## Chapter 1

## INTRODUCTION

Today cancer remains a primary concern in healthcare. In 2018, there were around 18.1 million new cases of cancer, with 9.6 million deaths, putting cancer into the top second cause of death worldwide (International Agency of Research on Cancer, 2018). This means that one in every five men and one in six women will develop cancer during their lifetime, with one in eight men and one in eleven women die from the disease. Without any breakthrough in cancer therapy, cancer incidence is predicted to increase by 63.4% by 2044 (World Health Organization, 2018). Meanwhile, the research on cancer etiology started back in the early 1700s, where epidemiology study showed that cancer is associated with certain occupations (Higginson, Muir, & Munoz, 1992). Nuns frequently developed breast cancer, while tar distillers developed scrotal cancer (Waldron, 1983; Wright, 1940). This initial observation leads to our current understanding that cancer is initiated by genetic mutation, environmental carcinogens, and lifestyle factors (Blackadar, 2016).

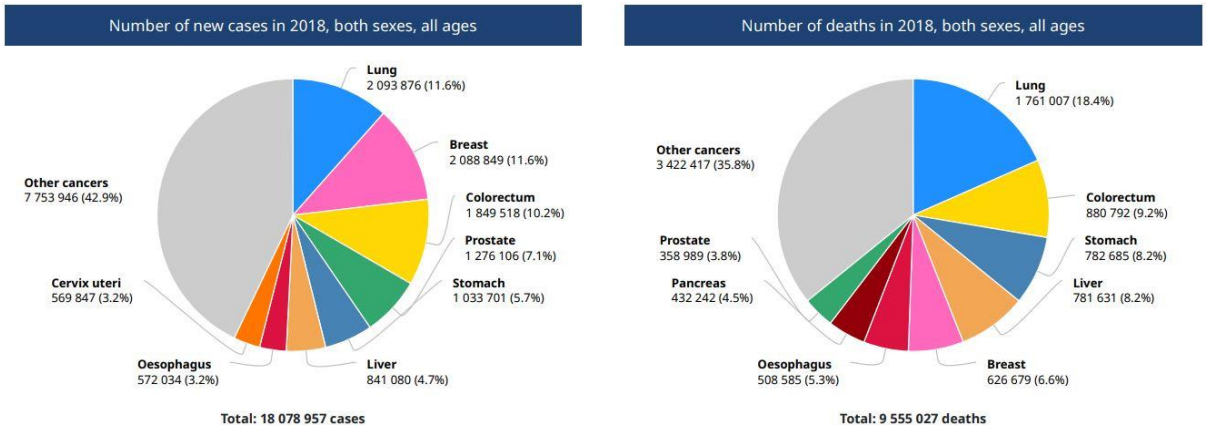


**Figure 1. Cancer incidence and mortality rate in both sexes worldwide 2018.** Breast cancer incidence is the second highest among all cancers, followed with approximately 30% deaths among these patients (International Agency of Research on Cancer, 2018).

Breast cancer is the second most common cancer worldwide (Figure 1). There were approximately 2.1 million new cases of breast cancer in 2018, with 630,000 deaths that year (International Agency of

Research on Cancer, 2018). Despite the fact that more than 90% of breast cancer cases are not metastatic by the time of diagnosis, the high mortality rate is mainly due to recurrence. After the benign breast tumor is surgically removed, it either reoccurs or metastasizes to other parts of our body (Waks & Winer, 2019). The female breast is made up of adipose tissues, mammary glands, and lymphatic glands (Figure 2). Healthy female breast is composed of 12-20 sections of mammary lobes and 20-30 lymph nodes (Ellis & Mahadevan, 2013). Each of this lobe is built up of small lobules. These lobules are connected to the nipple through milk ducts (Hassiotou & Geddes, 2013). Most breast cancers are originated from lobular or ductal cells. Both these lobular and ductal carcinomas are either benign (in situ) or malignant (infiltrating or invasive).

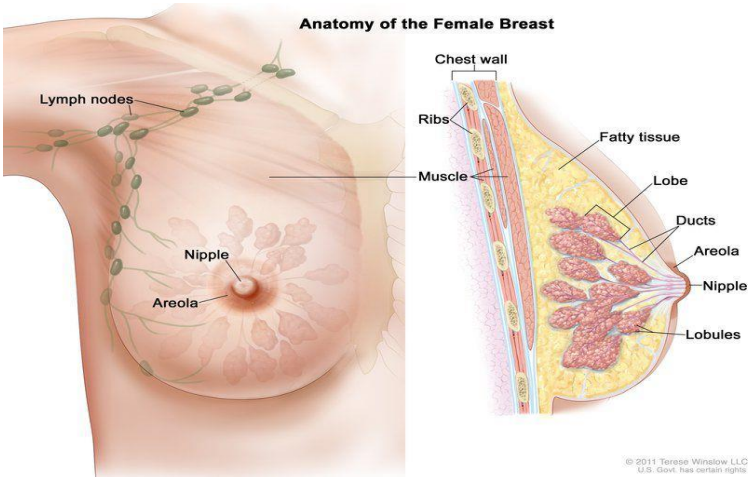


**Figure 2. Breast cancer anatomy.** Female breast is mainly composed of fatty tissue, surrounding the mammary glands and lymphatic vessels. There are milk ducts which connect the lobes to the nipple. Malignancies usually arise from either ductal or lobular cells (Marieb & Hoehn, 2019).

Based on National Comprehensive Cancer Network Guidelines version VIII, breast cancer diagnosis is started with examination of medical history and vital physical condition. The examination is followed by breast imaging; mammograph, USG, or MRI. If the tumor is confirmed, breast biopsy might be conducted to reveal the current status of the tumor. Other additional tests might be conducted to

check if there's any metastasis or other complications. If tumor is diagnosed, depending on its size and position, the patient might undergo mastectomy (removal of breast) or breast-conserving surgery (removal of lump/lumpectomy and radiation). Other procedures that might be conducted are breast-reconstruction surgery, lymph node surgery, radiation therapy, or systemic treatment. When surgery and radiation therapy is not sufficient, systemic treatment is prescribed depending on the stage and aggressiveness of the breast cancer. This treatment may include chemotherapy, hormone therapy, or monoclonal antibody therapy, depending on the subtype of breast cancer.

Histopathologists then differ the breast cancers based on the histologic features, tumor grade, lymph node status, and the presence of certain marker (Holliday & Speirs, 2011). Hormone-receptor positive (HR+) breast cancer is mainly treated with hormone therapies due to its better efficacy and less side effects compared to chemotherapy. Meanwhile, Human-Epidermal-Growth-Factor-2-positive (HER2+) breast cancer, which is HR-, should be treated with systemic therapy. However, this systemic therapy is still not as effective and safe as hormone therapies in HR+ breast cancer (Romond et al., 2005; Slamon et al., 2011). The challenge in HER2+ breast cancer treatment is to predict which pathways drive HER2+ breast cancer progression and to develop drugs that are effective in targeting these pathways.

Currently, there are epigenetic modulators that are being developed or utilized to target various pathways in cancer. These epigenetic modulators target different epigenetic-regulating molecules which control the expression of various oncogenes or tumor suppressor genes. For instance, 3-Deazaneplanocin A or DZNep showed anti-tumor activity in multiple myeloma, leukemia, NKT cell lymphoma, lung cancer, and colon cancer through the inhibition of a histone methyltransferase (HMT); Enhancer *zeste* homolog 2 or EZH2 (Lee & Kim, 2013; Miranda et al., 2009; Sha et al., 2015; Xie et al., 2011; Yan et al., 2013; Zhou et al., 2011). Previously, our group also reported that the anti-tumor activity of DZNep in prostate cancer is mediated by IFN-JAK-STAT pathway. By inhibiting EZH2, DZNep enhanced the expression of various IFN-related genes which sensitize the cancer cells towards IFN-γ-mediated tumor elimination (Wee et al.,

2014). Seeing its effectiveness against prostate cancer, various epigenetic drugs were then tested against breast cancer. RNA microarray was conducted with RNA derived from breast cancer cell line, MCF7, treated with various single and combination treatment of epigenetic drugs. The results showed that combination of I3 (an HMT inhibitor) and Trichostatin-A (TSA; an HDAC inhibitor) 12 of the upregulated genes are IFN-related genes (data not shown). The upregulation by I3-TSA combination is higher compared to all other single and combination treatments, including I3 alone and TSA alone. Since I3 is a DZNep analogue, we hypothesized that its anti-tumor activity of I3-TSA combination treatment is working through a similar pathway as well. We hypothesized that I3-TSA can sensitize breast cancer cells towards IFN-γ by upregulating IFN-related genes.

**Objective**

This study aims to;

1. validate the IFN-related genes upregulation induced by I3-TSA treatment in breast cancer,
2. elucidate the efficacy of I3 and TSA treatment in different breast cancer cell lines,
3. understand the underlying mechanism of action of I3-TSA in breast cancer.