Commentary

The immunogenicity of cancer mutations and implications for T cell therapy

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Comment on: Tran E, Ahmadzadeh M, Lu YC, *et al*. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science 2015;350:1387-90.

Submitted Sep 12, 2016. Accepted for publication Sep 19, 2016.
doi: 10.21037/tcr.2016.10.26
View this article at: http://dx.doi.org/10.21037/tcr.2016.10.26

Cancer cells can accumulate hundreds of mutations during tumor development. Some of these mutations can be recognized as "non-self" by the adaptive immune system and elicit an effective immune response (1,2). Increasing evidence suggests that T cell reactivity to these mutation-derived neoantigens has a critical role in the clinical response to immune checkpoint blockade therapies (3-8). For a mutation to be immunogenic, the protein has to be processed, and the resulting neopeptide must be able to bind to the major histocompatibility complex (MHC) in order to be presented on the cell surface. The neopeptide, once presented on the cell surface, has to be recognized by a T cell, which means that the T cell receptor (TCR) must interact strongly with the neopeptide-MHC complex (9). In a fascinating study, Tran and colleagues (10) examined how many mutations are immunogenic per tumor in nine patients with metastatic gastrointestinal cancers and whether these immunogenic neopeptides could potentially be used for the development of adoptive T cell therapies.

Tran *et al*. performed whole-exome or whole-genome sequencing of the metastatic tumors from the patients to identify putative mutations that might be expressed on the tumor cells. For each patient's mutations, the authors constructed minigenes or long peptides containing the mutation flanked by endogenous sequences (11). These minigenes were then introduced into autologous antigen presenting cells (APCs), which allows for the expression of neopeptides on the patient's HLA molecules. In order to detect T cell reactivity, the authors generated multiple co-cultures of these APCs expressing the tumor mutations and tumor-infiltrating lymphocyte (TIL) cultures from the metastatic lesions of each patient. They found that $CD8^+$ T cells predominantly recognized the mutation-derived neoantigens, and more importantly, that these T cell responses were mutation-specific. Of note, they found a specific immunogenic neoepitope derived from the known cancer driver mutation $KRAS^{G12D}$. This neoepitope was observed to be exclusively presented by the HLA-C*08:02 allele. Since the $KRAS^{G12D}$ driver mutation is usually expressed in pancreatic adenocarcinomas and colorectal cancers, adoptive T cell transfers using this $KRAS^{G12D}$-reactive TCR may have the potential to provide clinical benefit to eligible cancer patients. Importantly, the data from Tran *et al*. suggests that there are indeed shared neoantigens and refute the hypothesis by some that neoantigens are entirely private (2).

Tran and colleagues found that the number of neoepitopes recognized by $CD4^+$ and/or $CD8^+$ T cells varied between 1 and 3 in each patient's tumor, independently of the total number of mutations evaluated from the tumor sample, which ranged from 38 to 264 mutations (10,11). Importantly, it is critical to note that this number is in the absence of immune checkpoint therapy treatment. This number may be much lower than the potential number of mutations presented and potentially immunogenic in the setting of immune checkpoint therapy. Indeed, the number of immunogenic mutations depends on the immune context the measurements are being made. Here, immunogenicity of mutations and the number of mutations that elicit T cell responses clearly depends on the extent to which neoantigen-specific T cells are reinvigorated.

The process of immunogenic neoepitope formation can

be thought of a stochastic process where each occurring mutation in the tumor increases the probability that an immunogenic neoantigen is generated. It seems likely that the proportion of immunogenic mutations is small; however, theoretically, and in the absence of strong selection against mutations that generate neopeptides, the number of immunogenic neoepitopes should depend on the total number of clonal mutations present in the tumor. The findings from Tran *et al*. thus suggest that most "potentially" immunogenic neoepitopes in these patients may have gone undetected (10,12). In a recent study, Strønen *et al*. (13) found that the number of immunogenic neoepitopes from cancer patients recognized by naïve T cells from healthy donors with overlapping HLA haplotypes was higher than the number of immunogenic neoepitopes detected in the tumor-infiltrating lymphocytes from the cancer patients. Together, these two studies indicate that either (I) different immunosuppressive mechanisms may be actively impairing T cell responses against neoantigens; or (II) there is deficiency in T cell priming; or (III) there is strong immune tolerance against most neopeptides expressed in tumor cells.

In order to test this hypothesis, one would need to assess for immunogenicity of the tumor mutations using naïve T cells in the blood of cancer patients (12). Experimental evidence of a higher number of immunogenic neoantigens in a cancer patient's blood in comparison to tumor-infiltrating lymphocytes would indicate the presence of immune suppression mechanisms in the tumor (12). These results would further indicate that the numbers of immunogenic neoantigens that have been currently detected in cancer patients are underestimates.

Importantly, Tran and colleagues also determined the endogenous frequency of the mutation-reactive T cells infiltrating the metastatic tumors lesions. In order to do this, the authors performed deep sequencing of the TCR-Vβ region on all cryopreserved metastatic lesions. The authors found that only 4 out of the 17 identified TCRs reactive to neoantigens were ranked on the top 10 frequent TCRs across all cancer patients. The low ranking in frequency of most neoepitope-reactive TCRs suggests that the tumor microenvironment may also influence poor expansion and infiltration of the T cells (14). However, it is also possible that other TCRs present in the tumor may be reactive to other type of tumor antigens such as overexpressed non-mutated antigens (15).

Finally, in order to evaluate whether the identified patient-specific neoantigen-reactive T cells could be used as adoptive T cell therapy, Tran *et al*. treated four patients with expanded populations of T cells targeting mainly one immunogenic neoepitope expressed in their respective tumors (10,11). Two out of these four patients showed durable responses, with one patient with metastatic cholangiocarcinoma showing tumor regression at 20 months after treatment (11). The other patient had a transient regression of multiple lung metastases (10). It is worth mentioning that the average number of mutations ($\overline{n}$ =36) present in the tumors of the two durable responders was smaller than the average number of mutations ($\overline{n}$ =235) in the patients' tumors with no objective response. Theoretically, in vivo antitumor activity by T cells can be accomplished by targeting one immunogenic neoantigen clonally present in the tumor. Therefore, the use of adoptive T cell transfer alone may be more efficient for the treatment of human cancers with low intratumor heterogeneity. And, it is likely that therapies that overcome immunosuppression such as immune checkpoint blockade and/or vaccination with neoantigen peptides (16) that overcome T cell priming inefficiency may be required to enhance the efficacy of adoptive T cells (17) for the treatment of human cancers with high intratumor heterogeneity.

Overall, the study by Tran *et al*. is fascinating and presents important findings. Several challenges remain. For example, we must understand the dynamic interactions between T cells and tumor cells during immunotherapy. This will allows us to develop and apply the best possible strategy in order to avoid rapid evolution of cancer cells during immunotherapy. Furthermore, cancer mutations and the immune system are different between patients, which means that the process of identifying immunogenic neoantigens and their corresponding reactive TCRs may be complex and tedious. If the future of cancer treatment is immunotherapy, we must fully understand the rules that drive immunogenicity, which will allow us to develop models to efficiently identify immunogenic neoepitopes expressed in a patient's tumor.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Chen Qian (Center for Inflammation & Epigenetics, Houston Methodist Hospital Research Institute, Houston, USA).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2016.10.26). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Chowell D, Chan TA. The immunogenicity of cancer mutations and implications for T cell therapy. Transl Cancer Res 2016;5(Suppl 4):S872-S874. doi: 10.21037/tcr.2016.10.26