MDR-mediated efflux, and the (d) use of compounds competing with a drug for the MDR protein mediated efflux [159].

In order to overcome MRP and LRP resistance certain specific strategies have also been implemented. Reducing the MRP resistance can be achieved by affecting the intracellular formation of anionic drug conjugates. The formation of such conjugates with glutathione can be indirectly inhibited by buthionine sulfoximine

(BSO), a potent inhibitor of glutathione synthesis [163], or by the inhibition of glutathione reductase by N,N-bis(2-chloroethyl)-N-nitrosourea (BCNU) [164]. Inhibition of drug transport from the nucleus to the cytoplasm, mediated by LRP protein, was achieved with the PAK-104P inhibitor, a pyridine derivative [165].

First Generation inhibitors of P-glycoprotein include: verapamil, cyclosporin (cyclosporin A), tamoxifen, and several calmodulin antagonists and worked by competing with the cytotoxic compounds for efflux by the P-gp pump.

Second Generation inhibitors of P-glycoprotein include: dexverapamil, dexniguldipine, valspodar and biricodar.

Third Generation inhibitors of P-glycoprotein include: the anthranilamide derivative tariquidar, the cyclopropyldibenzosuberane zosuquidar , laniquidar and the substituted diarylimidazole [186].

Inhibiting P-gp as a way of reversing MDR has been extensively studied for more than 2 decades. Many agents that modulate the function of P-gp have been identified, including calcium channel blockers, calmodulin antagonists, steroidal agents, protein kinase C inhibitors, immunosuppressive drugs, antibiotics, and surfactants [136]. Perhaps the biggest impetus for pursuing the use of MDR modulators in the clinical setting was provided by the work of Chan et al [166-168] who first showed that the expression of P-gp was a significant prognostic marker in certain childhood malignancies.

In patients with retinoblastoma they then used cyclosporin in combination with chemotherapy and achieved a high cure rate (91% of previously untreated patients remained relapse-free, with salvage therapy combining cyclosporin and chemotherapy prolonging survival in those previously untreated with cyclosporin) [168] . Although these trials were limited in size, they raised substantial interest in the cancer research community. However, it is now widely acknowledged that the major limitation of many of the early agents is that they typically reverse MDR at concentrations that result in unacceptable toxicity [169, 170]. This, together with unfavorable pharmacokinetic interactions, prompted the development of a number of new molecules that are more potent and selective for the P-gp transporter.

Alternative ways of addressing the multidrug resistance

An approach to deal with MDR cells is based on Monoclonal antibodies. These were initially used to identify the expression of Pgp, however, they can also be applied to inhibit Pgp specifically because of their high selectivity against well defined epitopes, and the resulting ability to abolish the MDR phenotype of tumor cells [159, 171].

Combining the anti-Pgp monoclonal antibodies with Pgp modulators may also be useful in enhancing the reversal of Pgp-mediated multidrug resistance [170]. Monoclonal antibodies themselves also affect the proliferation of Pgp-expressing tumor cells. A combination therapy using a monoclonal antibody, chemosensitizers, and an antitumor drug was found to yield better results [159].

IV . Conclusion

Breast Cancer belongs among the most common types of cancer worldwide, furthermore the incidence of this disease is increasing in all countries. Comparing to previous years the discoveries on the treatment are in flare. Many drugs are used today and many others are under clinical trials.

The approach of oncologists in the treatment of breast cancer traditionally follows the sequence: surgery-radiotherapy-chemotherapy.

The specific receptors of cancer cells like estrogen receptor, progesterone receptor and human epidermal growth factor predetermine the choice of therapy. Clinical studies have demonstrated that the presence of such receptors formthe etiology and explaines why a number of women with Breast Cancer respond to hormonal therapy or immune therapy and why others do not.

The development of drugs that use the pathways of immune system to induce their effect, like interferon and monoclonal antibodies or vaccines have recently started to successfully contribute to the fight against cancer. For example the vaccine NeuVax is an example of immune therapy that reduces the recurrence in patients while it is administered with minimal local and systemic toxicity. The very novel approaches to treat breast cancer play on gene therapy as the strategy that effort to fix the mutations in genes that predestine women to fall ill with breast cancer. Studies also showed that the combination of some of the available drugs and therapies can maximize the effects of the treatment. For example, the combination of tyrosine kinase inhibitors and monoclonal antibodies with trastuzumab is a representative example of the possible combinations. Other combinations reported in the literature include the adjuvant therapy with letrozole which is more effective than tamoxifen in postmenopausal women with hormone-responsive early breast cancer, and extended adjuvant therapy with letrozole after the completion of adjuvant tamoxifen therapy which is more effective than placebo in this patient population. Furthermore, exemestane is used as an adjuvant treatment in postmenopausal women with estrogen receptor-positive early-stage BC that follows 2-3 years of adjuvant treatment with tamoxifen, and for the treatment of advanced BC in postmenopausal women, whose disease occurred by using tamoxifen or other antiestrogen therapy.

Despite there are many “weapons” available in the “arsenal” against cancer, these are not without side-effects. Every treatment available appears with related side-effects, which can be mild to severe. In fact in some cases, these can be so severe that can lead to the termination of the treatment followed and alternatives considered.

Anticancer therapy often fails through the multidrug resistance of tumor cells. This phenomenon can be caused by transport activity of efflux pumps localized to the cellular membrane. Therefore an intensive effort is put also into the search of substances or mechanisms that could reverse the resistance and make the tumor cells sensitive to the anticancer therapy.

Until the time when the cure of cancer will be discovered by scientists, the cancer journey continues, with its' ups and downs in the treatments available. However, the knowledge is the tool that healthcare workers need in their effort to choose the best available and individually tailored therapeutic approach to breast cancer for their patients.

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