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# Immune checkpoint blockade: timing is everything

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## ABSTRACT

Neoadjuvant immunotherapy effectively uses the in situ tumor as a reservoir of tumor antigens to promote systemic antitumor immunity. Studies indicate that intratumoral responses to immune checkpoint inhibitors (ICIs) are mediated by resident memory T cells cells that are sequestered in tumors and have specificity for a wide range of tumor antigens. ICI treatment produces de novo priming of $CD8^+$ T cells in tumor and in tumor-draining lymph nodes, and can boost the antitumor immune response by blocking inhibitory checkpoint proteins that can turn off T cells within the tumor. Neoadjuvant ICI treatment can enhance both intratumoral and systemic antitumor immunity, including expansion of intratumoral T-cell clones which is strongly associated with pathological treatment response. Recent data have shown high rates of pathological response to neoadjuvant immunotherapy with prolongation of survival compared with adjuvant ICI therapy alone in patients with unresectable or advanced melanoma. These data suggest that removal of the reservoir of tumor-specific T cells in the tumor and draining nodes by surgical resection may remove a significant proportion of the patient's antitumor immunity with the potential to compromise ICI outcomes.

Entry of T cells into tumors appears to be a critical step in the development of an effective antitumor T-cell response that confers protection against recurrence and metastasis. Immune checkpoint inhibitors (ICIs) boost the immune response against cancer cells by blocking certain inhibitory molecules that can turn off T cells within the tumor.[1] As an adjuvant treatment, ICIs have been shown to significantly improve clinical outcomes in patients with melanoma, non-small cell lung cancer (NSCLC), gastroesophageal cancer, urothelial cancer and renal cell carcinoma. Studies have evaluated neoadjuvant immunotherapy in locally advanced solid tumors in an effort to build on the success of ICIs in the adjuvant and/or advanced disease settings. In cancer treatment-naïve patients, neoadjuvant therapy is administered with the goal of downstaging tumors and improving their surgical resectability. Neoadjuvant immunotherapy effectively uses the in situ tumor as a reservoir of tumor antigens to promote systemic antitumor immunity. ICI treatment produces de novo priming of $CD8^+$ T cells in tumor and in tumor-draining lymph nodes, and can boost the antitumor immune response by blocking inhibitory proteins that can turn off T cells within the tumor.[1] Furthermore, neoadjuvant ICI treatment can enhance both intratumoral and systemic antitumor immunity, including expansion of intratumoral T-cell clones which are strongly associated with pathological treatment response.[1] In patients with resectable NSCLC, neoadjuvant programmed cell death protein-1 (PD-1) blockade plus chemotherapy resulted in significantly longer event-free survival and a higher percentage of patients with a pathological complete response than did chemotherapy alone.[2] Moreover, a study in patients with locally unresectable or metastatic melanoma showed that those who received neoadjuvant and adjuvant PD-1 blockade had a statistically significant prolongation of event-free survival compared with those who received adjuvant PD-1 blockade alone.[3] Total exposure to ICI was similar in both groups in the intention-to-treat population and the overall immune-related adverse event rate was similar by study arm. Of note, there were some patients (less than 10%) in the neoadjuvant group who did not undergo surgery due to either a toxic effect or disease progression.[3] These data establish that administration of an ICI prior to surgery has a beneficial impact on patient outcomes.

The adaptive immune response to cancer involves T-cell responses with an inherent capacity for memory of tumor antigens. Studies indicate that intratumoral responses to ICIs are mediated by resident memory T cells ($T_{RM}$) cells that are sequestered in tumors and have specificity for a wide range of tumor antigens.[4] $T_{RM}$ cells have emerged as an essential component of the immune response to cancer. $T_{RM}$ cells are long-lived, non-recirculating cells that are retained in tissue in the steady state.[4 5] Studies indicate that tumor-specific T cells with a $T_{RM}$ phenotype are sequestered in solid tumors and in draining lymph nodes (LN) among patients

with cancer.[1 6] The $T_{RM}$ program can promote adhesion of $CD8^+$ T cells in tumors making them less likely to egress and thereby, able to enhance the local response to ICI treatment.[4] The $T_{RM}$ gene program can also induce T cell-mediated effector functions implicated in the recruitment of other immune cell types.[1] Importantly, $T_{RM}$ cells in the tumor have been shown to be substantially increased by neoadjuvant ICI. $T_{RM}$ cells also have the potential to be mobilized into circulation and then to other locations during ICI treatment.[4] Intratumoral $T_{RM}$ express several inhibitory receptors including PD-1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), and their accumulation is associated with response to ICIs as well as with better patient survival.[5] In patients with oral cancer, $T_{RM}$ were found to be the predominant T-cell population responding to neoadjuvant ICI, shown using pretreatment and post-treatment datasets and integrated T-cell receptor (TCR) sequencing.[1] Following neoadjuvant PD-1 blockade, neoantigen-specific T cells showed transcriptional elevation of $T_{RM}$ markers and inhibitory receptors in patients with melanoma[7] as well as oral and lung cancers.[1 8] Furthermore, $T_{RM}$ responsiveness to neoadjuvant ICI was characterized by gene programs related to activation, cytotoxicity, and effector functions.[1]

While $T_{RM}$ display proliferation signatures and undergo clonal expansion, a substantial fraction of tumor-infiltrating T cells failed to respond to neoadjuvant ICI by clonal expansion suggesting that some T cells in $T_{RM}$ clusters may be in an exhausted state.[1 9] Markers of $T_{RM}$ include CD69 and CD103 of which the latter binds to E-cadherin expressed on epithelial cells and can enable exhausted $CD8^+$ T cells to reside in tumor tissue.[10] ICI treatment is believed to reverse an exhausted and dysfunctional state in T cells caused by chronic antigen exposure and characterized by the expression of inhibitory receptors including PD-1, CTLA4, lymphocyte-activation gene 3 (Lag-3), T-cell immunoglobulin and mucin domain 3 (TIM-3), and T-cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT).[11] Our profiling of intratumoral T cells from patients with melanoma who were durable ICI responders point to heterogenous transcriptional states for $T_{RM}$ cells. One $T_{RM}$ cluster expressed *TOX (thymocyte selection-associated HMG BOX)* along with other markers of exhaustion, but another lacked *TOX* and exhibited high expression of interferon $\Upsilon$ and other effector-associated transcripts.[9] In addition to these inhibitory immune checkpoints, concurrent expression of CD38 with exhaustion markers was detected on $CD8^+$ $T_{RM}$ within hepatocellular carcinoma, suggesting that CD38 can serve as a marker of T-cell exhaustion and as a potential therapeutic target for restoring cytotoxic $CD8^+$ T-cell function.[12] Inhibitory receptors are also upregulated during T-cell activation and are constitutively expressed by tissue-resident T cells, including treatment-responsive cells. ICI treatment in the neoantigen setting affords an opportunity to activate $T_{RM}$ cells residing within tumors to trigger an antitumor response. In patients with melanoma who experienced durable responses to immunotherapy, we found highly expanded, long-persisting $T_{RM}$ clonotypes in tumors with matched $T_{RM}$ counterparts in skin and in blood.[9] These broadly-distributed $T_{RM}$ clonotypes were more likely to be enriched in clusters with transcriptional evidence of function.[9] Among expanded $T_{RM}$ cell populations, multiple factors can influence antitumor T-cell responses that, in turn, influence patient outcomes. $T_{RM}$ cells may amplify the breath of cytotoxic $CD8^+$ T-cell responses through dendritic cells, thereby strengthening antitumor immunity.[13] However, $T_{RM}$ cells can be subject to regulation by regulatory T cells ($T_{regs}$) which may impair their longevity and/or induce a suppressed phenotype.[14] Furthermore, $T_{RM}$ cells can be suppressed by loss of major histocompatibility complex-I expression as an immune escape mechanism that was not reversed by anti-PD-1 treatment.[15] Another issue is the metabolic regulation of $T_{RM}$ cells that enables their persistence. $T_{RM}$ cells interact with tissue cells as part of an immune surveillance network and can encounter different metabolic environments. Instead of using glucose, $T_{RM}$ cells were shown to rely on fatty acid oxidation for their survival, and engage oxidative phosphorylation and glycolysis with the former being dependent on lipids.[16] Unlike circulating memory T cells, $T_{RM}$ cells preferentially acquire fatty acids from their local environment, and studies show that programmed death-ligand 1 (PD-L1) blockade enables $T_{RM}$ cells to compete for fatty acids within the tumor microenvironment.[16]

T-cell populations can be primed against tumor-associated antigens in tumor-draining LNs that can then traffic through the circulation into the tumor. In tumor-containing LNs, TCR clones frequently overlap with those in primary tumor tissues suggesting that enrichment of tumor-reactive T cells in these nodes are a good source of T cells for immunotherapy. In addition to the differentiation of memory precursor cells into mature $T_{RM}$ cells in tissue, evidence suggests that circulating T cells can give rise to $T_{RM}$ cells within the lymphoid compartment. In this regard, $T_{RM}$ cells may egress from peripheral tissues including tumor-draining LNs into circulation and other peripheral sites, and potentially into the tumor.[17] In a mouse model of melanoma where $T_{RM}$ populations were induced by neoadjuvant depletion of $T_{regs}$, $T_{RM}$ cells were shown to dominate in LNs where they afforded long-term protection against melanoma seeding whereas circulating memory T cells prevented melanoma growth in the lungs.[18] Expanded $T_{RM}$ populations were also present in melanoma-involved LNs from patients, and their transcriptional signature predicted better survival.[18] Thus, tumor-specific $T_{RM}$ cells persist in LNs where they can serve to restrict tumor metastasis. While it is yet to be determined if $T_{RM}$ cells can be mobilized from LNs, one could speculate that ICI treatment may also activate these populations. In preclinical models of colon cancer, immune activation was induced by ICI treatment predominantly in tumor-draining LNs whose resection at surgery was shown to abolish ICI-induced tumor regression.[19] Similar consequences from resection of tumor-draining LNs in mouse models were seen in another study in early-stage but not

advanced tumors. However, data also exist to indicate that LN metastasis may attenuate the tumor-specific CD8+ T-cell immune response and exacerbate immune suppression.[20] Given that neoadjuvant ICI treatment in patients with intact tumors can enhance both local and systemic antitumor immunity, surgical removal of the reservoir of tumor-specific T cells in the tumor and potentially in tumor-draining LNs, may compromise the patient's antitumor immunity directed at residual local and metastatic disease.

In summary, neoadjuvant immunotherapy effectively uses the in situ tumor as a reservoir of tumor antigens to promote systemic antitumor immunity and was shown to have a beneficial impact on patient survival.[3] ICI treatment can cause de novo priming of CD8+ T cells in the primary tumor and in tumor-draining LNs. Activation of tumor-specific $T_{RM}$ cells by ICI treatment may promote their egress into circulation to suppress metastasis and improve patient survival. In this manner, neoadjuvant ICI treatment in patients with intact primary tumors can enhance both local and systemic antitumor immunity. Data suggest that removal of the reservoir of tumor-specific T cells in the tumor and draining LNs by initial surgical resection may remove a significant proportion of the patient's antitumor immunity and thereby, compromise patient outcomes. While further study of the contribution of tumor-draining LNs, including those containing the tumor, to immunotherapy response and outcome in patients with solid tumors is awaited, existing data raise concern that initial surgical removal of the tumor prior to immunotherapy is counterproductive.

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