THE ANTICANCER EFFECT OF LOCAL *VERNONIA AMYGDALINA* IN COMBINATION WITH STANDARD CHEMOTHERAPY DRUG, TAMOXIFEN ON BREAST CANCER CELL LINE, MCF-7

By

CHIN TZE JIAN

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

A.D.	anno domini
AIDS	acquired immune deficiency syndrome
AKT	protein kinase B
ATP	adenosine triphosphate
B.C.	before christ
BRCA	breast cancer gene
BRCA1	breast cancer 1 protein
BRCA 2	breast cancer 2 protein
CAM	complementary and alternative medicine
cAMP	cyclic adenosine monophosphate
CCL4	chemokine ligand 4
CDK	cyclin dependent kinase
CD4+ T	T helper cells
CG5	estrogen sensitive human breast cancer cell line
CI	combination index
CO_2	carbon dioxide
DCIS	ductal carcinoma in situ

DMEM	Dulbecco’s modified Eagle's medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetra-acetic acid
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	estrogen receptor
ERK	extracellular signal-regulated protein kinase
FDA	Food and Drug Administration
g	gram
G_0/G_1	Gap 0 / Gap 1
GZL-8	ovarian carcinoma cell line
HER2/neu	human epidermal growth factor receptor 2
HMBA	hexamethylene bisacetamide
IC_{50}	50% inhibitory concentration
IGF-1	insulin-like growth factor 1
IL-2	interleukin-2
L	litre

LCIS	lobular carcinoma in situ
MCF7	human breast adenocarcinoma cell
MDA-MB-231	human breast adenocarcinoma cell
MDA-MB-468	human breast adenocarcinoma cell
MEK	Mitogen-activated protein kinase kinase
Mg	milligram
ml	millilitre
mRNA	messenger RNA
MRP1	multidrug resistance-associated protein 1
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADH	nicotinamide adenine dinucleotide
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NIH 3T3	normal mouse embryonic fibroblast cell line
OVCAR-3	ovarian carcinoma cell line
PBS	phosphate buffered saline
PEG	polyethylene glycol

PI3k	phosphatidylinositol-4,5-bisphosphate 3-kinase
PR	progesterone receptor
Rpm	revolutions per minute
S	synthesis
SD	standard deviation
TGF-β	tumour growth factor-β
UK	United Kingdom
USA	United States of America
v/v	volume / volume
VA	*Vernonia amygdalina*
WHO	World Heath Organisation
%	percentage
µg/mL	microgram / millilitre
ºC	degree Celsius
17-AAG	tanespimycin

KESAN ANTIKANSER DARIPADA *VERNONIA AMYGDALINA* TEMPATAN DALAM COMBINASI DENGAN STANDARD UBAT KEMOTERAPI, TAMOXIFEN ATAS SEL KANSER PAYUDARA, MCF-7

ABSTRAK

Vernonia amygdalina (VA) adalah tumbuhan tempatan yang kerap digunakan di Afrika untuk tujuan pemakanan dan terapeutik. Namun demikian, asal geographik, tempatan, keadaan cuaca dan masa menuai yang berbeza boleh mempengaruhi aktiviti biologikal tumbuhan. Walaupun bidang perubatan sudah menjadi lebih canggih, namun kelaziman kanser payu dara masih meningkat di seluruh dunia dan banyak data telah menunjukkan dalam semua jenis kanser, ia adalah salah satu daripada kanser yang menyumbang kepada kebanyakkan kematian. Selain itu, risiko dan kesan yang memudaratkan daripada kemoterapi makin mengimbangi faedahnya. Justeru, kajian ini menumpukan atas rawatan kombinasi dengan VA tempatan dan tamoxifen atas sel kanser payu dara, MCF7 dengan usaha untuk mengurangkan kesan bukan selektif atas sel normal, NIH 3T3. Aktiviti sitotosisiti dengan VA sahaja dan kombinasi dengan tamoxifen atas sel MCF7 dan NIH 3T3 telah dinilaikan dengan MTT asai. Nilai IC_{50} untuk MCF7 bagi VA (1.10 ± 1.78 mg/ml), tamoxifen (0.06 ± 0.04 mg/ml) dan kombinasi (0.09 ± 0.10 mg/ml) telah diperolehi. VA juga didapati tidak membahayakan sel normal dan meningkatkan nilai IC_{50} untuk NIH 3T3 dalam rawatan kombinasi (tamoxifen sahaja: 0.28 ± 0.22 mg/ml, kombinasi: 3.69 ± 2.74 mg/ml). Kombinasi antagonistik (CI>1) juga ditemui antara VA dan tamoxifen. Rawatan kombinasi mempunyai kesan anti proliferatif atas sel MCF7 dan kurangan toksisiti

atas sel normal. Pewarnaan nukleus dengan Hoechst 33258 mencadangkan kejadian apoptosis berdasarkan kehadiran pendarflour yang terang dalam sel yang dirawat dengan rawatan kombinasi. Kesimpulannya, VA menunjukkan potensi pilihan anti kanser atas sel MCF7 dengan kemungkinan melalui mendorong apoptotik kematian sel dan ia mempunyai peluang yang cerah untuk digunakan secara klinikal pada masa hadapan.

THE ANTICANCER EFFECT OF LOCAL *VERNONIA AMYGDALINA* IN COMBINATION WITH STANDARD CHEMOTHERAPY DRUG, TAMOXIFEN ON BREAST CANCER CELL LINE, MCF-7

ABSTRACT

Vernonia amygdalina (VA) is a broadly used local plant in Africa for both nutritional and therapeutic purposes. Nevertheless, different geographical origins, locality, climate conditions and time of harvesting could influence the biological activities of the plant. Albeit of the advancement in medical field, the prevalence of breast cancer still increases globally and many data have shown that among all the different types of cancers, it is one of the cancers that contribute to the majority of the mortality. Moreover, the risk and detrimental side effects of chemotherapy are fast compensating its benefits. Therefore, this study focused on combination treatment of local VA and tamoxifen towards breast cancer cell line, MCF7 in an effort to reduce the non-selective effect on normal cells, NIH 3T3. The cytotoxicity activities of VA alone and combination with tamoxifen towards MCF7 and NIH 3T3 cell lines were examined using MTT assay. The IC_{50} values on MCF7 for VA (1.10 ± 1.78 mg/ml), tamoxifen (0.06 ± 0.04 mg/ml) and combination (0.09 ± 0.10 mg/ml) were obtained. VA was also found to be non-deleterious towards normal cells and had the IC_{50} values of NIH 3T3 raised in combination treatment (tamoxifen alone: 0.28 ± 0.22 mg/ml, combination: 3.69 ± 2.74 mg/ml). Antagonistic combination (CI>1) was also discovered between VA and tamoxifen. The combination treatment managed to have an anti-proliferative effect on MCF7 cells and reduced toxicity on normal cells.

Nuclear staining by Hoechst 33258 suggested the occurrence of apoptosis based on the presence of bright fluorescence in the cells treated with combination treatment. In conclusion, VA exhibited potential selective anticancer properties in MCF7 cells by possibly inducing apoptotic cell death and it has a promising opportunity to be used clinically at the future.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer is a main public health burden in both developing and developed countries and is the second leading cause of death after cardiovascular disease. Six million out of ten million patients died annually. By 2030, the deaths from cancer worldwide are estimated to continue surging with a projected 12 million deaths (Alawode, 2013). Generally, all cancers are related to the faulty genes that control cell growth and death. This unusual growth of cells in body is lethal as they will invade and destroy normal cells (Prakash et al., 2013). Inheritance plays a bigger role in deciding the risk for a small proportion of cancers such as breast, colorectal and prostate compared with others. Familial cancers arise from the interplay between common gene variations and lifestyle or environmental risk factors like smoking, dietary imbalances, chemicals and radiation. BRCA1 or BRCA2 genes are examples of inherited mutations that cause the prevalence of breast and ovarian cancers to be much more typical in some families. However, most cancers are not the consequences of inherited genes (Howard et al., 2015).

Radiotherapy is a crucial modality for cancer cure and in the treatments of loco-regional growths that is impossible to be removed by surgery like those of advanced lung, neck and head cancers. It is gauged that around one half of the cancer patients are benefitted from it. Vomiting, nausea, diarrhoea and others are some of the

common pathological alterations in living system generated by radiation. Besides radiotherapy, chemotherapeutic agents used in latest clinical practice have been playing a remarkable role in lessening morbidity or mortality and in improving patient's quality of life (Pinto, Moreira and Simons, 2011). However, it has a myriad of downsides mainly because of the severe side effects produced as a consequence of damage to tissues. It is due to most anticancer drugs that are used in chemotherapy have slender therapeutic window, develop multi drug resistance, pose unspecific biodistribution upon intravenous administration resulting intolerable side effects to healthy tissues especially gastrointestinal tract and bone marrow (Pinto, Moreira and Simons, 2011; Tsakalozou, Eckman and Bae, 2012). Consequently, these drawbacks of conventional chemotherapeutic strategies often lead to treatment delay or discontinuance, suboptimal dosing and decreased patient compliance to therapy. Besides that, in a landmark UK study conducted by Wallington et al (2016), they discovered that people who receive chemotherapy are killed by the chemotherapy treatment and not by the cancer itself within 30 days of starting treatment. Furthermore, they also advised physicians to be more cautious in deciding chemotherapy to the patients especially to older and more infirm patients whom might be better without it.

A survey conducted by the National Centre for Complementary and Integrative Health on Complementary and Alternative Medicine (CAM) use depicted that a lot of people make use of CAM to improve their quality of lives. Rockwell, Liu and Higgins (2005) also added that its usage has escalated drastically in recent years. A survey of patients in clinical trials at NIH disclosed that 63% used at least one form

of CAM with an average of two CAM per patient. Furthermore, a study revealed that 10.6% of women that were being treated for early stage breast cancer had been using at least one CAM at the time of diagnosis while an extra 28.1% commenced using CAM after surgery (Rockwell, Liu and Higgins, 2005; Howard et al., 2015).

Vernonia amygdalina (VA) or more commonly known as bitter leaf is an ordinary medium sized shrub with numerous bitter principles in every part of the plant. It grows throughout the African tropics. It is a broadly used local plant in Nigeria for both nutritional and therapeutic purposes. The leaf decoction of the plant is traditionally utilised as an anti diabetic remedy apart from its numerous uses in ancient times which include treatment of gastrointestinal problems, schistomiasis and amoebic dysentery (Akah and Okafor, 1992; Erasto, Grierson and Afolayan, 2006). However, different geographical origins, locality, climate conditions and time of harvesting could influence the biological activities of the plant. Ngbolua et al (2011) explained that this quantitative variations are influenced by growing conditions like light intensity, temperature, humidity or other stress factors. In a research carried out by Wang et al (2014), they discovered that the chemical compositions of the leaves of same species from different countries vary which resulted in the variation in the antioxidant activities.

Therefore, this study focused on combination treatment of VA and tamoxifen towards breast cancer cell line in an effort to reduce the non-selective effect on normal cells. The analysis of combination index (CI) was conducted in order to confirm whether it acts synergistically or antagonistically.

1.2 Rationale of Study

Plants have been an ideal source of medicine since early times. The usage of plants in curing numerous human diseases has been recorded in Indian literature and Ayurveda. Besides that, medicinal plants have been serving as precious starting materials for drug development in both developing and developed countries and there has been a great deal of interest nowadays in the role of complementary and alternative medicines for the treatment of many acute and chronic diseases. Albeit of the advancement in medical field, the prevalence of breast cancer still increases globally and many data have shown that among all the different types of cancers, it is one of the cancers that contribute to the majority of the mortality. Moreover, the risk and detrimental side effects of chemotherapy are fast compensating its benefits. Thus, the anti cancer potential possessed by VA to work with the standard chemotherapy drug, tamoxifen in order to lessen the non-selective effect of chemotherapy is the reason to conduct this research project.

1.3 Objectives

1.3.1 General Objective

The general objective of this study is to determine the anticancer effect of VA and its combination with tamoxifen in human breast cancer cell line, MCF 7.

1.3.2 Specific Objectives

1) To investigate the cytotoxicity effect of VA towards malignant cell MCF-7.
2) To investigate the cytotoxicity effect of VA towards non-malignant cell, NIH 3T3.

3) To compare the effectiveness of cancer treatment between VA alone and in combination with tamoxifen.

4) To evaluate the mechanism of cell death induced by VA by assessing alterations in nuclear morphology.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer is the uncontrolled and abnormal growth of the normal cells. Changes to the DNA that pile up over time cause the transformation of a cell and the alteration in the genetic information, resulting in the inability of the cell to execute its functions properly. A main attribute of cancer cells is their capability to divide rapidly. The accumulation of cancer cells is known as a tumour. If the growing tumour does not occupy the surrounding tissue, it is denoted as benign (Figure 2.1a) while a malignant tumour is the one that spreads to nearby or distant tissues (Figure 2.1b). The malignant cells exhibit a myriad of biological features whereby they grow rapidly, invade nearby tissues and often metastasise to tissues that are unrelated to the primary location. Furthermore, increased abnormality of mitosis, vessel infiltration, nuclear atypic, neoangiogenesis, telomerase expression, genetic alterations and escape from apoptosis are some other attributes of a malignant cell. In contrast, benign neoplasms grow more slowly but may compress nearby normal tissues. Figure 2.2 illustrates the cytological and histological changes that happen during tumorigenesis (Almeida and Barry, 2010; Tanaka et al., 2013).

All tumours commence with mutations that amass in the DNA or genetic information of a single cell leading it and its offspring to behave abnormally. The mutations may affect different genes that control cell growth and division. Some of these genes are called tumour suppressor genes which are responsible to repair DNA mistakes, slow

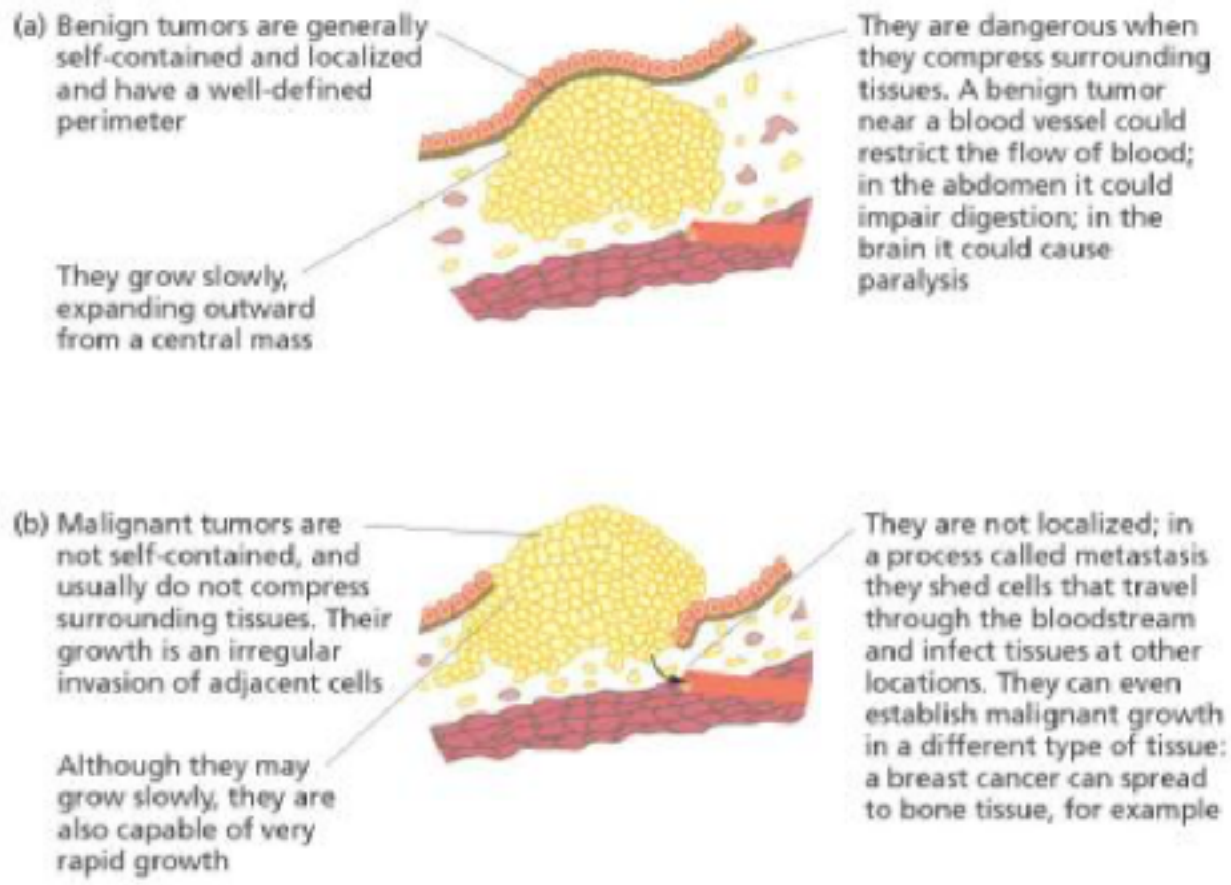

Figure 2.1 Benign vs malignant cancers. (a) A benign tumour is a bunch of cells that stays within the tissue in which it initially developed. (b) The intrusion of cancer cells into nearby tissues is the indication of a malignant tumour. Malignant cells may break away from the tumour and move to other sites in the body via the process of metastasis (Almeida and Barry, 2010).

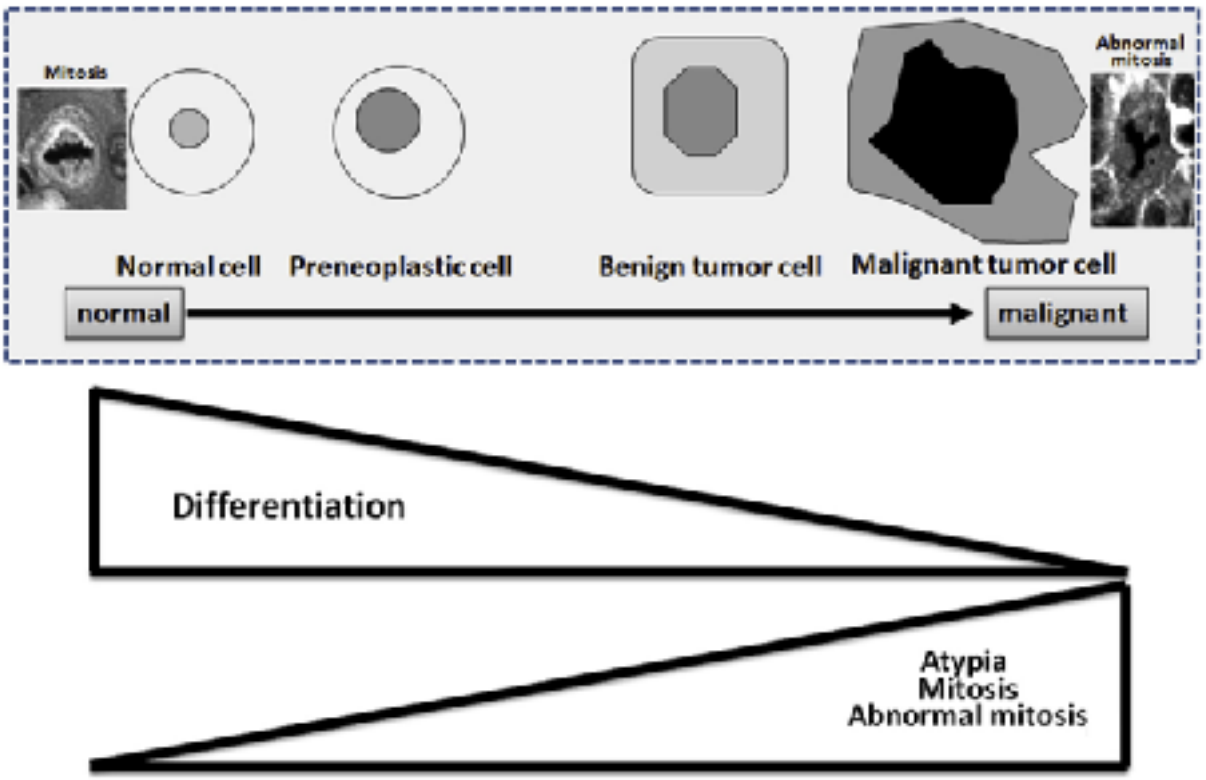

Figure 2.2 Differentiation and atypic of normal, preneoplastic and neoplastic cells. Cellular differentiation is reduced during carcinogenesis. Nuclear atypic and frequency of mitoses including uncommon mitoses rise during carcinogenesis. The abnormal mitosis in this figure is tripolar mitosis (Tanaka et al., 2013).

down cell division and induce apoptosis. When these genes do not work properly, cells can grow out of control and lead to cancer. Mutations can also prompt certain normal genes to turn into oncogenes. Proto-oncogenes are genes that help cells to grow but when it mutates or there are too many copies of it, it can be turned on indelibly or triggered when it is not supposed to be. Consequently, the cells grow out of control which can result in cancer. Furthermore, most cancer-causing mutations involving oncogenes are acquired and the oncogenes are generally activated by chromosome rearrangements and gene duplications. Nonetheless, as we have two copies of most genes and one from each chromosome in a pair, in order for a gene to stop functioning completely and potentially give rise to cancer, both copies have to be mutated (American Cancer Society, 2015).

DNA changes can be inherited or sporadic. Inherited mutations are present in the DNA conferred by the sperm and/or egg at the moment of fertilization and since all the cells in the body came from this first cell, this kind of mutation can be passed on to the next generation. This type of mutation is also known as germline or hereditary. Sporadic mutations happen spontaneously during the lifespan of a cell due to many reasons. It can be a result of a mistake made when a cell duplicates its DNA prior to dividing, the wrong repair of a damaged DNA molecule or chemical alteration of the DNA where each of which disrupts the expression of the genetic information. It takes place in one cell and then is passed on to any new cells. It is also termed as acquired mutation since it can be caused by substances that we are exposed to, such as radiation, cigarette smoke, diet and hormones. Generally, 90-95% of diagnosed

cancers seem to be sporadic in nature and have no heredity basis (Almeida and Barry, 2010; American Cancer Society, 2015).

Besides inheritance, environmental factors like lifestyle (nutrition, physical activity, tobacco use, excessive alcohol assumption), naturally occurring exposures (infectious agents, radon gas, ultraviolet light), medical treatments that comprise of radiation and medicines such as hormone drugs, chemotherapy or drugs that suppress the immune system as well as other exposures in workplace and household can contribute to the cause of cancer. In this case, substances and exposures that lead to cancer are termed as carcinogens. Moreover, the risk of developing cancer ultimately depends on various factors like the way, length and intensity of the exposure as well as the person's genetic makeup (American Cancer Society, 2015).

2.1.1 Types of Cancer

There are more than 100 identified types of cancers where all of them come with different symptoms and causes. Normally, the cancers are named based on the part of the body in which they emerge. Some tumours are distinguished to reflect the type of tissues they originate, with the suffix *-oma*, meaning tumour. Table 2.1 illustrates some of the common tumour terminologies. On top of that, sarcomas, carcinomas, lymphomas and leukemias are the major types of cancers. Around 90% of human cancers are carcinomas which arise in the epithelium or skin of the internal organs, body cavities and glands. Breast, lung, colorectal, skin and prostate are the tissues that habitually give rise to carcinomas. Sarcomas are less ordinary than carcinomas and incorporate the transformation of cells in connective tissue like bone, cartilage,

fat or muscle. There are a myriad of sarcoma subtypes and they can grow at any part of the body but most commonly in the legs or arms. Besides that, some types of cancers do not form solid tumours. For instance, leukemias are cancers of the bone marrow which causes overproduction and premature release of immature white blood cells. Another example is lymphomas, the cancers of lymphatic system that acts as the body's immune defence that consists of lymph vessels, lymph nodes and lymph (Almeida and Barry, 2010).

Table 2.1 Tumour terminology (Almeida and Barry, 2010).

Prefix	**Cell type**	**Benign tumour**	**Malignant tumour**	**Tissue affected**
Tumours of epithelial cells:				
Adeno-	Gland	Adenoma	Adenocarcinoma	Breast, colon/ rectum, lung, ovary, pancreas, prostate
Basal cell	Basal cell	Basal cell adenoma	Basal cell carcinoma	Skin
Squamous cell	Squamous cell	Keratoacanthoma	Squamous cell carcinoma	Esophagus, larynx, lung, oral cavity, pharynx, skin, cervix
Melano-	Pigmented cell	Mole	Melanoma	Skin
Tumours of supporting tissue origin:				
Hemangio-	Blood vessels	Hemangioma	Hemangiosarcoma	Blood vessels
Lipo-	Fat	Lipoma	Liposarcoma	Fat cells
Meningio-	Meninges	Meningioma	Meningiosarcoma	Brain
Myo-	Muscle	Myoma	Myosarcoma	Muscle
Osteo-	Bone	Osteoma	Osteosarcoma	Bone

Prefix	Cell type	Benign tumour	Malignant tumour	Tissue affected
Cancers of blood and lymphatic origin:				
Lympho-	Lymphocyte		Lymphoma Lymphocytic leukemia	Lymhocytes
Myelo-	Bone marrow		Myeloma Myelogenous leukemia	Granulocytes

2.1.2 Cancer Treatment

The most usual treatments for cancer are radiation, surgery and chemotherapy. Radiation is used to kill or at least slow down the growth of cancer cells. It can be used alone or combined with surgery or chemotherapy. Surgery is opted to remove some or all of the body parts that are affected but it is not useful for some types of cancer like leukaemia which is best treated with drugs (American Cancer Society, 2015). Chemotherapy is the usage of drugs to hinder their growth or kill cancer cells. It is administered either by intravascular or in a pill and since the drugs travel to nearly all parts of the body, they are essential for cancer that has spread (American Cancer Society, 2015). Since chemotherapies do not target specific genetic abnormalities related with tumour cells, the healthy dividing cells are also affected almost as critically as the tumour cells, resulting in the vastly debilitating side effects observed such as gastrointestinal toxicity, hair loss and myelosuppression. Occasionally, poor quality of life and declining effectiveness exacerbated the chances of patients to survive, particularly those that are in late stage cancer treatments. Table 2.2 illustrates some of the common examples of chemotherapeutic agents (Meyers, 2007).

Table 2.2 Examples of conventional cancer chemotherapeutic agents (Meyers, 2007).

Drug	Category
Methotrexate	Antimetabolite
Taxol	Spindle modulator
Cyclophosphamide	Alkylating agent
Cisplatin	Platinum-DNA complexes
Doxorubicin	DNA intercalating/topoisomerase inhibitor

2.2 Epidemiology of Cancer

2.2.1 Cancer Worldwide

Cancer is a major cause of morbidity and mortality with around 14 million new cases and 8 million cancer related fatality in 2012. It is also estimated that there are 182 and 102 per 100,000 cases of incidence and mortality respectively. For men, the five most common locations of cancer diagnosed in 2012 were lung (16.7%), prostate (15.0%), colorectum (10.0%), stomach (8.5%) and liver (7.5%). For women, breast (25.2%) is the highest incident sites of cancer followed by colorectum (9.2%), lung (8.7%), cervix (7.9%) and stomach (4.8%). In addition, the highest incidence rates are related with the high income countries of western Europe and North America alongside Japan, South Korea, New Zealand and Australia. World Cancer Report 2014 reveals that the cancer burden is projected to rise by approximately 70% globally in 20 years but the greatest impact will be on the lowest income nations with the least developed cancer services. Figure 2.3 and Figure 2.4 below show the estimated global annual numbers of of new cases and cancer deaths for the most vital types of cancer for men and women (World Health Organization, 2014).

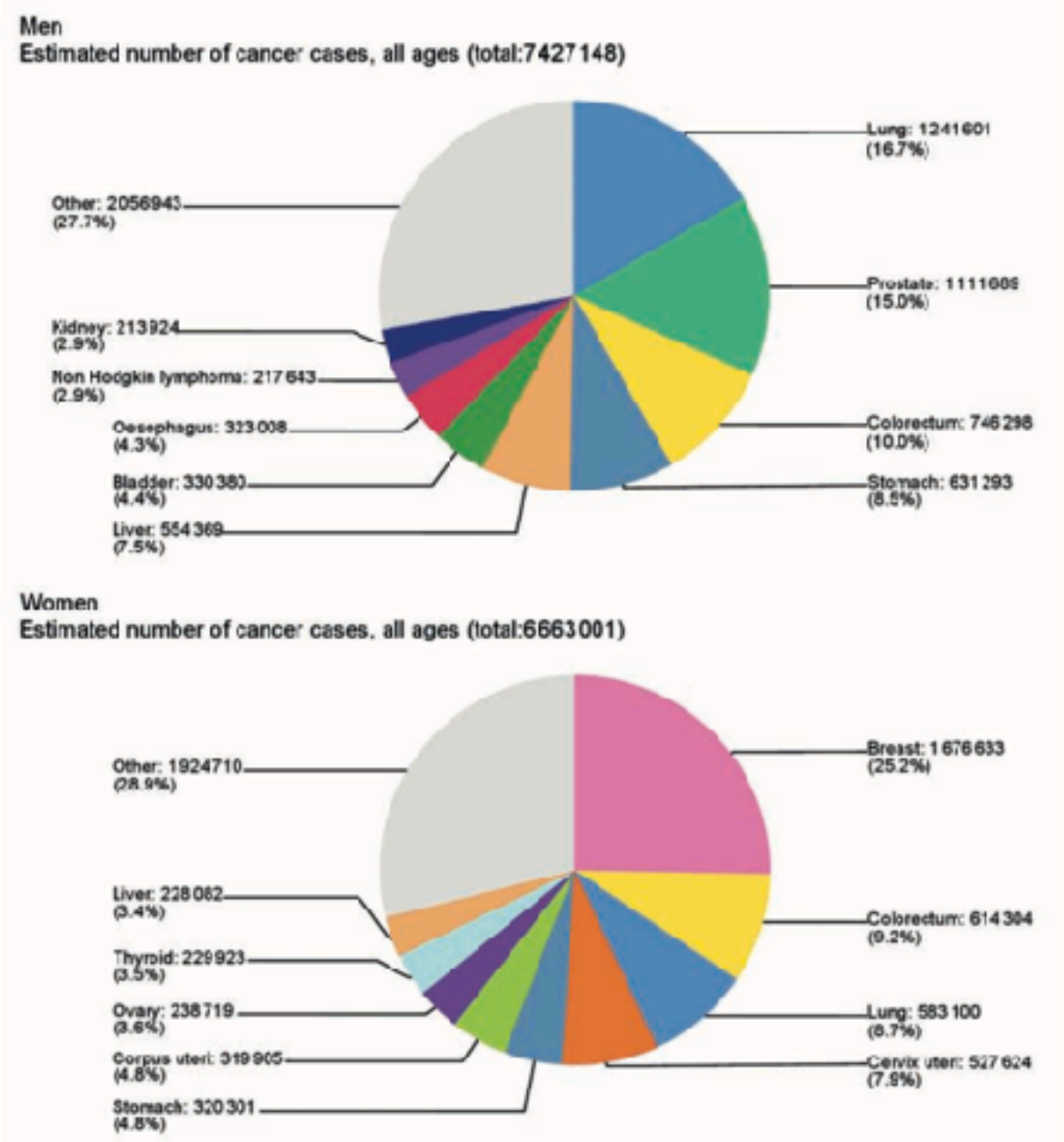

Figure 2.3 Estimated world cancer incidence proportions by major sites in men and women, 2012 (World Health Organization, 2014).

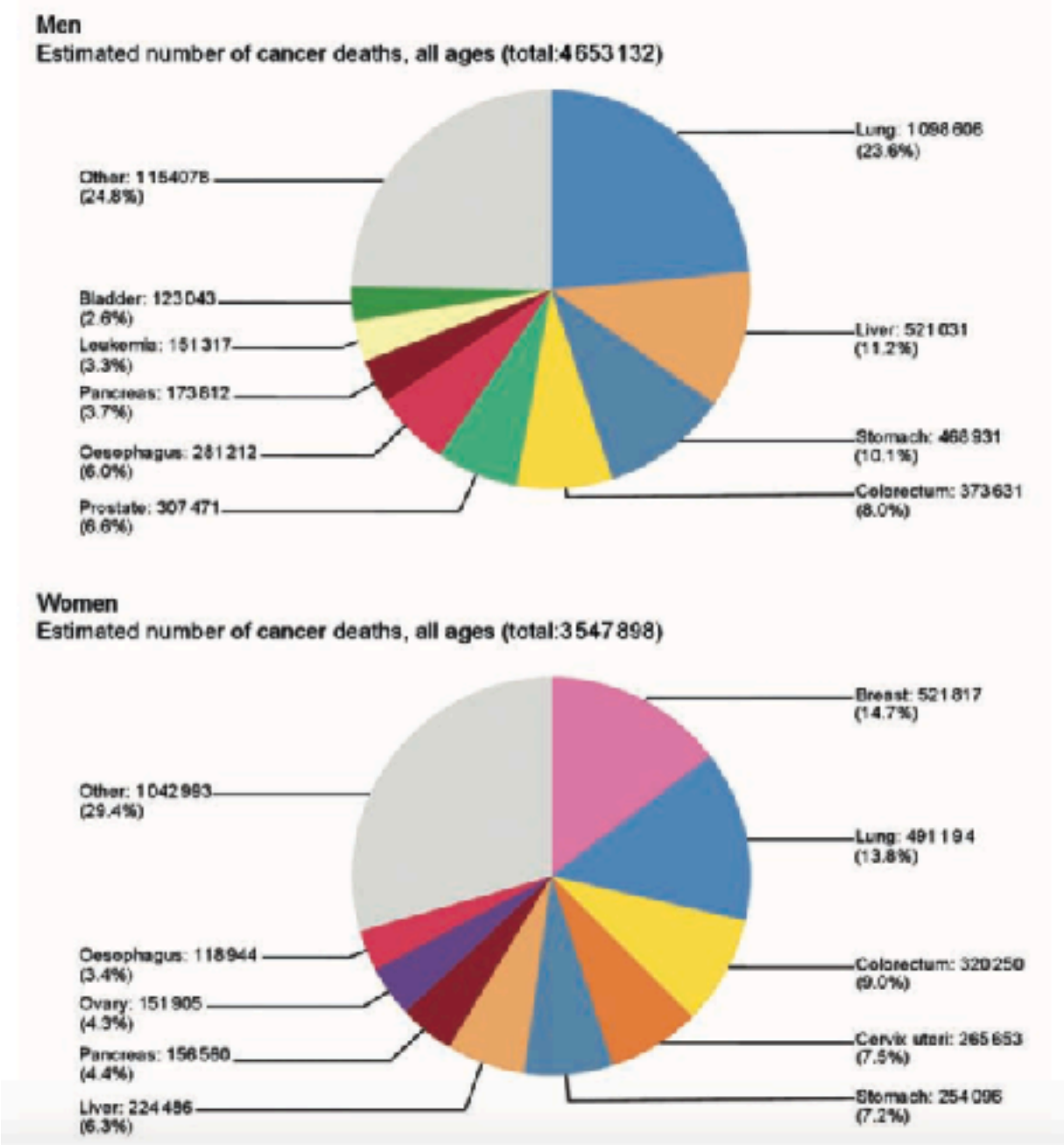

Figure 2.4 Estimated world cancer mortality proportions by major sites in men and women, 2012 (World Health Organization, 2014).

2.2.2 Cancer in Malaysia

In Malaysia, National Cancer Institute (2016) reported that cancer contributed 13.56% of all deaths occurred in the Ministry of Health Hospitals in 2015. It was the third most usual cause of death followed by diseases of circulatory system (22.77%) and respiratory system (18.54%). A total number of 103,507 newly developed cancer cases were diagnosed in Malaysia between 2007 to 2011 of which 56,713 (54.8%) were reported in females and 46,794 (45.2%) in males as announced by the Malaysian National Cancer Registry. Moreover, as mentioned in the report, the risk of females getting cancer was 1 in 9 and for males was 1 in 10. The five most common cancers among males were cancers of colorectum (16.3%), lung (15.8%), nasopharynx (8.1%), lymphoma (6.8%) and prostate (6.7%). For females, the five most common cancer were breast (32.1%), colorectum (10.7%), cervix uteri (7.7%), ovary (6.1%) and lung (5.6%).

2.3 Breast Cancer

Breast cancer is the most frequently diagnosed cancer and cause of cancer death among women in which an estimated 1.7 million new cases (25% of all cancers in women) and 0.5 million cancer deaths (15% of all cancer deaths in women) in 2012 (World Health Organization, 2014). Since 1989, the mortality rates for breast cancer have been decreased steadily with the largest drop in younger women from 2006 to 2010, probably thanks to the improvements in early detection and treatment. Yet, breast cancer still remains the highest incidence of all organ sites of cancers in adult women in the USA and health discrepancies prevail in that there are huge gaps in breast cancer survival by race (Kumar, Abbas and Aster, 2013). The American

Cancer Society (2017a) estimated that in 2017, about 252,710 new cases of invasive breast cancer will be diagnosed in women. Moreover, around 63,410 new cases of carcinoma in situ will be diagnosed and approximately 40,610 women will succumb to breast cancer.

In Malaysia, female breast cancer was accounted for 32.1% of all cancer among females. The incidence was highest among Chinese followed by Indian and Malay in which the lifetime risk for Chinese was 1 in 22, Indian 1 in 24 and Malay 1 in 35. Figure 2.5 and Figure 2.6 below illustrate age-specific incidence rate of female breast cancer from 2007-2011 and the stages respectively (National Cancer Institute, 2016).

2.3.1 Breast Cancer Treatment

Owing to the availability of mammographic screening, carcinomas often are recognised even before they become observable. The mean invasive carcinoma detected by mammographic screening is around 1 cm in size and only 15% of these have yielded nodal metastases. Besides that, DCIS is also detected prior to the development of invasive carcinoma during screening most of the time (Kumar, Abbas and Aster, 2013). There are few alternatives to treat breast cancer depending on its stage and type. Certain treatments are local, meaning the tumour is treated without affecting the rest of the body. Surgery and radiation therapy are two examples of local therapy used for breast cancer. Most women with breast cancer have some type of surgery as part of their treatment. It may be done to remove as much of the cancer as possible (mastectomy or breast-conserving surgery), determine

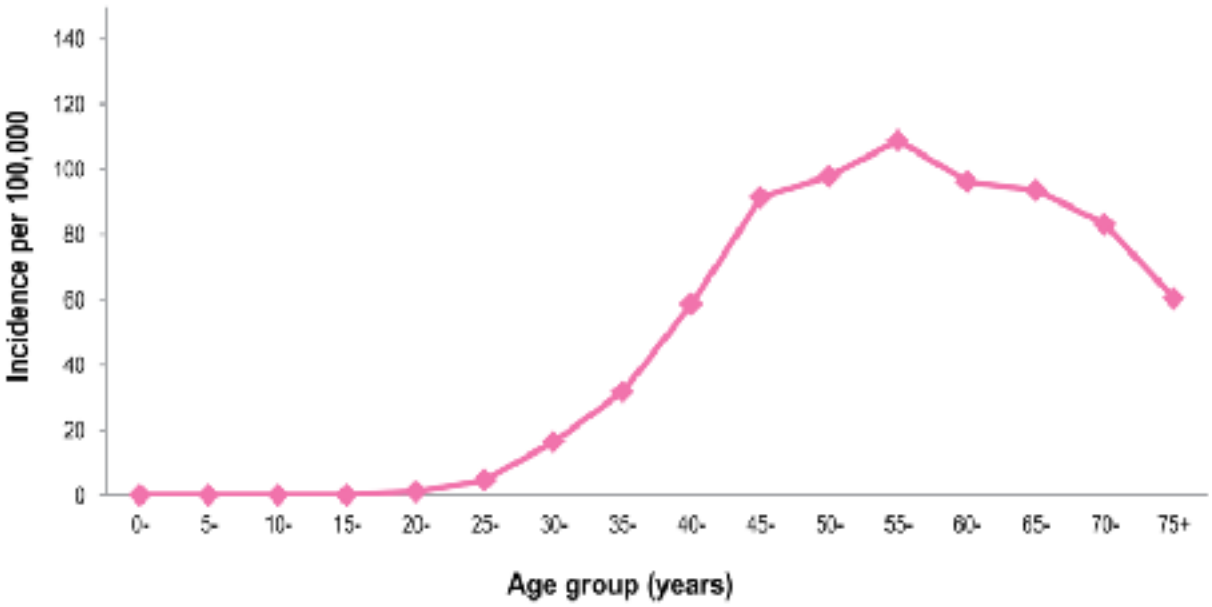

Figure 2.5 Female Breast: age-specific incidence rate, Malaysia, 2007-2011 (National Cancer Institute, 2016).

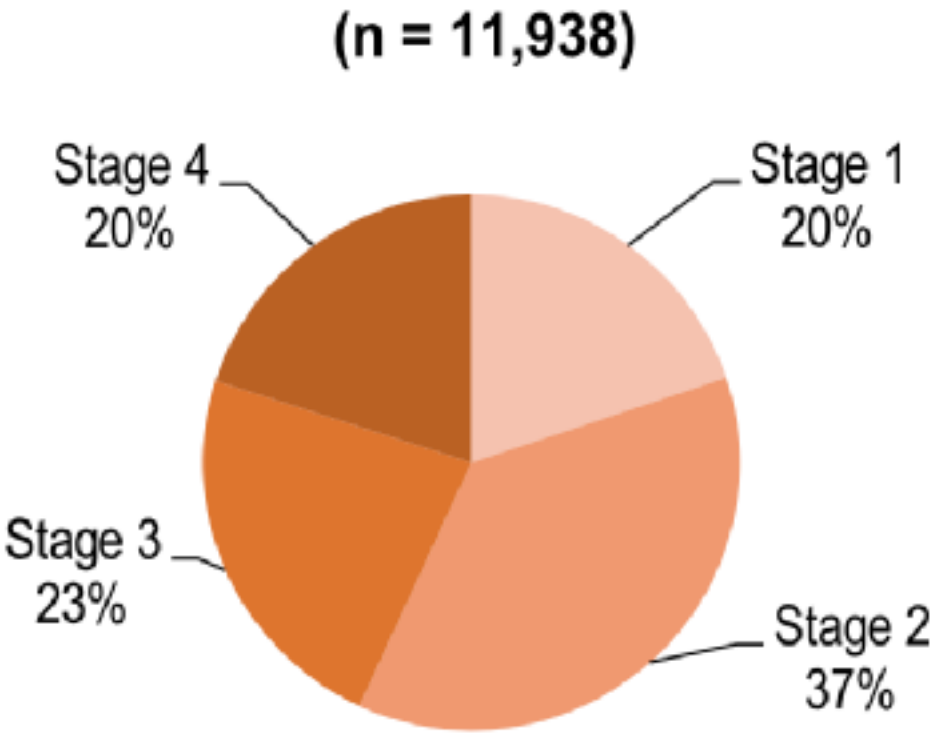

Figure 2.6 Female Breast Cancer in Malaysia: Stage (National Cancer Institute, 2016).

whether the cancer has spread to the lymph nodes under the arm, breast reconstruction reconstruction and relieve symptoms of advanced cancer. Besides that, bilateral prophylactic mastectomy is a highly effective approach to prevent the development of breast cancer in women with *BRCA* mutations. This procedure is linked with a 90% reduction in the development of breast cancer in these women and an even higher (95%) decrease in breast cancer among those who had their ovaries removed (World Health Organization, 2014).

Lumpectomy and mastectomy are two main types of surgery. Lumpectomy or sometimes known as quadrantectomy, partial mastectomy or segmental mastectomy is a surgery where only the part of the breast containing cancer is removed while mastectomy is to remove the whole breast including all of the breast tissue and sometimes other nearby tissues (American Cancer Society, 2017b). For radiation therapy, it is a treatment with high energy rays or particles that demolish cancer cells. Two major types of radiation therapy are external beam radiation and internal radiation (brachytherapy). The external radiation comes from a machine outside the body while internal radiation requires a radioactive source to be put inside the body for a short period. Normally it can be used after mastectomy to lessen the chance of cancer reoccurrence or when the cancer has spread to other parts of the body (American Cancer Society, 2017b).

Drugs used to cure breast cancer are considered systemic therapies since they can reach cancer cells almost anywhere in the body. They can be administered via mouth or directly into the bloodstream. Chemotherapy, hormone therapy and targeted

therapy can be used to treat different types of breast cancer. Chemotherapy is recommended after surgery as an adjuvant to kill any cancer cells that might have spread or left behind but cannot be detected even on imaging tests. It also can be used before surgery to shrink the tumour so that it can be removed more easily. Last, it is preferred to treat advanced breast cancer where it has spread outside the breast and underarm area. Some of the most common drugs used for adjuvant and neoadjuvant chemo include anthracyclines (doxorubicin and epirubicin), taxanes (paclitaxel and docetaxel), 5-fluorouracil, cyclophosphamide and carboplatin. Normally, a combination of two or three of these drugs are used. Platinum agents (cisplatin, carboplatin), vinorelbine, capecitabine, gemcitabine, eribulin, taxanes and anthracyclines are the drugs for advanced breast cancer (American Cancer Society, 2017b).

Some types of breast cancer are influenced by hormones in the blood. ER-positive and PR-positive breast cancer cells have receptors that attach to estrogen which assists them to grow. Hormone therapy is recommended for women with hormone receptor-positive breast cancers. It is often used after surgery to help decrease the risk of reoccurrence of cancer. It is also initiated prior to surgery or treat cancer that has returned after treatment. Hormone therapy is normally taken for at least 5 years (American Cancer Society, 2017b). As the ovaries are the prime source of oestrogen in premenopausal women, by suppressing or eliminating their functions, the oestrogen levels can be lessened. Ovarian ablation or blocking ovarian function can be carried out surgically or via radiation and this is normally irreversible. Alternatively, ovarian function can be subdued temporarily by gonadotroprin-

releasing hormone agonists or also termed as luteinising hormone-releasing hormone agonists. This drug acts by interfering the signals from the pituitary gland that trigger the ovaries to secrete oestrogen. The examples of ovarian suppression drugs are leuprolide and goserelin (National Cancer Institute, 2017).

Furthermore, aromatase inhibitors like anastrozole, letrozole and exemestane are used to halt the activity of aromatase, an enzyme that the body utilises to make oestrogen in other tissues and in the ovaries. They are used predominantly in postmenopausal women since their ovaries produce a huge amount of aromatase. Another mechanism to treat hormone-sensitive breast cancer is impede with the estrogen's ability to activate the growth of breast cancer cells. Tamoxifen and toremifene are selective oestrogen receptor modulators that bind to oestrogen receptors thereby hindering the binding of oestrogen. Besides blocking oestrogen activity by binding to estrogen receptors, they can also mimic the effects of oestrogen by serving as oestrogen agonists or antagonists depending on the types of tissues (National Cancer Institute, 2017).

Targeted therapy is another type of treatment for breast cancer. In this treatment, the targeted drugs will restrict the growth and spread of cancer cells. The cancer cells of about 1 in 5 women with breast cancer have excessive HER2/neu on their surfaces. These cancers tend to grow and spread more aggressively. A number of drugs including trastuzumab, pertuzumab, ado-trastuzumab emtansine, lapatinib and neratinib have been designed that target this protein (American Cancer Society, 2017b).

The side effects of hormone treatment depend mostly on the type of treatment or the specific drug. The most typical side effects are symptoms of menopause such as night sweats and hot flashes. In addition, tamoxifen can result in vaginal discharge and dryness. Pre-menopausal women that take tamoxifen occasionally will experience menstrual changes in which the menstrual periods can become irregular or even come to a halt. Furthermore, since tamoxifen behaves like oestrogen in the uterus, the risk of uterine sarcoma and endometrial cancer can be surged. Raloxifene can increase the risk of blood clots especially in the legs and lungs and stroke in some subgroups. Ovarian suppression can lead to bone loss, loss of libido, mood swings and depression while aromatase inhibitors raise the risk of angina, heart attack, hypercholesterolemia and heart failure, besides causing bone loss, joint pain, depression and mood swings. In addition, fulvestrant can result in gastrointestinal symptoms, loss of strength and pain (American Cancer Society, 2016; National Cancer Institute, 2017). On top of that, many drugs like fluorouracil-epirubicin-cyclophosphamide, FEC-tamoxifen and cisplatin-methotrexate fluorouracil have been used in combination with the aim of increasing efficacy and reducing side effects but patients still experience phlebitis, fatigue, nausea, alopecia, anaemia, mucositis and myelosuppression together with the well-known long term side effects. Table 2.3 depicts the conventional or approved drugs used in breast cancer chemotherapy alongside their main mechanisms of action and side effects that are most commonly seen (Liao, Apaya and Shyur, 2013).

Table 2.3 Summary of major mechanisms of action and common side effects of chemotherapeutic drugs approved for breast cancers

Chemotherapeutic agent	Mechanism of action	Side effect
Cyclophosphamide	Apoptotic cell death	Pulmonary toxicities, weight gain
Cisplatin	DNA damage, apoptosis	Nephrotoxicity
Doxorubicin	DNA damage	Impaired cognitive function, anaemia
Docetaxel	Mitotic inhibition	Pulmonary toxicities, colitis, diarrhoea
Epirubicin	DNA damage	Nausea, cardiotoxicity
Fluorouracil	Thymidylate synthesise inhibition; DNA synthesis inhibition	Cardiotoxicity, anemia, GI tract toxicity
Gemcitabine	Nucleic acid synthesis inhibition	GI tract toxicity
Methotrexate	Cell cycle arrest	Anemia, weight gain, jaundice, diarrhoea, loss of bone density
Mitomycin	DNA alkylating agent	Myelotoxicity, fatigue, systemic toxicity
Mitoxantrone	Topoisomerase inhibition	Alopecia, systemic toxicity

2.4 Tamoxifen

2.4.1 Chemistry and Pharmacology

Tamoxifen marketed under the name of Nolvadex, emerged from a research programme targeted at developing an antioestrogen oral contraceptive about 50 years ago. Nowadays, interest in the use of tamoxifen as oral contraceptives has mainly been substituted with interest in the use for prevention and treatment of breast cancer. Tamoxifen has intricate pharmacological characteristics and can act as either a pure oestrogen agonist, an antagonist or a partial agonist depending on the target organ and species examined and the endpoint assessed. It has been discovered that it was oestrogenic in the mouse and partial oestrogen agonist in the uterus of the rat. Further