Chapter 9

Summary and general discussion

SUMMARY

The research in this thesis has used a well described retrospective breast cancer cohort including all women with non-metastasized breast cancer who were primarily operated in the Leiden University Medical Center between 1985 and 1996. This cohort has been used for all studies, which made comparisons and combinations of various markers possible. Elderly breast cancer patients were considered patients aged 65 years or more, according to the world health organization definition (www.who.int).
The main results found in this thesis were that (1) we found strong independent prognostic effects for biomarkers involved in immunosurveillance and tumor immune escape, especially when accounted for various interactions between these markers and (2) key prognostic biomarkers differed in their distribution and prognostic effect in elderly breast cancer patients compared to their younger counterparts.

Part I: Prognostic biomarkers in the interactions between the host's immune system and breast cancer

In the first part of this thesis we investigated the expression and prognostic effect of various crucial immunological markers and their interactions in breast cancer patients.
In **Chapter 2** frequent down regulation or loss of expression of classical HLA class I and high tumor infiltration of Treg were seen in tumors of breast cancer patients. Prior studies indicate that breast cancer is immunogenic and induces tumor associated antigen (TAA)-specific CTL[1]. Our finding of HLA class I down regulation in more than half of all tumors, which was concordant with results found in previous studies [2-4], therefore implies a common phenomenon in breast cancer of selective outgrow of these cells which were able to escape from immune destruction[5]. The frequent presence of immunosuppressive Treg in the tumor microenvironment supports the hypothesis that tumors may attract these immune-suppressing cells in order to evade attack from effector T cells. These data are strongly suggestive for immune escape mechanisms in breast cancer tumors [5]. Further supporting this was the specific prognostic effects found for HLA class I and Treg among chemotherapy-treated patients. Cyclophosphamide, which was included in all chemotherapy regimens of our studied population, is known to positively influence host immune responses against cancer through selective elimination of Treg [6-8]. Ceasing of Treg, reduces immunosuppressive effects, resulting in an enhanced expansion and function of responding of CTL [6-8]. This restored CTL functioning leads to an increased anti-tumor response and therefore might lead to a better patient outcome. We explain the specific prognostic effect found for HLA class I and Treg among chemotherapy-treated patients by this restored CTL functioning, which logically specifically takes place in patients with Treg presence in the tumor microenvironment before chemotherapy administration and in patients whose tumors

had not lost expression of HLA class I. This hypothesis was further supported by a previous study that found a decline in absolute numbers of tumor infiltrating Treg after preoperative chemotherapy, which was associated with a pathological complete response in combination with presence of CTL infiltration [9]. Additional to the fact that our results strongly support the immunoediting hypothesis and add to the current knowledge of the interactions between breast cancer and the immune system, HLA class I and Treg are one of the few predictive factors for chemotherapy response in breast cancer and these markers could therefore be applied in response prediction to chemotherapy in breast cancer patients [10]. Contrary to some previous reports however, no unfavorable prognostic effect was found for classical HLA class I down regulation or loss [3, 4, 11]. An explanation to this might be the increased susceptibility to NK cell recognition and attack of cells with loss of HLA class I expression. The following stages of in tumor immune escape after classical HLA class I down regulation or loss are therefore focused on the escape of NK cell recognition.

Non-classical HLA class I molecules, HLA-E and HLA-G, play an important role in controlling auto-immune NK cell reactions. Under normal circumstances, expression of the HLA-E molecule is found in most tissues that express classical HLA class I or HLA-G molecules and is thought to provide an important "self-signal" to the immune system by accommodating and presenting peptide fragments from leader sequences of these molecules [12, 13]. HLA-G expression, on the other hand, has very restricted tissue expression and has been mostly found in extravillous trophoblastic cells, where it mediates semi-allograft immunotolerance during pregnancy [14]. Expression of HLA-E and HLA-G on the cell surface can respectively bind with the inhibitory receptors CD94/NKG2A and KIR2DL4/p49 of NK cells, and thereby cause inhibition of their proliferation and cytotoxic effector functions [15, 16].Tumors may acquire or up regulate expression of HLA-E and HLA-G as protective property against immune recognition and elimination by NK cells [12]. HLA-E is regularly expressed in various healthy tissues and correlates with expression of classical HLA class I molecules. This physiological correlation with classical HLA class I molecules has been found to be disturbed in tumors, suggesting that malignant cells which escape T cell immune recognition by down regulation of classical HLA class I expression, may further escape immune recognition by up regulation of HLA-E [17]. In addition, expression of HLA-G protects against "missing self" recognition of NK. Expression of this molecule, which is rarely found in healthy tissues, is frequently observed in pathological conditions such as in tumors [18, 19]. Both HLA-E and HLA-G expression showed an association with a worse clinical outcome in various tumor types [20-27]. In **Chapter 3** we showed that HLA-E and HLA-G expression were of independent statistically significant similar influence on outcome of breast cancer patients with high discriminative power, however, specifically in tumors devoid of classical HLA class I expression. This suggests that specifically in tumors devoid of classical HLA class I expression, up regulation of HLA-E and HLA-G expression counteracts the resulting NK cell susceptibility, leading to immune

escape of tumor cells. Supportive for a specific NK cell inhibition of the non-classical HLA class I molecules, for both HLA-E and HLA-G an inverse correlation was found with NK cell infiltrate in a colorectal cancer and gastric cancer study respectively [28, 29], while other studies demonstrated that overexpression of HLA-E and HLA-G directly inhibited NK-mediated cell lysis [29].

Aside from non-classical HLA class I, the activating receptor NK cell lectin-like receptor gene 2D (NKG2D) ligands have great influence on NK cell recognition of pathological cells. NKG2D ligands bind to the NKG2D receptors on NK cells, NKT cells, $\gamma\delta^+$ T cells and CD8+ T cells and provide a stimulatory activating response[30]. Ligands which bind NKG2D receptors comprise major histocompatibility complex class I chain-related proteins A and B (MIC-AB) and unique long 16 (UL16) binding proteins 1-6 (ULBP1-6)[31, 32]. Expression of these ligands may be induced upon infection and other inducers of cellular stress, such as malignant transformation, and may initiate an immune response by binding to the NKG2D receptors on NK and T cells [33]. In **Chapter 4** we show that NKG2D ligands are frequently high expressed in breast tumors and that a statistically significant association exists between expression levels of these ligands and that this expression influences patient's prognosis. We were able to statistically prove that high expression levels of MIC-AB, ULBP-2, and mostly a combination of high expression of both markers, resulted in a beneficial relapse free period with high discriminative power, comparable to results found in previous studies on colorectal cancer [34, 35]. Altogether, these results indicate a cooperation of NKG2D ligands with each other and further add to the hypothesis that low expression of these ligands is a result of selective pressure by the immune system that results in cancer immune evasion or immunoediting. Additional analyses were performed in our study with two different variables that represented combined number of highly co-expressed ligands and amount of co-expression of all ligands. The results of these analyses revealed no patterns of any cooperative functioning between all ligands, as both variables showed no consistent and significant relationship with clinical outcome of disease. Supported by the results found in previous studies [30, 34-37] this shows that each NKG2D ligand analyzed separately does not show equal effects on clinical outcome, that different ligands show varying prognostic effects in different tumors and that a simple additive effect of all NKG2D ligands cannot be assumed. This indicates the complexity of NKG2D ligands functioning and emphasizes again the importance of interactions between various immune markers.

The above mentioned results show that loss of classical HLA class I, up regulation of classical HLA-E and HLA-G expression, induction of Treg in the tumor microenvironment and low expression of certain NKG2D ligands are frequent events in breast cancer, supporting the hypothesis that breast tumors are capable of evading immune recognition. In addition, they highlight the importance of accounting for these interactions within and between the immune system and breast tumors and therefore studying combinations of markers of immune surveillance together with markers of

tumor immune escape. This lead to the construction of tumor immune subtypes based on tumor susceptibility for cellular immune responses (**Chapter 5**) (Figure 1).

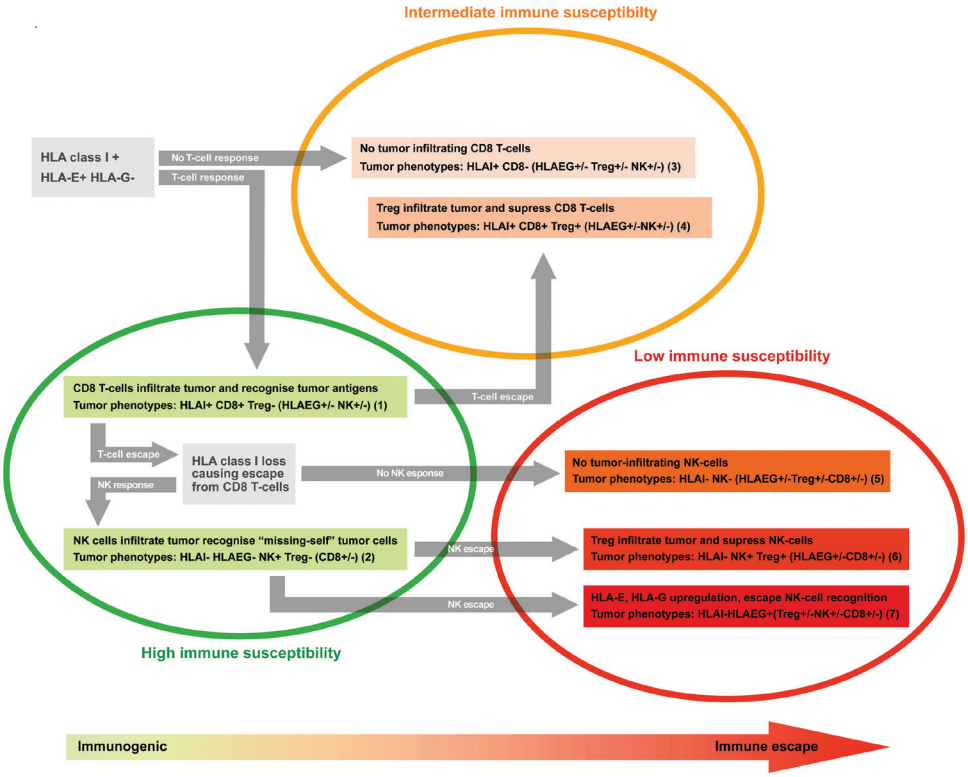

Figure 1 Tumor immune subtypes showing a schematic overview of different stages of immune surveillance and tumor immune escape classified into 7 tumor immune subtypes, graded from (1) to (7) in ascending order from highly immunogenic and therefore high immune susceptibility (green) to high immune escape and low immune susceptibility (red), concerning combinations of CTL infiltration, NK cell infiltration, Treg infiltration, classical HLA class I tumor expression and HLA-EG tumor expression. Tumor immune subtypes were clustered by combining from the original tumor immune subtypes groups as shown in by encircled groups (high immune susceptible) clustered (1) and (2)(green circle), (intermediate immune susceptible) clustered (3) and (4)(orange circle), (low immune susceptible) clustered (5), (6) and (7) (red circle).

Outcome analyses of the immune subtypes revealed strong associations with patient outcome where tumors defined as being highly susceptible to immune system attack showed a favorable outcome for breast cancer patients compared to patients with tumors defined having a low immune susceptible profile. These prognostic effects were shown in this study to be independent of known clinicopathological prognostic parameters and were additionally validated in an independent breast cancer patient cohort confirming the high discriminative power on patient outcome stratification. The study showed that a successful anti-tumor immune response depends not only on the level of expression of a single marker such as classical HLA class I, but on the variety of factors involved

in the multifaceted immune response. Due to this complexity of the balance between immune surveillance and tumor immune escape, it is not a single marker that is able to reflect outcome of the interaction, but a set of key markers. While most studies focus on the effect of single parameters and thereby many contradictory results have been published on immune biomarkers, we showed that it is combinations of these markers which are able to reflect the multifaceted interaction between immune cells and tumor cells, thereby filtering out understating or confounding impacts of immunosurveillance and therefore predict outcome with high stratification capacity. The results found for the tumor immune subtypes are not only concordant with prior evidence on tumor immune biology in breast cancer[38, 39], but additionally join together the conclusions of prior studies by linking single tumor-immune markers to functional tumor-immune interaction.

Part II: Prognostic biomarkers in elderly breast cancer patients

In the second part of this thesis we studied differences in the distribution and effect on outcome of prognostic biomarkers in elderly breast cancer patients.

First, in **Chapter 6** we demonstrated that the presence of ALDH1, a representative marker for cancer stem cells, expression is significantly higher in young breast cancer patients than in elderly patients and demonstrated that ALDH1 expression is an independent risk factor for decreased survival in young breast cancer patients, but not in elderly patients. Cancer stem cells, defined as a small subset of tumor cells with stem cell-like features, including epithelial-to-mesenchymal transition, have the capacity of self-renewal and differentiation; giving rise to a heterogeneous tumor cell population[40]. A biological explanation of the qualitative age-interaction of the prognostic effect of ALDH1 expression might be that of a changing micro-environment in elderly patients, which may result in hampered signal transduction between tumor stem cells and the micro-environment. Moreover, changes in metabolic processes might limit the role of tumor stem cells in elderly patients. Increasing evidence from the field of epigenetics demonstrates that hypermethylation-induced repression of genes required for stem cell differentiation is linearly associated with age.[41] This suggests that, with increasing age, the role of tumor stem cells becomes more limited.

In **Chapter 7**, using gene expression validated IHC surrogates of molecular subtypes, we demonstrated that elderly breast cancer patient tumors show a different distribution of molecular subtypes where were more Luminal A and Luminal B subtypes are found compared to their younger counterparts. These data are concordant to previous studies that showed more ER and/or PR positivity and less overexpression of EGFR, HER2 and ki67 in tumors of elderly breast cancer patients [42-44]. Results also showed no statistically

significant association for molecular subtypes and patient outcome in elderly breast cancer patients in contrary to young breast cancer patients. We sought an explanation to this finding in competing risks of death in elderly breast cancer patients; elderly breast cancer patients compared to their younger counterparts have shown in absolute sense to develop more relapses [45], however proportionally due to higher risk of dying earlier and from other causes they show less breast cancer relapses and breast cancer specific deaths [46-48]. Only about 60% of elderly breast cancer patients die as a consequence of breast cancer, compared to almost 100% of young patients. It is important to realize that this has major implications on the impact and value of prognostic biomarkers in elderly breast cancer patients. Prognostic biomarkers, identifying patients with low versus high risk of breast cancer progression and breast cancer related death will show limited to no prognostic effect in the 40% of elderly patients which have a short-term prognosis due to breast cancer un-related causes, especially in those who are considered frail. These elderly patients are also unlikely to benefit from systemic treatment, since their cause of death will be other than due to breast cancer. Therefore, the clinical value of prognostic biomarkers, which aid at distinguishing between patients who might and might not benefit from systemic treatment, is also limited in this patient population.

As described in the first part of this thesis, the immune system plays an important role in the battle of the host against cancer development and progression. With aging, there are well-known alterations occurring in the immune response affecting both innate and adaptive immunity. It has been suggested that this process of immunosenescence might contribute to cancer development and progression, however this relation is nowadays still poorly understood [49]. In **Chapter 8** we evaluated the distribution and impact on patient outcome of anti-tumor immune response and tumor immune evasion in elderly breast cancer patients compared to their younger counterparts. This showed no differences in number of infiltrating CTL, NK cells or Treg, but a trend towards less classical HLA class I down regulation and statistically significant less HLA-E or HLA-G up regulation of tumors. These differences were also reflected, though not statistically significant, in less "low immune susceptible" tumors in elderly breast cancer patients. These results suggest that tumors in elderly patients have less need to down regulate expression of classical HLA class I and up regulate expression of HLA-EG, because of less immune selective pressure is given by respectively CTL and NK cells. Our results strongly suggest a decreased need for immune escape strategies in higher aged patients compared to their younger counterparts suggesting a left skewed tumor-immune equilibrium. During advancing oncogenesis, tumor immune recognition and attack by the immune system, causes immunoselection of target cancer cells, whom on their turn evolve variants able to resist immune attack. This results in the appearance of new tumor cells variants in order to maintain a state of equilibrium between the immune system and the tumor. The immune system must now exert new powerful selective pressures on the tumor cells, which will evolve again new variants able to resist this immune response, finally leading to tumor immune escape [38, 50]. It therefore is likely

that a comprised immune system, as seen with aging, may lead to a left skewed shift in this tumor-immune equilibrium, where less tumor immune attack correlates with lower stages of tumor immune escape variant phenotypes [49, 49]. Comparable numbers of infiltrating CTL of NK cells between tumors of elderly and young breast cancer patients does not contradict the theory of immunosenescence, since immunosenescence seems to be identified by a disfunctioning in immune recognition and cytotoxicity of CTL or NK cells, rather than by a non-capability of migration and infiltration in inflamed or carcinogeneous environments [49]. Comparable to ALDH1 and molecular subtypes, again no statistically significant association was found between tumor immune subtypes and outcome in elderly breast cancer patients, contrary to their younger counterparts. The fact that elderly breast cancer patients have comparable outcomes independently of the immune susceptibility of tumors is again highly suggestive for a less effective immune system in elderly patients and supports the hypothesis of immunosenescence and its contribution to cancer progression.

Conclusions and future perspectives

Not only did our results provide further evidence supporting an immunoediting hypothesis; we also found various biomarkers with the ability to stratify breast cancer patients according to their predicted prognostic outcome with high discriminative power, especially when accounted for the various interactions between these markers. Differences in distributions and prognostic effect of these tumor immune subtypes in elderly breast cancer patients compared to their younger counterparts, provided further prove for immunoediting and immunosenescence theories. Differences in distributions and prognostic effects of stem cells marker ALDH-1 and molecular subtypes in these elderly breast cancer patients further suggest that it is underlying biological differences in the micro-environmental changes which might influence differences in tumor behavior. We also consider competing risk of death in elderly to be an important factor in fading prognostic effects with increasing age. We finally conclude that biomarkers need validation in elderly breast cancer patients, since results from young patients cannot be simply extrapolated to this patients group.

Immunoediting and immunosenescence hypothesis:

The concept that the immune system can recognize and eliminate primary developing tumors has existed for more than a 100 years [51]. Clearer insight of interactions between the immune system and malignant tumors during the first years of the 21st century gave rise the "cancer immunoediting" hypothesis, characterized by three phases: elimination, equilibrium and escape [38]. Tumor cells can be successfully eradicated by the immune system during the elimination phase. On the other hand, some tumor cells may be capable of escaping from these first line mechanisms of host tumor immune elimination and enter the next phase of cancer immunoediting; equilibrium. During this phase it

is suggested that there is a constant interaction between the tumor, which consists of rapidly mutating and genetically unstable cells, and the immune system. Many tumor cells, susceptible for immune recognition and attack, are eradicated by the immune system, but new tumor cell variants arise which have increased resistance to immune attack. Following these interactions, the tumor constitution is constantly shaped by the immune system. Following the equilibrium phase, tumors can transit to the final escape phase of cancer immunoediting [38]. During this final phase the balance of the battle between the immune system and the malignant tumor becomes favorable to the latter. Following this balance shift, the tumor growth proceeds and tumors become clinically detectable. Further development of significant immune escape mechanisms by the tumor are suggested to result in further tumor progression and spread. Tumor immune escape mechanisms are various and complex, but briefly characterized by: classical HLA class I down regulation, HLA-E and HLA-G up regulation, down regulation of stress molecules NKG2D ligands and attraction of immunosuppressive regulatory T cells in the tumor microenvironment. Evidence supporting the cancer immunoediting theory has been described in various mice studies [38, 52-57]. Human data is limited. Though our results on the distribution and prognostic effect do not prove the existence of the immunoediting theory in human breast cancer, it is strongly supportive for this hypothesis, where we show higher immune susceptible tumors to be associated with a beneficial patient outcome while on the other hand more tumor immune escape variants result in a worse patient prognosis. Importantly, our results highlight the importance of considering various factors in investigating the interplay between tumors and the immune system, accounting for the significant complexity and interactions in these processes.

With increasing age, the immune system declines in functional innate and adaptive immunity leading to a reduced ability to respond to infection and vaccinations [58-61]. There has been increasing evidence that age associated immunosenescence might contribute to cancer development and progression [49]. The phenomenon of immunosenescence and its possible effects on cancer development and progression in humans is still hypothetical and especially our results on less immune escape variants and fading prognostic effect with increasing age in elderly breast cancer patients provides evidence for this.

It appears that unravelling the complex interactions of the immune system in tumor development and progression leads to valuable new insights in the tumor biology. These new insights have a high potential to lead to prognostic or predictive biomarkers and might form the basis of new immunotherapeutical strategies. Future research providing new evidence for the immunoediting and immunosenescence hypotheses is needed.

Tumor immune subtypes as prognostic biomarker in breast cancer:

Many prognostic biomarkers have been identified for breast cancer. Of these, the ASCO guidelines advised the use in clinical practice of urokinases plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1) and gene profiles detected

with multiparameter gene expression assays[62]. The clinical value of microarray-based prognostic tools, like the MammaPrint, a 70-gene expression profile, and Oncotype DX, a 21-gene expression profile is currently being debated[63, 64]. One major critique is that these gene prints were constructed using top-down analyses and were not defined based on a biological rationale. Therefore, it is unclear what tumor types are represented by the various patient risk-groups[65]. One can imagine that such methods contain a high proportion of "noise" due to the involvement of unknown chance in the statistical construction and the optimalisation of the factor during creation and that these prognostic factors almost definitely lose discriminative power due to these reasons. On the other hand, bottom-up analyses, where a biological factor is correlated to patient outcome has a high chance of not showing any prognostic association and therefore a high chance of failure. In addition, it generally shows lower discriminative power than the prior mentioned technique, since it is not optimized on prognostic outcome of patients. However, when a prognostic biomarker is found with this method it provides reliability since it is based on well-founded biological facts. In the first part of this thesis we provided various prognostic biomarkers using bottom-up analyses based on well-founded biological hypotheses on breast cancer immunoediting. The final tumor immune subtypes that were constructed lead to an independent prognostic variable with high discriminative power, comparable to ones found in gene expression prognostic arrays. In addition, independent validation of this biomarker lead to similar results. Moreover, as shown by the predictive effect of HLA class I and Treg on chemotherapy response and as suggested before in literature reports, treatment response is in part regulated by the immune microenvironment, which gives the tumor immune subtypes potential for a predictive effect on existing adjuvant systemic treatment next to potential for development of immunotherapies [66]. Such predictive effects of tumor immune subtypes are not yet investigated and therefore this still remains speculative. A drawback of tumor immune subtypes is the fact that it is still an elaborative test, where many stainings and quantifications need to be performed in order to get the subtype. In addition, it does not show prognostic significance in elderly breast cancer patients. This might in part be due to immunosenescence as explained above and, as explained below, due to competing causes of death in the elderly population.

Biological differences in elderly versus young breast cancer:

Patient, tumor and treatment characteristics have been found to differ considerably between elderly and young breast cancer, [67-69]. This may be indicative for differences in underlying tumor biology and it has indeed often been suggested that elderly breast cancer is a biologically different tumor type of a more indolent character compared to young breast cancer [68-70]. This hypothesis contradicts the fact that increased breast cancer specific mortality is seen with increasing age [45]; elderly breast cancer patients were found to decease more often due to breast cancer regardless of a higher risk of mortality from other causes and independent of known tumor and patient characteristics. In our

studies we did find differences in distributions of key biomarkers, where less aggressive phenotypes with low numbers of ALDH-1, luminal molecular subtypes and less tumor immune escape were seen in elderly breast cancer patients compared to their younger counterparts. However, an explanation to the contradictory findings can be sought in the interactions between tumor and its microenvironment (Figure 2).

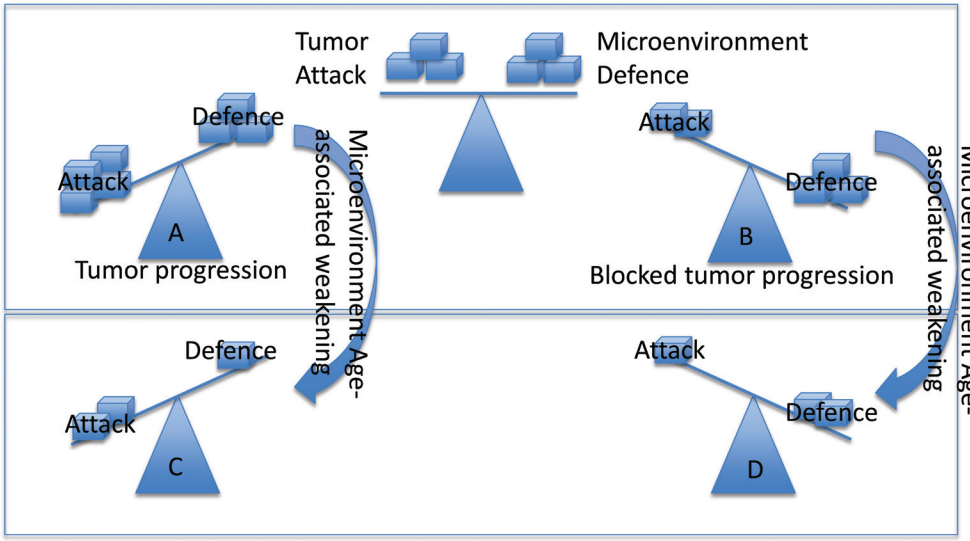

Figure 2 schematically representing the balance between tumor aggressiveness and host microenvironmental defence against the tumor. The number of cubusses represents the weight or strength of the attack, or the progression and invasion of the tumor (left on the balance scale) and the microenvironmental defence against this attack (right on the balance scale). The final equilibrium resulting from this balance results in either: (1) the tumor attack dominates (shown in (A) and (C)), resulting in tumor progression or (2) host microenvironmental defence dominates (shown in (B) and (D)), resulting in a blocked tumor progression. Situations (C) and (D) show the hypothetical situations in elderly patients, where the tumor host microenvironment has weakened compared to the microenivronment in younger patients as shown in (A) and (B).

The outcome of the balance between the tumor and the microenvironment results in changes in patients' outcome, where high tumor aggressiveness should result in more tumor progression and therefore a worse outcome of patients (A), whereas a low aggressive tumor should results in a blocked tumor progression by a well-functioning microenvironment and therefore a beneficial outcome of patients (B). However, usually not the case in young breast cancer patients, but a relevant factor which should be taken into account in elderly breast cancer patients, is the fact that host's defenses against tumors might have deteriorated due to ageing. Herein the suggestion that elderly breast tumors are of a more indolent character and therefore should lead to a favorable outcome compared to younger breast tumors are depicted in Figure 2 by situation B, in case of a host defense comparable to young patients, or D, where the host defense has deteriorated, but the tumor aggressiveness is reduced even more. The combination of a less aggressive tumor with an even more lowered host defense results in tumor progression and therefore a worse patient outcome (C). Less aggressive tumors combined

with the finding of a worsened breast cancer specific outcome with increasing age can be explained by the fact that host defenses deteriorate faster than the aggressiveness of tumors. Changes in the surroundings of the tumor, i.e. the tumor microenvironment, with increasing age seems to be the bridge between the contradictory findings of less aggressive tumor types but worse patient outcomes in elderly breast cancer patients. In this scenario it is not so much the increased malignancy of tumors as it is the weakening of the host defense against cancer, in determining tumor progression and therefore patient outcome. As explained, a changing micro-environment might lead to a hampered signal transduction between tumor stem cells and the micro-environment in elderly breast cancer patients, causing differences in prognostic effects of ALDH1 compared to young breast cancer patients. Moreover, the differences in distribution and prognostic effect for tumor immune subtypes suggest processes like immunosenescence to influence tumorigenesis in elderly breast cancer patients.

Prognostic biomarkers in elderly breast cancer patients:

None of the key biomarkers we investigated showed a statistically significant prognostic effect in elderly breast cancer patients. A combination of underlying differences in tumor biology and behavior, loss of statistical power due to differences in distribution of the biomarker subcategories and competing causes of death in elderly breast cancer patients might explain the fading prognostic significance of biomarker. The fact remains that the clinical value of prognostic biomarkers, which aid at distinguishing between patients who might and might not benefit from adjuvant systemic treatment, is limited in the elderly breast cancer population. Therefore we conclude that validation of biomarkers in elderly is required, since possible differences in the tumor microenvironment and in addition competing causes of death in elderly might results in a significant differing prognostic value and which therefore significantly interferes with patient treatment modalities. Importantly, considering competing causes of death in elderly breast cancer patients, breast cancer prognostic biomarkers can only have a prognostic value in elderly patients whose life expectation will be long enough for the cancer to progress and cause patient death. It is only in these fit enough patients that prognostic biomarkers may show differences in outcome between elderly breast cancer patients and may aid clinical decision making on systemic treatment. In order to improve tailored treatment in elderly with the aid of prognostic biomarkers, the first step would therefore be to identify these fit elderly patients.

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