Evaluating differences in lymphocyte subsets and gene expression profile in anti-PD1 responders and non-responders

By Le Min

In clinical trials, monoclonal antibodies against immune checkpoint proteins have demonstrated promising and durable anti-cancer effects. In 2014, the FDA approved pembrolizumab and nivolumab, antibodies against PD1, for the treatment of advanced melanoma. In addition, positive responses to immune checkpoint inhibition therapies have been documented in numerous advanced malignancies.

In human studies, CTLA-4 blockade induces a proliferative signature, predominantly in a subset of transitional memory T cells, whereas PD-1 blockade instead leads to changes in genes implicated in cytolysis and NK-cell function. There have been no studies to compare such changes between immune checkpoint blockade responders and non-responders. Unveiling the changes in lymphocyte subsets and gene expression profiling in lymphocytes between responders and non-responders will provide an in-depth understanding of the differential responses to these novel immunotherapies. The overarching hypothesis underlying this project is that the peripheral lymphocyte subsets and cancer immune related gene expression profiles are distinct in anti-PD1 responders and non-responders. Since only one third of patients respond to immune checkpoint blockade therapy, the identification of responders by signature profiles of lymphocyte subsets and immune-related gene expression profiling will offer a precision medicine approach to target these therapies to the patients who will achieve the greatest benefit, while avoiding unnecessary, expensive treatments to non-responders and saving time to implement alternative treatments for these subgroups.

To characterize the unique profiles of lymphocyte subsets and the immune gene expression profiles in responders and non-responders, patients with advanced malignancies who are scheduled to receive anti-PD1 at the Dana-Farber Cancer Institute (DFCI) will be enrolled in this study. We have established a strong collaborative relationship with physicians at DFCI who treat patients with immune checkpoint blockade therapy, which will enable us to have sufficient patient enrollment to complete this project. IRB approval for the project will be obtained, and informed consent will be obtained from all patients prior to enrollment. Blood samples will be collected prior to treatment and 1, 8, and 24 weeks after the first treatment. The rationale for this proposed timing of blood collection is to look at the immediate response, at the time of peak adverse events (8 weeks) and after tumor response can be fully assessed to identify responders and non-responders (24 weeks). The criteria for responders and non-responders will follow Response Evaluation Criteria in Solid Tumors (RECIST) protocol. Lymphocyte subsets and
cancer immune-related gene expression profiles will be measured. We will use a BD cell analyzer (BD FACSCalibur™) to study lymphocyte subsets. Prior to performing flow cytometry, the collected blood samples will be processed by the BD FACS™ Lyse Wash Assistant. We will use the BD™ Human Regulatory T-lymphocyte separation set and BD IMag™ Human B lymphocyte, T lymphocyte, CD8, CD4, monocyte, and NK cell enrichment sets to separate and enrich lymphocyte subsets, monocytes and NK cells. The cancer immune related gene expression panel in isolated lymphocytes and subsets of lymphocytes, monocytes and NK cells will be evaluated using the Nanostring nCounter PanCancer immune profiling panel.

The results of the baseline profiles and the changes in lymphocyte subsets and gene profiles after the first treatment may reveal significant differences between responders and non-responders, which may provide guidance to identify the responders and non-responders prior to or shortly after the initiation of the immunotherapy. As a secondary goal, by correlating the findings with the occurrence of autoimmune adverse events, the results of this study may also have applications to advance our understanding of immune-related gene profiles in autoimmunity.