Chapter 1

General introduction

INCIDENCE AND ETIOLOGY

With an estimated 1.67 million new cases diagnosed in 2012, breast cancer is the most common malignancy and the leading cause of cancer related death in women of the western world [1;2]. Currently it is estimated that one in eight women will develop breast cancer at some point in life. However, with a growing aged population, and an increased adoption of cancer-causing behaviors, it is expected that the global burden of (breast) cancer will further increase in the coming decades [3;4].

In 2000, Hanahan and Weinberg proposed that carcinogenesis is embodied in defects of regulatory circuits governing cell proliferation and homeostasis. It was suggested that the comprehensive cancer cell genotypes are a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth [5]. These six biological alterations, induced by genomic instability which a tumor acquires during a multistep development pathway, are also known as 'the hallmarks of cancer' and consist of: 1. sustaining proliferative signaling, 2. evading growth suppression, 3. activating tissue invasion and metastasis, 4. enabling replicative immortality, 5. inducing angiogenesis and 6. resisting cell death. In 2011, after recognition of the importance of tumor microenvironment, Hanahan and Weinberg added two additional hallmarks, namely, reprogramming of energy metabolism and evasion of immune recognition [6].

TREATMENT AND PROGNOSIS

In general, treatment of breast cancer employs a multidisciplinary approach involving surgery, radiation, and systemic treatment. Today, treatment choices are mainly influenced by the tumor, node and metastasis (TNM) classification. The main aim of the TNM classification is to provide an estimation of the prognosis in order to guide therapy choice and create treatment uniformity in oncologic disease [7;8].

Generally, patients with early stage breast cancer undergo primary surgical resection (lumpectomy or mastectomy) of the tumor and regional lymph nodes, with or without radiation therapy. Subsequently, adjuvant systemic treatment may be offered based on patient and tumor characteristics such as tumor size, tumor grade, number of affected lymph nodes, age at diagnosis, co-morbidities, hormone receptor and human epidermal growth factor-2 (HER-2) status as well as patient preference.

Breast cancer mortality rates have been steadily declining since the early 1990's [9]. Survival of breast cancer patients largely depends on disease stage at diagnosis, in which a great inter-stage difference is seen. Currently, a five-year survival rate of 95% is seen in stage I breast cancer, which, regardless of current onco-pathology knowledge and treatment modalities, drastically drops to 18% in stage IV breast cancer patients [10;11].

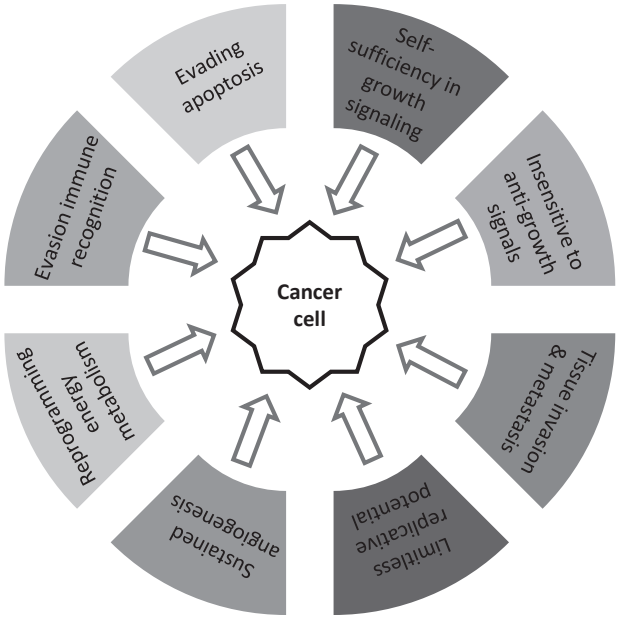

Figure 1: Global overview of the hallmarks of cancer as proposed by Hanahan and Weinberg.

BREAST CANCER IN THE ELDERLY

For most women, increasing age is the primary risk factor for breast cancer. Currently, almost half of the annually diagnosed breast cancer cases arise in women above the age of 65 years [4;9;12]. With the continuously increasing life expectancy and the decreased birth rates of the last decades, a larger proportion of the general population will be categorized as older. Consequently, the number of older women diagnosed with breast cancer will likely rise in the coming years, increasing the burden on society and on already overtaxed health care systems.

Elderly breast cancer patients differ from their younger counterparts in several aspects. For instance, with regard to tumor biology, it has been shown that breast tumors of older patients have lower proliferation rates, which result in slower tumor growth. Furthermore, they are genetically more stable and are more likely to be hormone-sensitive [13]. On the other hand, older patients tend to be diagnosed with larger tumors and increased nodal involvement, which may partly be the result of delayed diagnosis [14;15]. In addition to tumor biology differences, age-related physiological changes might affect metabolism, which may drive oncogenesis and also alter drug functionality and tolerability (Figure 2) [16].

With higher age, women with breast cancer not only have a higher risk of dying from other causes than breast cancer, known as competing mortality, but, compared to younger counterparts, also have an increased risk of breast cancer mortality [17].

Consequently, absolute benefits of anti-cancer therapy may be less clear in this specific subset of breast cancer patients. Furthermore, in contrast to the younger breast cancer patients, breast cancer survival in the older population has not improved in recent years, further increasing the survival gap between young and old breast cancer patients [18].

If the functional status of the older breast cancer patients is not sufficiently taken into account, the result may be both undertreatment (not treated with adjuvant therapy or treated with drugs of insufficient additive value) and over-treatment (cured with solely local therapy or limited adjuvant treatment) of this specific breast cancer population. This could explain the lack of survival gain for older patients, emphasizing the importance of individualized treatment strategies to improve breast cancer care in the older breast cancer population.

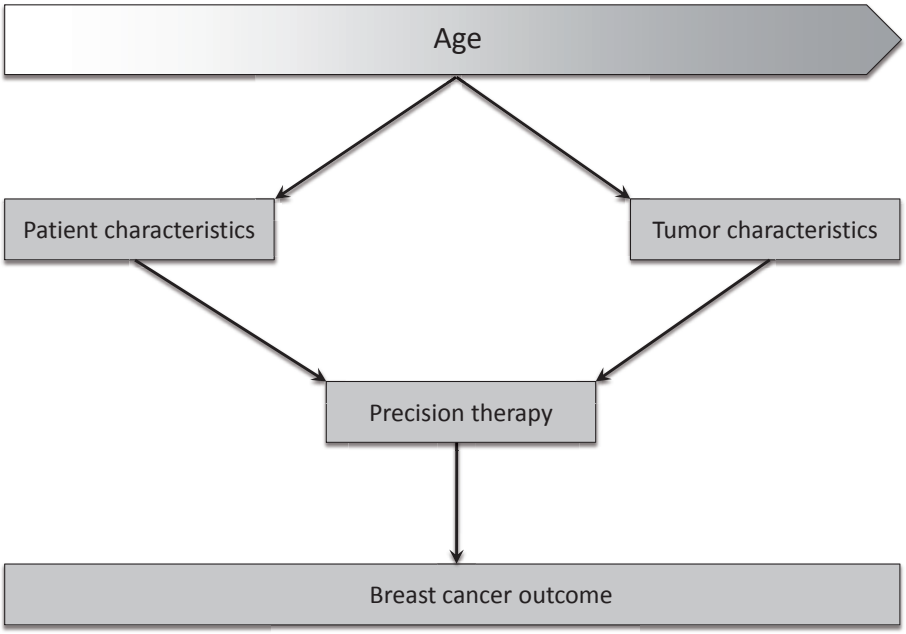

Figure 2: Global overview of the effect of age on patient and tumor characteristics, consequently leading to different treatment modalities with a focus on personalized care, aiming for the best clinical outcome for each patient.

NON-EVIDENCE-BASED MEDICINE IN THE ELDERLY

Despite the high cancer incidence and cancer-related mortality in the elderly [19], our knowledge about aging and its role in oncogenesis, and about optimal treatment for older patients is still far from adequate. The international society of geriatric oncology (SIOG) has established guidelines for breast cancer treatment in the elderly, but confirms that in many areas, solid evidence is lacking [12]. This is mainly due to underrepresentation of older breast cancer patients in clinical trials, due in large part to eligibility criteria

that have excluded the elderly for different reasons [20]. Therefore, current breast cancer treatment guidelines are largely based on studies performed in younger breast cancer patients [12]. However, given the aforementioned differences between older and younger breast cancer patients, guidelines for younger patients are not automatically applicable to elderly breast cancer patients. It is for this reason that the use of currently available online decision making tools, such as Adjuvant! Online, which are mainly based on research-data from studies performed in younger breast cancer patients, which estimate clinical outcome and assist in making treatment choices, should be interpreted with caution for this specific subset of breast cancer patients.

As a result, there is a lack of evidence-based guidelines to inform the most appropriate treatment of breast cancer disease in the older breast cancer population.

One of the major characteristics of the older cancer population treated in everyday clinical practice is the heterogeneity observed among patients of the same calendar age. Consequently, older breast cancer patients often receive less standard therapy compared to their younger counterparts [21-23];older patients presenting with breast cancer have less surgical resection, less frequently receive adjuvant radiation therapy following breast conserving surgical intervention and have an overall higher rate of primary endocrine therapy [21]. These differences in treatment among older and younger patients are largely due to co-morbidities or the declining general health status of the older women which is also associated with an increased risk of treatment-related complications and death [24]. It is for this reason that oncogeriatric breast cancer research is increasingly focusing on individualized, tailored treatment for the older breast cancer patient. The ultimate aim is to find the most appropriate care for each individual in the heterogeneous elderly breast cancer population by predicting who will die *with* (those harboring a low risk of recurrence and a high risk of competing mortality) and who will die *from* (those with a high risk of recurrent disease) breast cancer.

Currently, usage of a comprehensive geriatric assessment (CGA) is widely accepted to guide therapeutic decision making in the elderly breast cancer patient [25]. However, a systematic review published in 2012 showed that frailty screening by the clinician was not sufficient to qualify patients for a CGA [26]. Furthermore, the performance of a CGA is laborious, with high observer bias risk. Therefore, prognostic markers distinguishing between and taking into account the functional status of a patient would be of great value in clinical decision making with regard to breast cancer treatment in the elderly population.

PROGNOSTIC AND PREDICTIVE MARKERS

By definition, a *prognostic* factor is capable of providing information on clinical outcome at the time of diagnosis independent of therapy. Usually these markers are indicators of growth, invasion and metastatic potential. A *predictive* factor is capable of providing information on the likelihood of response to a given therapeutic modality [27;28]. Although often separated, in breast cancer several factors are both prognostic and predictive. As explained above, it is highly desired to have reliable *prognostic* markers that could help select those patients most at risk of recurrence or cancer-related death. In addition, clinically applicable predictive markers would aide in the tailoring of adjuvant therapy by identifying of which treatment a patient would most optimally benefit, thus saving them from unnecessary exposure to potentially toxic and expensive therapies.

To date, tumor stage has had the greatest influence on treatment decisions. However, new insights and advances in the molecular biology of breast cancer have started to influence prognostication and treatment decisions. The cellular and molecular heterogeneity of breast cancer, as well as the large number of genes involved in controlling cell growth, death, and differentiation emphasize the importance of studying multiple genetic and epigenetic alterations in concert. Over the last decades gene expression profiling studies have identified several molecular breast cancer subtypes, also called the intrinsic breast cancer subtypes, with greatly differing prognosis. In short, this subtype shows that estrogen receptor (ER)-positive and ER-negative tumors are fundamentally distinct molecular diseases [29]. There are two predominantly ER-positive intrinsic molecular subtypes (luminal A and luminal B, which carry the best prognosis) and two predominantly ER-negative intrinsic subtypes (HER-2-enriched and basal-like). The intrinsic molecular subtypes are largely distinguished by the expression of genes involved in luminal epithelial differentiation (ER and progesterone receptor (PR) genes), proliferation (Ki67 gene), human epidermal growth factor receptor-2 pathway (HER-2 gene), and basal differentiation [29]. Other promising molecular prognostic assays are the 21-gene Recurrence Score (RS) (Onco*type* DX Breast Cancer Assay (Genomic Health, Redwood City, CA, USA)), the Amsterdam 70-gene profile (Mammaprint (Agendia, Amsterdam, the Netherlands)), and the PAM50 Risk of Recurrence score assay (Prosigna, Nanostring Technologies, Inc., Seattle, USA)) [30-32]. In all breast cancer patients, but especially in the increasingly frail elder patient, predicting the clinical behavior of a tumor through a combination of clinical, pathological and biological characteristics is of great value as it may lead to tailored, optimally beneficial treatment.

AIM OF THIS THESIS

The work presented in this thesis is part of the collaborative FOCUS project (**F**emale breast cancer in the elderly; **O**ptimizing **C**linical guidelines **US**ing clinico-pathological & molecular data), seeking insight into breast cancer disease in the elderly population in order to improve care in this often affected but frequently neglected patient group. As it cannot be expected that clinical trials focusing on older patients with breast cancer will abate the current knowledge-gap in tumor-biology and treatment in the near future, the aim of this thesis is to define normal tissue, breast cancer, and therapeutic sensitivity differences in observational, population-based cohorts consisting of elderly breast cancer patients. The ultimate goal is to improve risk stratification and consequently treatment benefit for the individual patient, paving the way for the clinical introduction of precision medicine, especially in the older breast cancer population.

The FOCUS project consists of four domains; analysis of a large observational cohort of elderly patients; age- specific analyses of clinical trial data; a prospective study investigating patient preferences; and a pathology study aiming to elucidate and unravel the differences and/or similarities in tumor biology of elderly breast cancer patients compared to younger counterparts. The studies presented in this thesis consist of analyses of pathology studies combined with the observational cohort data and clinical trial data.

USED PATIENT COHORTS

JANE cohort

Data from the JANE cohort was used in chapters 2, 3, 4, and 9. The JANE cohort is a population-based cohort consisting of 822 breast cancer patients. JANE is comprised of heterogeneous, non-metastasized, primarily surgically treated breast cancer patients, without a history of previous malignancy, who were treated at the Leiden University Medical Center (LUMC) between 1985 and 1996. Breast tissue was collected from the department of pathology in the LUMC, after which all samples were histologically confirmed malignant according to current pathological standard. All samples were handled in a coded fashion, according to national ethical guidelines: "Code for Proper Secondary Use of Human Tissue" of the Dutch Federation of Medical Scientific Societies. Information on patient and tumor characteristics, treatment, follow-up and outcome were recorded for all patients by medical record review. The main advantage of this cohort is that we were able to collect detailed information of a large number of unselected patients, reflecting the large heterogeneity among the general breast cancer population.

TEAM trial

Data from the Dutch Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial were used in chapters 5 and 7. Originally, the TEAM trial was a randomized, phase 3, multinational, open-label study conducted between January 2001 and January 2006 in postmenopausal women with hormone-receptor positive breast cancer. In short, postmenopausal patients with histologically confirmed breast carcinoma who completed local therapy with curative intent (i.e., without evidence of metastatic disease) and no history of previous malignancy (with a disease-free interval of less than 5 years), were eligible. Overall, 9.766 patients were randomized to receive either exemestane, 25 mg once daily for 5 years, or tamoxifen, 20 mg once daily for 2.5 to 3 years, followed by exemestane, 25 mg once daily for 2 to 2.5 years, for a total of 5 years within 10 weeks of completion of surgery and, if indicated, chemotherapy. Appropriate approvals from the ethical committees and written informed consent from all patients were obtained. Patients were assessed every 3 months during the first year of treatment and at least once a year thereafter. Clinical outcome data was retrieved, and vital status was established by medical record review or through linkage with the municipal population registries. For the studies performed in this thesis, only tumor material from the patients enrolled in the TEAM trial in the Netherlands was available for experimental purposes. A large advantage of using data and material from the TEAM trial, was the structured follow up on recurrence and cause of death, which provided a unique opportunity to study associations between age, tumor characteristics and breast cancer outcomes.

FOCUS cohort

Data of the FOCUS cohort was used in chapters 6, 8, and 10. The FOCUS cohort is a population-based cohort of breast cancer patients aged 65 years or older, who were diagnosed in the geographically defined Comprehensive Cancer Center Region West in the Netherlands, between 1997 and 2004. Overall, 3.672 patients were included. Information on patient characteristics, tumor characteristics, treatment, follow-up and outcome were recorded for all patients. Co-morbidity was defined as presence of co-morbidity at time of diagnosis, and categorized by the 10th edition of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Vital status was established either directly from the patient's medical record or through linkage with the municipal population registries. The main advantage of this cohort is that we were able to collect detailed information and tumor and normal tissue samples of a large number of unselected older patients, reflecting the large heterogeneity among elderly breast cancer patients in the general population.

OUTLINE OF THIS THESIS

Four major topics will be discussed in this thesis; for overview purposes this thesis is therefore subdivided into overarching parts.

Molecular differentiation, immune evasion, and sustaining proliferative signaling and resisting cell death are important mechanisms that cancer cells acquire during tumor development [5;6] and are therefore studied in ***part I*** of this thesis. ***Part II*** discusses the predictive value of the biomarkers HER-2 and the insulin growth factor-1 receptor (IGF1R) in relation with treatment. ***Part III*** investigates the effect of aging on tumor development, and the functional status of the patient. Ultimately in ***part IV***, the use of predictive and prognostic biomarkers in clinical practice, its utility and the road to precision medicine are discussed.

Deregulation of the proliferative and apoptotic signaling pathways are two important hallmarks of tissue homeostasis disturbance, ultimately leading to tumor development [5]. Previous studies have shown contradicting results with respect to the relation of apoptosis or proliferation in tumor specimens and patient outcome in breast cancer [33;34]. As tumor growth is characterized by a fine balance between cellular multiplication and cell death, we hypothesize in **chapter 2**, that the level of imbalance between these two signaling pathways might indicate tumor aggressiveness more accurately than single marker studies.

Over the last two decades, it was shown that the immune system has a substantial effect on tumor development and spread [35]. It is believed that under certain conditions, tumors possess the ability to edit themselves, in order to improve their survival through a selection process, leading to a poorly immunogenic tumor variant which is able to evade immune recognition, consequently leading to tumor progression [36-39]. Research aimed at unraveling the tumor cell mechanisms leading to immune evasion showed multiple potential target points in order to obtain the diminished immune susceptible phenotype; First, down-regulation of classical human leukocyte antigen (HLA) class I expression, which minimizes the level of tumor-associated antigen (TAA) expression on the tumor cell surface, leads to less immune recognition and subsequently less destruction by cytotoxic T-cells (CTL) [40]. Second, expression of non-classical HLA class I molecules, HLA-E and HLA-G, on the tumor cell surface: under normal circumstances HLA-E is found in most tissues that express classical HLA-I and is thought to provide an important 'self-recognition-signal' to the immune system [41]. In contrast, HLA-G is rarely expressed in healthy tissue but is shown to be frequently up-regulated in extravillous trophoblastic cells, where it mediates immunotolerance during pregnancy, and in tumor tissue [42]. Simultaneous expression of both non-classical HLA class I subtypes, HLA-E and HLA-G, has been associated with evasion of natural killer (NK) cell recognition, resulting

in further escape from immune attack [42;43]. A third mechanism is the attraction of immunosuppressive regulatory T-cells (Tregs) into the tumor microenvironment, leading to suppression of CTL activity [44].

Overall, a complex association was seen between these known immune markers, highlighting the need for combined marker analyses [45-47]. Therefore, in **chapter 3** we evaluated the association of these immune markers, separately and combined, with the clinical outcome of the breast cancer patients. In **chapter 4**, we performed the same analysis in breast cancer patients stratified for tumor histology, to investigate whether there is a difference in tumor immune escape between invasive ductal carcinoma and invasive lobular carcinoma. This was of particular interest due to the fact that these two histologically different breast tumors tend to present with different clinical properties. Finally, in **chapter 5** we studied the tumor immune characteristics in relation to clinical outcome in a large, clinical trial controlled hormone receptor-positive (HR+ve) breast cancer cohort, in which the effect of endocrine therapy was investigated, as previous research hinted at a possible immuno-modulatory effect of endocrine therapy [48].

Identification of breast cancer molecular subtypes has proven that breast cancer is a heterogeneous disease, requiring different adjuvant treatment [49-51]. In the older breast cancer population, where a large part of the tumors are HR+ve, have lower proliferation rates and patients have an increased risk of dying of other causes than breast cancer, we investigated the prognostic value of the molecular subtypes in this specific subgroup of breast cancer patients (**chapter 6**).

In ***part II,*** **Chapter 7** of this thesis, the benefit of aromatase inhibiting treatment in high IGF-1R expressing HR+ve breast tumors compared to estrogen receptor-blocking therapy was noted. This effect was committed to the activating capacity of IGF-1R by estrogen and insulin growth factor [52]. This beneficial effect was further enhanced when metformin, a well-known reducer of hepatic glucose production and insulin, due to improvement of the peripheral insulin sensitivity, was added to the breast cancer-related endocrine treatment.

With the dreaded side effects of anti-HER-2 treatment, its use in the already frail elderly population is reluctant. Currently, no literature can be found to support this clinical decision. Furthermore, recent studies show that HER-2-positive breast carcinomas with a PIK3CA mutation are less likely to respond to anthracycline-taxane-based chemotherapy plus HER-2 treatment [53]. Therefore, in **chapter 8** the clinical consequence of HER-2 overexpression on the breast tumor surface of elderly (≥65 years) patients, with or without PIK3CA mutations, and the effect of chemotherapy, was investigated. The aim of this study was to define whether we could identify a subgroup of elderly breast cancer patients who could potentially still benefit from anti-HER-2 treatment, despite the risk of the dreaded side effects.

Still a matter of ongoing debate, and an important question to address, is 'Why does cancer risk increase as we age?' The current attribution that cancer risk increases due to the so-called multi-hit hypothesis, stating that time is necessary for cells to accumulate sufficient genetic mutations to push them over a certain mutagenic threshold and into full-blown carcinogenesis [54;55], fails to explain why cancer risk is greatly reduced by calorie restriction and physical exercise, even in situations where chemical carcinogens would normally evoke a 100% cancer penetrance, and why a high-fat diet and a sedentary lifestyle has the opposite effect [56]. Recent work proposed that it is not simply the time necessary to accumulate sufficient hits that account for the increased rate of cancer with age, but the decline in metabolic homeostasis and gene regulation that occurs normally as we age [55;57]. A hallmark of cancer is a shift away from oxidative phosphorylation (OXPHOS) toward anaerobic glycolysis, to provide cells with sufficient substrates for biomass [57]. This reprogramming, also known as the Warburg-effect [58], is driven by several pathways, of which hypoxia-inducible factor-1 (HIF1α) is an important component [59]. Recent evidence has emerged, from studies performed in *C. Elegans* and mammals [57;60], for an important role of HIF1α in aging, supporting the proposition of a decline in metabolic homeostasis as a driver of aging, which also primes for a carcinogenic environment. ***Part III*** of this thesis will focus on the difference in young and old breast cancer patients with regard to HIF1α targets in the tumor (**chapter 9**) and in normal breast tissue (**chapter 10**), in relation with the functional status of the patient and clinical outcome parameters.

Over the last decades the public health sector witnessed a vast and rapid development of genomic profiling techniques, with the promise of precision medicine as a strong driving force. Prediction of pathway deregulation coupled to molecular target identification using genome-wide approaches may provide an opportunity to guide treatment [61]. ***Part IV***, **Chapter 11** discusses the impact of current clinically approved multi-gene assays such as the Onco*type* DX Breast Cancer Assay (Genomic Health, Redwood City, CA, USA) and the Mammaprint (Agendia, Amsterdam, the Netherlands) on surgery.

Finally, an overall summary and discussion on the content of this thesis are presented in **chapter 12**.

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