A SUBSET OF MATURE NEUTROPHILS CONTAINS THE STRONGEST PMN-MDSC ACTIVITY IN BLOOD AND TISSUE OF PATIENTS WITH HEAD AND NECK CANCER

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Background A high neutrophil-to-lymphocyte ratio in the circulation and high frequencies of tumor-associated neutrophils (TAN) in malignant tissue are associated with poor outcome and tumor progression in patients with cancer. It is hypothesized that immuno suppressive neutrophil activity (aka PMN-MDSC activity) contributes to this effect. In addition, this MDSC activity represents a major resistance mechanism in different types of immunotherapy. The exact cellular identity of human PMN-MDSC is still under debate. Improved immunomonitoring and functional characterization of MDSC is needed in order to exploit these cells as novel biomarkers and targets for combination immunotherapy.

Methods In this study, we sought to identify the neutrophil subset that contained the highest T cell suppressive activity. To this end, we purified different subsets of circulating neutrophils by FACS and performed multi-parameter immunofluorescence together with digital pathology on 2-D and 3-D tumor tissue samples.

Results We found that a population of circulating, mature, arginase 1+ neutrophils that co-purified with mononuclear cells in density gradients, most potently suppressed T cell function in multiple in vitro assays. These PMN-MDSC were also superior to monocytes MDSC in T cell suppression. Using a novel technology of tissue whole mount labelling, clearing and imaging we derived 3-D spatial maps of neutrophil – T cell interaction in human tumors. We found that T cells, which were conjugated to arginase I+, myeloperoxidase + TAN, had significantly reduced expression of proliferation and cytotoxicity markers. In patients, frequent conjugation of T cells to those PMN-MDSC was associated with poor prognosis. In contrast to circulating PMN-MDSC, tissue PMN-MDSC expressed high amounts of LOX-1 (oxidized low density lipoprotein receptor 1) and a high intratumoral frequency of LOX-1+ PMN-MDSC was associated with poor survival.

Conclusions We identified and characterized PMN-MDSC activity in human cancer patients. Our findings will facilitate and improve MDSC immunomonitoring and MDSC targeting in combination therapies.

Ethics Approval Use of patient material was approved by the Ethics committee of the Medical Faculty of the University of Duisburg-Essen.

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Figure 1 After activation by antigen-presenting cells in the lymph nodes, viable CD8+ T cells express high levels of phosphatidylserine, which coincides with a highly proliferative and cytotoxic state. As they migrate towards tumour cells in the serous body cavities, they are sequestered by Tim-4+ resident macrophages which impede their anti-tumor cytotoxicity. Tim-4 abrogation can alleviate this sequestration and enhance anti-tumor immunity.

PShigh CD8+ T cells expressed genes associated with cytotoxicity, activation/exhaustion, and proliferation, and mediated greater cytotoxicity. Mechanistic studies revealed that Tim-4 mediates sequestration of PShigh CD8+ T cells by macrophages which subsequently impede CD8+ T cell cytotoxicity of tumor cells.

Conclusions We demonstrate that Tim-4+ resident macrophages impair anti-tumor CD8+ T cell immunity in the serous body cavities and Tim-4 blockade represents a novel therapeutic strategy to overcome resistance to immune checkpoint blockade (figure 1).

Ethics Approval The retrospective clinical analysis was approved by Memorial Sloan Kettering Cancer Center IRB #16-1366. The human biospecimen analyses were approved by Memorial Sloan Kettering Cancer Center IRB #06-107 and 14-091.

REFERENCES


Abstract 525 Figure 1 KIT mutation and MS4A1/CD20 expression - overall survival
Low MS4A1/CD20 expression with concurrent KIT mutation is associated with poor overall survival

Background

Melanoma has high response rate to immune checkpoint inhibitors. KIT, a driver mutation in melanoma seen in ~10% of the patients. However, the role of the KIT mutation in immune microenvironment of melanoma is not well established yet. Here we report a case with KIT mutation and a likely impaired B cell activity with poor response to Immune-checkpoint inhibitor therapy (ICI). We also describe the overall survival of melanoma depending on KIT mutation and MS4A1/CD20 expression, which encodes CD20, B-lymphocyte-specific membrane protein that plays a role in the development, differentiation, and activation of B-lymphocytes.

Methods

A case with poor response to ICI with KIT mutation and monoclonal B cell lymphocytosis was identified. Clinical and molecular characteristics of melanoma in TCGA was analyzed using cBioPortal web page. TCGA data were analyzed to determine KIT mutation status and MS4A1/CD20 expression in melanoma cohort. Samples in the upper 33 percentile of MS4A1 expression were identified as high expression, and the lower 33 percentile were identified as low expression. Mantel-Cox method was used for overall survival (OS) comparison between the cohorts.

Results

69-year-old male with initial diagnosis of stage III-B melanoma of the left thumb with local recurrence in the resection site and then lung metastases. Patient was then started on nivolumab/ipilimumab with rapid progression on immunotherapy. He was found to have KIT mutation (exon 13K642EMT), and started on imatinib, but he continued to have progression. He was switched to temozolomide with no response. He also had history of leukopenia, pre-dating the metastatic melanoma and was diagnosed with monoclonal B cell lymphocytosis. With the hypothesis that the patient’s dysfunctional B cells may have impaired ability of ICI and poor prognosis; we analyzed TCGA database for KIT mutation and MS4A1/CD20 expression - which was used as marker for B

Abstract 525 Figure 1 KIT mutation and MS4A1/CD20 expression - overall survival

Low MS4A1/CD20 expression with concurrent KIT mutation is associated with poor overall survival