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The ratio of peripheral regulatory T cells to Lox-1^{+} PMN-MDSC predicts the early response to anti-PD-1 therapy in non-small cell lung cancer patients

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To the Editor:

Immune checkpoint inhibitors (ICI) have emerged as a promising treatment modality in non-small cell lung cancer (NSCLC) patients. However, ICI monotherapy has a relatively low response rate (approximately 20-50% in NSCLC patients) (1), and as a consequence, the development of predictive biomarkers of response are important to inform clinical decisions regarding ICI use. While PD-L1 expression on tumor cells is currently used as a predictive determinant for anti-PD-1 therapy responses, the accuracy of this test is relatively poor. Therefore, better predictive biomarkers for anti-PD-1 therapy in NSCLC patients are needed.

Myeloid derived suppressor cells (MDSC) and regulatory T cells (T_{reg}) play crucial immune suppressive roles in cancer patients (2). In addition to promoting tumor growth, the suppressive actions of MDSC and T_{reg} hinders the efficacy of cancer immunotherapy. Therefore, we hypothesized that frequency of immune suppressive cellular subset might be correlated with the response to anti-PD1 therapy. The fact that MDSC and T_{reg} can be easily detected and affordably quantified in the peripheral blood of NSCLC patients make them excellent candidates for predictive biomarkers to ICI.

To identify correlations between suppressive cell subsets and the response to anti-PD-1 therapy, we longitudinally analyzed the frequencies of immune suppressive cells, including T_{reg} and MDSC, in the blood of an exploratory cohort (n=34) of pre- and post-nivolumab in NSCLC patients. The data were validated in an independent cohort of NSCLC patients (n=29). We prospectively enrolled NSCLC patients who had failed platinum-based chemotherapy.

Patients were categorized as responders (complete response, partial response or stable disease for more than 6 months) or non-responders and blood samples were collected both pre- and post-1st nivolumab (3mg/kg every 2 weeks). The frequency of T_{reg} and polymorphonuclear (PMN)-MDSC, which are the dominant population of MDSC in NSCLC patients, were quantified using flow cytometry. Expression of the lectin-type oxidized LDL receptor-1 (Lox-1) in PMN-MDSC was additionally analyzed to distinguish PMN-MDSC from neutrophils (3). At baseline, the percentage of T_{reg} was higher in responders than in non-responders while there was no significant difference in the frequency of Lox-1^{+} PMN-MDSC (not shown). After the 1st treatment, the median percentage of T_{reg} was also higher in the responders than non-responders, whereas the median

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percentage of Lox-1^{+} PMN-MDSC was significantly lower in responders than in non-responders (not shown). Interestingly, an inverse correlation was observed between the percentage of T_{reg} and Lox-1^{+} PMN-MDSC (not shown).

To optimize the cut-off value, we evaluated the ratio of T_{reg} to Lox-1^{+}PMN-MDSC, which was defined as TMR. The difference between the TMR of responders and non-responders was significantly greater than either frequencies of T_{reg} or Lox-1+PMN-MDSC alone, with an area under the receiver operator characteristic (ROC) curve (AUC) of 87% (not shown). Patients with a TMR ≥ 0.39 (the cut-off value that optimized both sensitivity and specificity) had a likelihood ratio for being a responder of 3.17. The sensitivity and specificity of using this cut-off to predict response was 87.5% (95%CI 63.5-98.5%) and 72.2% (95%CI 46.5-90.3%), respectively. Moreover, patients with a TMR ≥ 0.39 had significantly longer median progression-free survival (PFS; 103 *vs*. 35 days; *P*=0.0079) than those with a TMR < 0.39 (Fig. 1A). The validation cohort confirmed that the TMR predicts the treatment outcome of nivolumab in NSCLC patients (Fig. 1B). To further evaluate the performance of the TMR as a predictive marker for anti-PD-1 therapy, we analyzed all of the data available (n=63). The positive and negative predictive value of the TMR 0.39 were 69.2% and 91.8%, respectively.

To confirm the presence of PMN-MDSC, Lox-1 expression and reactive oxygen species (ROS) production were analyzed in PMN-MDSC compared to neutrophils. Lox-1 was previously reported as a PMN-MDSC specific marker expressed on immune suppressive PMN-MDSC but not on neutrophils (3). High ROS production is one of the immune suppression mechanisms in PMN-MDSC (4). In our patients, Lox-1 was prominently expressed on PMN-MDSC compared to neutrophils (Fig. 2A). Moreover, the level of ROS production was significantly higher in PMN-MDSC than in neutrophils (Fig. 2A), indicating that the PMN-MDSC in our study showed common features of PMN-MDSC which can be distinguished from neutrophils.

To elucidate the mechanism involved in the response to nivolumab, we analyzed 40 cytokines and chemokines in plasma from patients after the 1st nivolumab by multiplex immunoassay. The levels of three chemokines (CXCL2, CCL23, and CX3CL1) and HMGB1 were significantly higher in non-responders than in responders (Fig. 2B). CXCL2, CCL23, and CX3CL1 were reported to promote the recruitment of MDSC (5-7) and HMGB1 is also involved on MDSC differentiation (8).

MDSC accumulation is linked to a poor prognosis in NSCLC patients (9), but their specific role in anti-PD1 therapy has not been studied. Our data demonstrate that factors involved in MDSC proliferation and recruitment are markedly higher in non-responders than in responders after anti-PD1 therapy, which might impair the efficacy of anti-PD-1 therapy. Lox-1$^+$ PMN-MDSC numbers increased to anti-PD-1 therapy in non-responders suggesting that Lox-1$^+$ PMN-MDSC are a specific subset of MDSC with immunosuppressive function in NSCLC patients.

Interestingly, the frequency of peripheral T_{reg} was significantly higher in responders than in non-responders. Presence of T_{reg} in tumors has been associated with poor survival, but recent report had demonstrated that higher frequencies of circulating T_{reg} are associated with a favorable response to anti-PD-1 therapy (10). In addition, we observed that tumor T_{reg} numbers are inversely correlated with peripheral T_{reg} numbers in the mouse tumor model (not shown). These data support our observation of high T_{reg} numbers in peripheral blood associated with a favorable response to anti-PD1 therapy.

In conclusion, the ratio of T_{reg} to Lox-1$^+$PMN-MDSC in blood after the 1st nivolumab predicts the early response in NSCLC patients, and may provide clinicians with vital information on whether to continue or stop further anti-PD-1 therapy.

Figure Legends

Figure 1. Quantifying of peripheral immune suppressive cells after 1st anti-PD-1 therapy in NSCLC patients. Comparison of T_{reg} to Lox-1$^+$ PMN-MDSC ratio between non-responder and responder and progression free survival (PFS) cut-off value 0.39 of T_{reg} to Lox-1$^+$ PMN-MDSC ratio in the exploration cohort (A) and validation cohort (B). PFS was measured from the first day of anti-PD-1 therapy to tumor progression or death. Patients were censored on June 5th, 2018, if alive and progression-free. NR: not reache.

Figure 2. Characterization of PMN-MDSC in NSCLC patients. (A) Comparison of Lox-1 level and reactive oxygen species (ROS) production between PMN-MDSC and neutrophil in peripheral blood of NSCLC patients. PMN-MDSC was isolated from the peripheral blood mononuclear cells and neutrophils isolated from the pellet after density gradient centrifugation of blood. ROS

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production was measured using the oxidation-sensitive dye DCFDA (Dichlorodihydrofluorescein diacetate). (B) Analysis of chemokine and HMGB1 in the plasma of NSCLC patients after the 1st therapy. *p<0.05, **p<0.01 (two-tailed Student's t-test).

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Figure 1

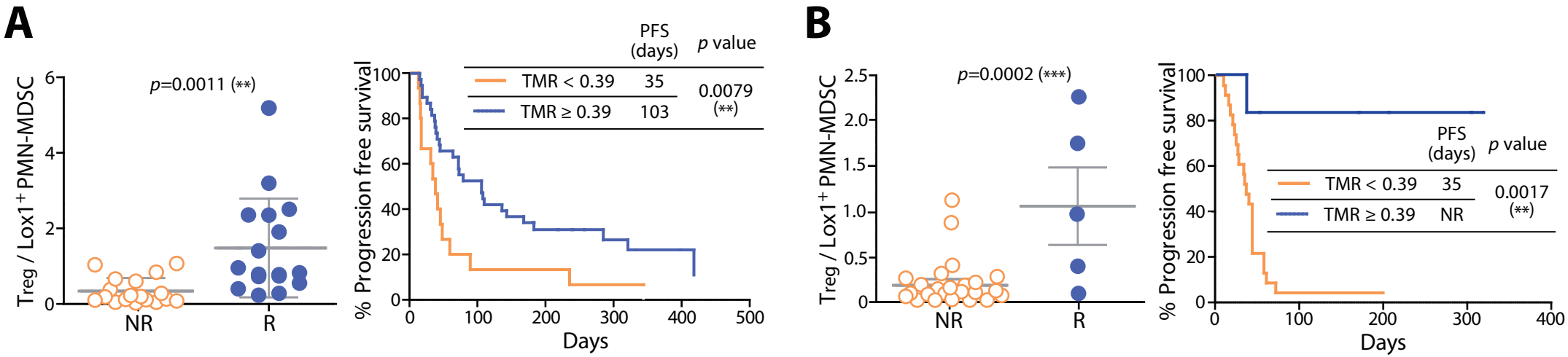

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Figure 2