Single-cell spatial analysis may help predict response to neoadjuvant immunotherapy in triple-negative breast cancer

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A next-generation technology that allows the study of protein expression at the single-cell level and the location of the cells within the tumor microenvironment (TME) was feasible and provided information on the

benefit of adding the immune checkpoint inhibitor atezolizumab (Tecentriq) to chemotherapy as neoadjuvant treatment for patients with early high-risk and locally advanced triple negative breast cancer (TNBC), according to results presented at the San Antonio Breast Cancer Symposium, held December 7-10, 2021.

"We are experiencing a revolution in the technologies available for characterizing the molecular complexity of tumors," said presenter Giampaolo Bianchini, MD, head of the Breast Cancer Group in the Department of Clinical Oncology at IRCCS Ospedale San Raffaele, Milan. "Among these, imaging mass cytometry allows us to collect unprecedented information about the heterogeneity of tumors and their surrounding microenvironment."

Through imaging mass cytometry (IMC), it is possible to simultaneously analyze more than 40 markers in a single tissue section to identify the set of proteins present on individual cells, while accounting for their precise location within the tissue, Bianchini explained. IMC combines the principles of flow cytometry, which analyzes single cells or particles as they flow past single or multiple lasers, and mass spectrometry, which identifies the molecules present in a sample by accurately measuring their mass.

Emerging evidence has shown that TNBC tumors are infiltrated with mononuclear cells and lymphocytes. Combining immune checkpoint inhibition and chemotherapy demonstrated a significant benefit for high-risk TNBC patients in the KEYNOTE-522 trial, leading to the FDA approval of pembrolizumab (Keytruda) in combination with chemotherapy as neoadjuvant therapy in this setting.

"Unfortunately, one size does not fit all patients and it is possible that some of them may have responded to chemotherapy alone, while others who originally benefited from immunotherapy will eventually relapse. In

addition, although immunotherapy is overall well tolerated, some rare but potentially serious immune-related side effects have been reported," commented Bianchini. "For these reasons, biomarkers are urgently needed to help us identify the patients who will benefit the most from the addition of immunotherapy—potentially leading to chemotherapy de-escalation or chemo-free strategies, and those who will do well just with chemotherapy."

Bianchini and colleagues investigated whether IMC could assist in the identification of ideal candidates for this therapeutic approach. They performed IMC analysis in the context of the phase III NeoTRIPaPDL1 trial, which was designed to evaluate the addition of atezolizumab (Tecentriq) to the chemotherapeutics carboplatin and nab-paclitaxel (Abraxane), compared with carboplatin and nab-paclitaxel only, as neoadjuvant therapy in patients with early high-risk and locally advanced TNBC who underwent surgery within six weeks of finishing the treatment.

"We assessed the predictive value of identifying the different phenotypes present within the tumor and the TME through single-cell analysis, and the relevance of the cell-cell interactions," said H Raza Ali, MD, Ph.D., group leader at Cancer Research UK Cambridge Institute and University of Cambridge and a leading contributor to the study. "Physical interactions among cells are required for both immune activation and tumor cell killing, so information on the spatial organization of the tumor tissue is critical when studying the response to immunotherapy."

The investigators successfully analyzed 43 proteins expressed on more than 1 million single cells identified in tissue samples collected through pre-treatment biopsies from 243 patients (representing 86.8 percent of the study population). For each sample, they generated three high-dimensional images that encompassed the tumor, tumor-stroma

interface, and adjacent stroma. They investigated the association of protein expression on tumor and TME cells, cell phenotypes, and the spatial tissue organization with pathological complete response rate (pCR), defined as the absence of invasive cancer cells in tissue samples collected during surgery.

According to the authors, bulk protein expression analysis might deliver limited predictive information because it does not take into account the cell compartment in which each protein is expressed. For instance, assessment of Ki67 on TME cells and HLA-DR on epithelial cells seemed to provide more predictive information than the same biomarkers assessed in the whole tissue specimens.

By allowing for a precise identification of the different cell phenotypes, including cell type and functional state, this approach revealed the potential predictive role of the density of certain cell populations: high density of antigen presenting cells with high expression of PD-L1 and the immunosuppressive molecule IDO and of epithelial cells with high expression of the CD56 neuroendocrine marker was associated with higher pCR in patients who received atezolizumab plus chemotherapy but not in patients who only received chemotherapy.

In addition, high degree of spatial connectivity between epithelial cells and specific TME cells, for instance, CD8+ T cells with granzyme B or PD1 expression and features of exhaustion, correlated with a significant increase in the pCR rate after atezolizumab, whereas lower expression of these markers was associated with similar pCR rates between the atezolizumab arm and the chemotherapy only arm.

"Our results demonstrated that spatial data on the interactions among specific cells in the TME might be very informative about the benefit provided by an immune checkpoint inhibitor such as atezolizumab in addition to chemotherapy," Bianchini commented. "This type of

information can only be provided by technologies that allow us to simultaneously characterize the single cells and their spatial localization with precision."

This approach also confirmed the extreme heterogeneity of TNBC, both in terms of tumor cell composition and in the amount, type, and functional state of the cells present in the TME.

"The predictive information we obtained through IMC complemented what can be derived with commonly used immune biomarkers such as PD-L1 expression or the amount of stromal tumor-infiltrating lymphocytes. In addition, we found that several immune-related gene expression signatures that capture immune cell types and function were less informative than the corresponding biomarkers assessed by IMC," Bianchini said.

The complexity of the IMC technology led to questioning whether it could be applied to large series of tumor samples, such as those collected in routine practice. "In our study, we demonstrated that this disruptive technology can be successfully applied to samples prospectively collected in large clinical trials, paving the way for its broad implementation in cancer research to aid precision immunology," added Bianchini.

According to the authors, all the findings of this study will require independent validation. In addition, a formal adjustment for multiple comparisons was not applied, calling for caution in the interpretation of the results. Finally, the reproducibility and applicability of this technology outside of the research setting must still be investigated.

More information: Abstract: GS1-00. Single-cell spatial analysis by imaging mass cytometry and immunotherapy response in triple-negative breast cancer (TNBC) in the NeoTRIPaPDL1 trial