CHAPTER 7

Summary, general discussion
and future perspectives
SUMMARY

Breast cancer is a heterogeneous disease affecting many women worldwide. In clinical practice, breast cancer is currently divided into subtypes based on immunohistochemical expression of the estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). These subtypes are crucial for treatment choice and outcome. However, even within these subgroups there is great variability in tumor behavior, which has led to further subdivision in for instance triple-negative breast cancer (TNBC). This variability within breast cancer subtypes should presumably have clinical implications for treatment decision-making and the potential of novel therapeutic targets. However, conducting trials to investigate treatment efficacy and validate diagnostics are costly, labor-intensive and time-consuming. Therefore, it takes a long time to translate the knowledge regarding tumor variability within breast cancer subtypes into clinical implications for patients. To speed up this translation, using low-cost tools for hypothesis-generation could be very convenient. The aim of this thesis was to gain novel insights into how to select the best treatment for the right patients and to find potential new therapeutic strategies for difficult to treat subtypes of breast cancer. To this end, we used a large set of publicly available gene expression profiles from patients with nonmetastatic breast cancer.

In chapter 1 a brief introduction to the aim of this thesis is given. In chapters 2-4, we gained more insight into improving patient selection for current systemic therapies and upcoming treatment options in subgroups of breast cancer patients. Clinical trials have shown that extending endocrine therapy beyond 5 years benefits a subset of patients with ER-positive breast cancer. However, thus far it has been unclear how to identify these patients. Gaining insight into the biology of late recurrences could optimize patient selection for extended endocrine therapy. Therefore, in chapter 2, we summarized the current knowledge of late recurrence biology, clinical trials on extended endocrine therapy, and tools for predicting late recurrence and benefit from treatment extension in ER-positive breast cancer. Our review of reports of clinical trials shows that extending 5 years of tamoxifen with 5 years of tamoxifen or aromatase inhibitor (AI) reduces late recurrence risk by 2% to 5%, while results of extending AI-based therapy are inconsistent. Multiple tools predict late recurrence risk, but those at risk do not necessarily benefit from extended endocrine therapy. More insight into the biology of late recurrences could improve patient selection for extended endocrine therapy. We additionally performed a retrospective pooled analysis using 2,231 publicly available mRNA profiles of primary tumors of patients with ER-positive/HER2-negative breast cancer. Our data shows that patients with higher expression of estrogen-responsive genes in the primary tumor who did not receive any systemic treatment had a high recurrence risk persisting beyond 5
years. While 5 years of endocrine therapy reduced the risk for early recurrence in these patients, their risk for late recurrence remained. This suggests that patients with higher expression of estrogen-responsive genes might benefit most from extended endocrine therapy.

Previously, it was thought that breast cancer was not an immunogenic cancer type, in contrast to melanoma or renal cell cancer. Yet recent data suggests that also in case of breast cancer, new options such as immunotherapy can be potentially effective in certain subgroups of patients. Selection of patients, however, remains an unsolved issue. Increased insight in the impact of immune cells on breast cancer for rational therapy decisions is clearly needed. In chapter 3, we performed unbiased in silico analyses on gene expression profiles of 7,270 unrelated tumor samples of non-metastatic breast cancer patients with known clinical follow-up. Many immune cell fractions were associated with highly relevant outcome measures such as treatment response and survival, independent of clinicopathological parameters currently used in the decision-making on systemic therapy. Depending on breast cancer subtype, a higher fraction of regulatory T cells, M0 macrophages and activated mast cells was associated with worse disease outcome, while a higher fraction of γδ T cells and M1 macrophages was associated with a better disease outcome. These results are of particular interest in light of the current clinical developments of immune-modulating therapies in breast cancer. In addition, these insights might support a next step into improving patient selection for systemic therapy.

In chapters 4-6, we explored new potential therapeutic targets for breast cancer and its subtypes. The androgen receptor (AR) is overexpressed in the majority of breast cancers and is considered to be an interesting drug target. We therefore summarized the role of AR in breast cancer based on preclinical and clinical data. Our review shows that response rates to AR-targeted therapies have been relatively low in unselected patient populations. Preclinical and clinical data indicate that patients with ER-negative/AR-positive breast cancer could benefit from treatment with AR antagonists. Studies on the prognostic role of AR in breast cancer have shown that AR expression in ER-positive tumors, measured immunohistochemically, is associated with improved disease outcome. However, in patients with HER2-positive tumors, the role of AR remains unclear. In addition, interpreting the role of AR has been hampered by the broad range of cut-offs and the various antibodies used in immunohistochemical assessment of AR. We therefore also performed a retrospective pooled analysis using 7,270 publicly available mRNA profiles from primary breast tumors. We showed that associations between AR mRNA expression and disease outcome vary between receptor status-based- and intrinsic molecular subtypes. These data indicate that patient selection using additional tumor characteristics could enlarge the role of AR-targeted therapy in breast cancer treatment.
In chapter 5, by reanalyzing >34,000 gene expression profiles we revealed the degree of transcriptional adaptation to CNAs in a genome-wide fashion. The degree of transcriptional adaptation strongly associated with distinct biological processes. We observed that ~10%, ~50%, and ~40% of genes have a low, moderate, and high degree of transcriptional adaptation to CNAs, respectively. Furthermore, we observed strong associations between distinct biological processes and the degree transcriptional adaptation to CNAs. Building on our analysis and findings, we developed a platform-independent method – ‘transcriptional adaptation to CNA profiling’ (TACNA profiling) – that extracts the transcriptional effects of CNAs from gene expression profiles without requiring paired CNA profiles. TACNA profiling was applied to >28,000 patient-derived tumor samples to define the landscape of transcriptional effects of CNAs. The utility of this landscape was demonstrated by the identification of four genes that, when transcriptionally affected by CNAs, are predicted to be involved in tumor immune evasion. Together, these findings provide novel tools to gain insight into how CNAs drive tumor progression via altered gene expression levels. Ultimately, these insights might lead to the discovery of new therapeutic strategies, particularly for copy number-driven malignancies such as breast cancer.

The membrane bound glycoprotein mesothelin (MSLN) is a highly specific tumor marker, which is currently exploited as target for drugs. There are only limited data available on MSLN expression by human tumors. Therefore, in chapter 6 we determined overexpression of MSLN across different tumor types with Functional Genomic mRNA (FGmRNA) profiling of a large cancer database. We found that MSLN is overexpressed in gastrointestinal cancers, gynecological cancers, non-small cell lung cancer, synovial sarcomas, thyroid cancer and renal cell cancer. Subtype analysis in breast cancer revealed MSLN overexpression in 28% of TNBCs and 33% in basal-like tumors. Within TNBC subtypes, MSLN amplification rates were highest in the basal 1-like subtype (42%) and lowest in the luminal androgen receptor subtype (9%). These findings could facilitate prioritization of tumor types for future research to assess the possible clinical benefit of targeting MSLN with immunotoxins or antibody-drug conjugates.

DISCUSSION AND FUTURE PERSPECTIVES

Despite major improvements in breast cancer management over the last decades, a substantial subset of patients experience undertreatment or overtreatment of their disease. In addition, for some subtypes of breast cancer, targeted therapy is still unavailable, leaving systemic chemotherapy as the only treatment option for patients.

Improving patient selection for systemic therapy

One of the major hurdles in improving patient selection for systemic therapy in breast
cancer is tumor variability within breast cancer subtypes. In multiple chapters in this thesis, our results underline the extensive variability present across and within breast cancer subtypes.

In chapter 2, we provide insight into which patients with ER-positive/HER2-negative breast cancer are most likely to benefit from extended endocrine therapy to reduce their risk for late recurrence. In the absence of validated predictive tools, patients who are willing to consider extended endocrine therapy currently rely on assays combing clinical tumor characteristics with molecular data to providing late recurrence risk estimates. However, a subset of patients will develop a late recurrence despite extended endocrine therapy. With dormant tumor cells being the likely underlying cause of late recurrences, these patients will generally not benefit from adjuvant chemotherapeutic regimens which target actively dividing cells. A more promising approach to reduce late recurrence risk might involve the targeting of dormant tumor cells themselves. Current therapies being tested in a preclinical phase are mainly focusing on targeting signaling pathways driving tumor cells to maintain a dormant state. In addition, efforts are currently ongoing in which whole genomic sequencing of paired primary breast tumors and metastases are being analyzed, which ultimately could lead to new insights into the molecular traits that give rise to late recurrences.

In chapter 3, we hypothesize that fractions of multiple specific immune cell subtypes, depending on breast cancer subtype, could be useful as independent predictive and prognostic biomarkers for breast cancer management. As our study was hypothesis-generating, any future use of tumor tissue immune cell type fractions as biomarkers warrants additional tissue validation in well-designed studies. Our data are also of interest in light of the current clinical developments of immunotherapy in breast cancer. Although response to immune checkpoint monotherapy in breast cancer has been modest, combining immune checkpoint inhibition with chemotherapy has yielded better response rates, particularly in patients with triple-negative, PD-L1-positive tumors. These findings have led to drug approval in the U.S. Whether upfront chemo- or radiotherapy can improve immunogenicity and response to immune checkpoint inhibition, is currently being investigated in clinical trials (NCT03417040, NCT02499367). Also, combination strategies with for instance macrophage-targeted treatment are of interest in this setting. Ultimately, the data presented in chapter 3 could contribute to the identification of the subgroup of breast cancer patients that are most likely to benefit from these approaches.

Potentially relevant therapeutic targets in breast cancer subtypes

The awareness is increasing that the relevance of many therapeutic targets in cancer is not confined to merely one tumor type, or subtype. By using a large amount of publicly
available gene expression profiles, it is possible to gain insight into the relevance of several therapeutic targets in cancer. This could contribute to increased insight in how to improve patient selection for novel treatment strategies. The vast amount of publicly available data that could be accessed through data sharing may be used for guiding interventional studies, pharmaceutical companies, and regulatory agencies to improve patient selection for current and upcoming treatments.

In this thesis, we used various approaches to gain insight into potentially relevant therapeutic targets in breast cancer subtypes. In chapter 4 we explored different thresholds for AR-positivity based on mRNA expression to explore the prognostic impact of AR on disease outcome, which could potentially aid the selection of patients for AR-targeted therapy. In chapter 5, we provide a tool – TACNA profiling – which allows for large transcriptomic-wide associations studies to investigate relations between the effects of CNAs on gene expression levels with tumor phenotypes. Ultimately, this could lead to the identification of novel therapeutic targets in different tumor types, including breast cancer. In chapter 6, we predict amplifications of MSLN and highlight its potential relevance in several tumor types including basal-like breast cancer. Phase I trials assessing MSLN-targeted treatment with antibody drug conjugates and chimeric antigen receptor T cells are currently ongoing (NCT02792114, NCT02414269, NCT02580747).

To further improve insights into potentially relevant therapeutic targets in breast cancer, ideally the number of randomized controlled trials, that generate high-throughput sequencing data should be expanded. This is becoming increasingly feasible due to rapidly declining costs and shorter sequencing durations of high-throughput sequencing. Moreover, mining these large amounts of sequencing data and analyzing them in the context of clinical parameters could greatly aid the identification of patients who will benefit from novel or existing treatment strategies. Importantly, in the future considerable effort needs to be invested in transforming these complex, molecular-based insights into straightforward decision-making tools for physicians and their patients.

CONCLUSION

In this thesis, we used a large database of publicly available gene expression profiles as a low-cost tool to gain insight into how to improve patient selection for systemic therapy and to explore potential new therapeutic targets for difficult to treat subtypes of breast cancer. This has led to the generation of multiple hypotheses which require further study in sets of tumors from patients participating in larger prospective, preferably randomized trials. Ultimately, these findings could contribute to the further improvement of patient outcome in early-stage breast cancer.
References
