Summary of conducted research 2016 – 2017, Alexios Matikas

During the academic year 2016-2017, I worked (and continue to do so) at a postdoc appointment at Karolinska Institutet, Jonas Bergh group under the supervision of associate professor Theodoros Foukakis, supported by the European Society for Medical Oncology (ESMO) Georges Mathé translational research scholarship 2016. My work is dedicated on deciphering the predictive role of immune function for chemosensitivity in the various disease settings of breast cancer within a patient subset that is traditionally not associated with eliciting a strong immune response, that of estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) negative disease. With our work we suggest the use of immune based predictive biomarkers and of different ways to grasp the totality and complexity of the immune-host interactions, gene expression (GE) analysis instead of the widely used tumor infiltrating lymphocytes (TILs) enumeration.

The first part of my work concerned the search for predictors for chemosensitivity in patients with metastatic breast cancer. Metastatic biopsies had been obtained from patients enrolled at the academic TEX trial (epirubicin and paclitaxel with or without capecitabine at the first line, NCT01433614). Gene expression analysis using previously published immune modules (Denkert 2015, Sota 2014) and modules related to ER signaling, proliferation, *PIK3CA* and *TP53* mutations demonstrated that only the two immune signatures were correlated with objective responses, % tumor decrease and time to chemotherapy failure in ER+, HER2- and Luminal breast cancer patients, but not in other immunohistochemical or molecular subgroups. An independent gene set enrichment analysis confirmed our results, since only immune related gene sets were enriched in responders and only in the aforementioned subgroups. The quantitative and qualitative analysis of the immune infiltrate using immunocytochemistry and GE-based

methods (MCP-counter) did not outperform the immune modules and neither did a novel signature derived by the differentially expressed genes of the two modules. The findings were summarized in a manuscript which was published at the British Journal of Cancer (Br J Cancer. 2018 Feb 20;118(4):480-488, impact factor: 6.176).

The second part of my work during the previous year at Karolinska Institutet concerned the neoadjuvant setting of breast cancer. Focusing again on the ER+, HER2- subgroup, we analyzed longitudinal tissue samples (at baseline and after 2 cycles of chemotherapy) from patients enrolled at the academic PROMIX trial, a phase 2 study of neoadjuvant epirubicin, docetaxel and bevacizumab (the latter for cycles 3-6; NCT00957125). GE analysis using a previously published immune module (Denkert 2015, also used within the frame of the TEX correlative study), enumeration of TILs by standard hematoxylin and eosin, as well as quantification of specific immune cell populations via immunohistochemistry (FOXP3+ T regulatory lymphocytes, CD163+ M2 subtype macrophages) was performed. Our results demonstrated that baseline immune function as assessed by GE analysis, but not TIL enumeration or immune cell subpopulations, was predictive for chemosensitivity (pathologic complete remission and % decrease in tumor size) at the neoadjuvant setting in patients with ER+, HER2- BC. Furthermore, after short-term exposure to chemotherapy (2 cycles), the predictive power of the immune signature was stronger, while TIL enumeration also predicted the tumor response to chemotherapy. Interestingly, at the multivariate analysis, only GE after 2 cycles of chemotherapy, and not at baseline, remained predictive for tumor response. These results were reported in poster form at the recent ESMO annual meeting in Madrid and were awarded the best poster award for its category. In addition, they were summarized in a manuscript which was published in Oncoimmunology (In press, Impact Factor 7.719).

The possible implications regarding the treatment of breast cancer are obvious: at the metastatic setting, no other predictive factor has been demonstrated to be associated with chemosensitivity in ER+, HER2- patients with advanced disease. Our

uniquely novel study of therapy prediction according to immune GE analysis from metastatic biopsies, if validated in a second study, confirms the relevance of metastatic biopsies, suggests the differential use of cytotoxic chemotherapy in this population by selecting patients according to the underlying tumor – host interactions and offers a basis for patient selection in future trials of combinations of chemotherapy with novel immune checkpoint inhibitors.

On the other hand, at the neoadjuvant setting, our observations are consistent with the hypothesis that priming the anti-tumor immune response after an exposure to antineoplastic therapy may be a critical step towards the induction of immunogenic cancer cell death. This hypothesis generating study, with its dual novelty of comparing different aspects of immune function in a less studied breast cancer subtype and doing so longitudinally after short-term exposure to chemotherapy, serves as a proof of principle for the feasibility of serial biopsies at this setting and the potential predictive value that the evaluation of immune function holds. In addition, another possible application could be the selection of patients enrolled in studies evaluating agents that modulate the immune system, by treating patients that either initially harbor an immunologically “hot” microenvironment or that demonstrate a shift in the microenvironment after short-term exposure to cytotoxic therapy.

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