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EDITORIAL – TRANSLATIONAL RESEARCH

Increased Lymphocyte Infiltration in NSCLC Neoadjuvant Chemo-Immunotherapy Non-responders: A Biomarker of T-Cell Dysfunction and Prognosis?

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After proven efficacy in the advanced and metastatic setting in non-small-cell lung cancer (NSCLC), the use of immune checkpoint inhibitors that target the programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) pathway is rapidly becoming standard-of-care in the perioperative setting for locally advanced patients. The Checkmate-816 trial changed the treatment paradigm for stage II and stage III disease by demonstrating improvements in complete pathologic response rates and improved event-free survival rates at 24 months among patients treated with 3 cycles of neoadjuvant nivolumab plus chemotherapy versus chemotherapy alone prior to surgical resection.[1]

PD-L1 expression and tumor mutational burden (TMB) are commonly used, but imperfect biomarkers, to predict clinical response to anti-PD therapy in NSCLC.[2–4] While increased PD-L1 protein expression in tumors correlates with higher overall response rate,[5,6] fewer than half of advanced NSCLC patients with PD-L1 levels ≥ 50% will have even a partial response to pembrolizumab, and objective responses are observed in patients with PD-L1 negative tumors.[6,7] Better biomarkers to accurately identify patients who will benefit from PD-1 pathway blockade, and better understanding of the mechanisms underlying immunotherapy response and resistance are needed.

In the study "Neoadjuvant Chemo-immunotherapy Increases Tumor Immune Lymphocytes Infiltration in Resectable NSCLC," Shun Lu, Ziming Li, and colleagues, studied pre-treatment biopsies and NSCLC surgical specimens from 32 patients for tumor-infiltrating lymphocyte (TIL) infiltration and the presence of tertiary lymphoid structures (TLS) before and after neoadjuvant chemo-immunotherapy (NCIT) and correlated the immune profile with pathologic immunotherapy response. In pre-treatment biopsy samples, a lack of TILs as measured by CD3+, CD8+/PD-1+, and FOXP3+ on multiplex immunofluorescence was the strongest predictor of non-response to NCIT.[8] This important finding in the neoadjuvant setting is concordant with multiple other studies that have evaluated predictors of response to immunotherapy in advanced NSCLC. In cases where cytotoxic T lymphocytes are absent, even tumors with high PD-L1 expression are unlikely to respond to anti-PD pathway therapy.[9] The degree of cytotoxic TIL infiltration is a promising predictive biomarker, especially when used in combination with PD-L1 expression.[10,11] A meta-analysis of 33 studies of NSCLC patients treated with immune checkpoint inhibitors found that high levels of TILs were associated with better overall response rate to anti-PD therapy and longer progression-free survival and overall survival.[12] Similarly, a study from the Netherlands demonstrated that stromal CD8+ TILs were the strongest predictor for progression-free survival and overall survival in advanced NSCLC patients treated with anti-PD therapy.[13]

Lu et al. then compared the TIL profile following treatment with NCIT and demonstrated that TIL infiltration generally increased post NCIT in both immunotherapy responders and non-responders; however, the frequency in T-cell subpopulations differed between the two patient groups. Responders saw a decrease in FOXP3+ T-cells while non-responders saw an increase in CD8+T/PD-1+ TILs, suggesting that NCIT inhibited the number of regulatory T-cells in responders while increasing CD8+ T-cells in

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non-responders.[8] Improved TIL infiltration and increased TLS within both immunotherapy responders and non-responders raises interesting questions about the function, or dysfunction, of TILs in non-responders. It is likely that the CD8+ TILs populations that expanded in the tumor and stromal environment in NCIT non-responders are dysfunctional.[14]

Dysfunctional or exhausted T-cells are characterized by defective effector function, reduced proliferative capacity, reduced cytokine production, and persistent high expression of inhibitory receptors, including PD-1.[15] There is also evidence for subsets of exhausted T-cells that vary in function and expression of molecular markers. We have described a subset of effector burned-out CD8+ T-cells (Ebo) in NSCLC tumors that correlate with poor response to PD blockade.[14] These Ebo cells are simultaneously highly proliferative and apoptotic, and hyperactive, yet unable to produce IFNγ. Moreover, Ebo cells decreased in response to anti-PD therapy and predict poor response to anti-PD therapy.[14] Perhaps the CD8+/PD-1+ T cells found in post-NCIT tumors in the Lu study,[8] which infiltrate tumors without a response to anti-PD therapy, represent a similar subset of TILs. If non-responder tumors are infiltrated with dysfunctional TILs, is it unlikely that they will respond to additional cycles of adjuvant anti-PD therapy, but perhaps may benefit from therapies that target other co-inhibitory molecules.

What can we learn from these results to appropriately design perioperative immunotherapy treatment regimens that maximize survival benefit in responders and non-responders without overtreating patients? The latest wave of trials, KEYNOTE-671 (phase 3, neoadjuvant and adjuvant pembrolizumab in stages II–IIIB),[16] NADIM II (phase 2, neoadjuvant and adjuvant nivolumab in IIIA and IIIB),[17] and AEGEAN (phase 3, neoadjuvant and adjuvant durvalumab in stages II–IIIB)[18] all explore the use of combined neoadjuvant plus adjuvant immunotherapy in resectable NSCLC. All three trials have demonstrated significant improvements in complete pathologic response rates relative to chemotherapy alone and improvements in event-free survival compared with the placebo group.

However, most patients treated with NCIT do not have a complete pathologic response. In Checkmate-816,[1] KEYNOTE-671,[16] NADIM II,[17] and AEGEAN[18] 76%, 81%, 63%, and 83% respectively, of all NCIT patients have residual tumor; and certain tumors within the non-responder group will fail to respond to PD-1 pathway blockade no matter how many cycles of additional adjuvant therapy are given. In KEYNOTE-671 event-free survival was dramatically different between patients treated with pembrolizumab who experienced a major pathologic response (MPR) (~ 90% at 24 months) compared with those treated with pembrolizumab without a MPR (~ 50% at 24 months).[16] This difference in survival among non-responders is an opportunity to improve outcomes with an individualized treatment strategy after surgery for non-responders. Instead of continuing the same drug, an improved adjuvant treatment regimen might include adding dual immune checkpoint blockade with combined CTLA-4 or a Siglec-15 inhibitor.[19] Data from NEOSTAR suggest that the addition of CTLA-4 blockade with ipilimumab (anti-CTLA-4) to nivolumab (anti-PD-1) in the neoadjuvant setting increased rates of MPR up to 50% over that of nivolumab alone[20,21] with many other treatment combinations in clinical trials.

Another unanswered question is: If a patient does have an MPR to NCIT on the surgical specimen, does giving additional adjuvant treatment improve outcomes? We lack randomized data to show that additional adjuvant immunotherapy has a survival benefit over observation alone. While we cannot make cross-trial comparisons, 24-month event-free survival data between Checkmate-816 (neoadjuvant nivolumab) and KEYNOTE-671 (neoadjuvant and adjuvant pembrolizumab) was essentially the same (63.8% vs. 62.4%) and does not suggest that additional adjuvant immunotherapy improved survival.[1,16] An immune profile of the MPR surgical specimens, like Lu et al.[8] describe, could be used in the future to identify patients with a long-term response to NCIT versus those that would obtain additional benefit from adjuvant immunotherapy, with some early exploratory work already being done in this space.[22]

Important follow-up questions for Lu et al.[8] include following the study population for disease recurrence and overall survival to determine whether the degree of lymphocyte infiltration, as quantified by TILs or TLS on the surgical specimen, correlates with long-term response to therapy and survival, and to look for biomarkers of improved outcomes among responders and non-responders. Non-responders with higher immune-cell infiltration in the surgical specimen may prove to have better long-term outcomes than non-responders with less immune-cell infiltration. Many unanswered questions remain and highlight the need for ongoing investigation in this space.

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