Can Molecular Imaging Measure T-cell Activation?

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Successful immunotherapy usually depends on activation of T cells in the tumor microenvironment. However, ascertaining whether T-cell activation has occurred *in vivo* is difficult without invasive tissue sampling. Inducible T-cell costimulator (ICOS) is a specific marker of T-cell activation that can be imaged by radiolabeling an anti-ICOS antibody and performing PET scanning. Hopefully, this agent will be the first of many molecular imaging constructs that can determine whether T-cell activation has occurred and could be used in drug development and clinical trials of cancer immunotherapy.

See related article by Xiao et al., p. 3023

Cancer immunotherapy, comprising an array of drug- and cell-based methods has transformed cancer management. Among the various forms of immunotherapy, immune checkpoint inhibitors have had the greatest clinical impact with the approval of multiple mAbs against such targets as CTLA-4, PD-1, and PDL-1. Checkpoint inhibitors have produced impressive responses in some patients especially in such cancers as melanoma, bladder, and lung, but overall, only a minority of patients respond. The tantalizing results in a few patients contrasted with the disappointing results in the majority have prompted interest in predictive markers including assessments of the tumor microenvironment, mutational burden, gene expression profiling, T-cell repertoire sequencing, and IHC. In contrast to these serum or tissue assays, molecular imaging offers advantages as a predictive marker (1). For instance, there is evidence that *in vitro* and *in vivo* expression of immune checkpoints may differ so that *in vivo* molecular imaging may be more predictive (2). Imaging also offers a whole-body perspective rather than a single sample, thus, revealing the predictable heterogeneity of expression among different sites. Moreover, it localizes and quantifies the volume of disease, potentially enabling focal therapy for nonresponsive sites. Thus, there has been interest in developing molecular imaging agents for immunotherapy.

One limitation of such agents as a predictive marker is that there are a plethora of antibodies and cell therapies now deployed in the clinic that would require an equal number of labeled agents as companion diagnostics. Given the epitope specificity of checkpoint inhibitors, for instance, no one imaging probe could predict for the entire class (1). Nor is it likely that the needed number of agents would be sustainable by the medical market. Finally, it will be hard for clinicians to deny an agent with curative potential to a dying patient based on a molecular imaging study. There is no existing precedent for this.

An alternative approach to the problem is to evaluate the effect of immunotherapy, rather than try to predict it. The ultimate goal of most immunotherapies is to activate tumor-specific effector T cells. Thus, a method that detected T-cell activation could find use across a broad range of immunotherapies, albeit after their administration. The phenomenon of pseudoprogression, wherein exuberant immune reactions lead to early enlargement of the tumors simulating tumor progression, is one potential application of such an agent.

Resting T cells can be identified by their characteristic-specific cell surface markers such as CD4, CD8, and FOXP3, which is the basis for several targeted imaging agents (3), however, these markers are present whether or not the T cell is activated. Thus, accumulation of radioactivity could represent a nonspecific inflammatory response instead of a specific antitumor response. Markers uniquely associated with T-cell activation are unusual (4). Activated T cells express granzyme beta, IFNγ, and CXCL9/10, which are potential targets but they tend to be transient, diffusible, and are located in the extracellular matrix rather than on the T cell. OX40, a T-cell surface costimulatory receptor, is a promising marker because it is only expressed upon antigen-specific recognition (5). It can reveal T-cell activation in tumor draining lymph nodes but is generally limited to CD4 cells, although it is found on other cell types as well. In their article, Xiao and colleagues in this issue present yet another target of activated T cells, inducible T-cell costimulator (ICOS), also known as CD278 (6). ICOS mediates immune response across multiple therapy strategies and is primarily expressed on activated cytotoxic T cells, both CD4 and CD8 T cells, memory T cells, and regulatory T cells. Ligation of ICOS and its ligand, ICOS-L, expressed on B cells, macrophages, and dendritic cells, initiates the ICOS pathway and triggers a series of intermediates, which regulate T-cell proliferation and survival, as well as secretion of IL4, IL10, and IFN (7). OX40 and ICOS cooperate in a nonredundant manner to maximize and sustain Th cell immune responses.

In their article, Xiao and colleagues radiolabeled an anti-ICOS antibody with Zirconium-89 (^{89}Zr), a positron-emitting radioisotope with a 3-day half-life. The relatively slow clearance of the antibody is therefore matched with a positron emitter of comparable physical half-life. This compound, ^{89}Zr-DFO-ICOS mAb, can noninvasively quantify activated CD4 and CD8 T-cell responses in the rodent Lewis lung cancer model. This agent proved successful in monitoring T-cell activation after several immune therapies.

Because it is based on PET, this method is inherently more quantitative than most imaging methods. In fact, in their study, the authors compared the effectiveness of two different treatments (antiPD-1 and STING) with regard to the uptake of ^{89}Zr-DFO-ICOS mAb, reflecting differing levels of T-cell activation. From a drug development perspective, being able to compare the degree of T-cell activation in two different treatments *in vivo* might be helpful in choosing drug candidates. Of course, the normal caveats apply that there are only a limited number of syngeneic mouse models in which immunotherapy can be tested and this is limiting both for drug and imaging agent development. However, from a clinical perspective one could predict that such an agent might be extremely useful (8, 9). While not predictive of response per se, it could quantitate response after each

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drug cycle to detect early signs of drug resistance. An immediate application could be in distinguishing tumor progression from pseudoprogression. One could also imagine an imaging method that reads out immune activation as highly useful in guiding a patient through the increasing complex drug landscape of combinatorial immunotherapies wherein each combination could be compared for its ability to activate T cells in each patient. If used early enough, such information could lead to treatment modifications before completion of a templated treatment course. Although there is not yet validation, it is reasonable that where there is low or no activation of ICOS, the likelihood of success is similarly low and another line of treatment might be considered. ICOS imaging could improve clinical patient management by allowing earlier decisions regarding drug dosing or switching drug regimens. ICOS imaging, thus, may lead to improved patient outcomes by enabling the selection of optimal therapy prior to tumor progression and potentially decrease costs associated with unnecessary therapies of no benefit, yet still posing risks to patients.

While highly encouraging, enthusiasm has to be tempered by the formulation of this agent, a radiolabeled antibody. Antibodies accumulate in the liver and the long circulation time allows for ^{89}Zr to be released by the antibody and find its way to bone, causing nonspecific uptake. Antibody-based imaging necessarily means that there is prolonged background activity due to slow clearance reducing sensitivity. In addition, antibody imaging usually takes place over several days to reduce background activity sufficiently to detect the target. This is time consuming and inconvenient for the patient and delays treatment decisions. Moreover, larger molecules such as antibodies do not diffuse freely in tumors, resulting in incomplete penetration. These problems could be overcome if a smaller molecule–targeting ICOS with equal or higher affinity could be found. Single-domain antibodies or nanobodies, peptides, and other antibody fragments are examples of such agents. There has never been a better time to discover suitable low molecular weight targeting ligands due to emerging platform technologies. Such an agent could enable imaging on the same day of injection, making the agent much more practical.

Another potential limitation of ICOS-based imaging is that the nature of immune responses is transient and somewhat unpredictable so timing of the scan may be critical. To compare cycles or different therapies where quantitation will be important, each of the various parameters affecting uptake must be controlled including the timing of the scan with reference to the time of administration of therapy, the time from injection to scanning (uptake time), and scanning conditions (vendor and technology used), which may introduce variables into the ability to measure and compare activity. History tells us that such consistency is difficult to achieve in clinical practice.

Even though ICOS PET is not envisioned primarily as a predictive agent, it is possible that an ICOS-based imaging agent could ultimately be predictive if it were combined with a test dose of therapy. Although it would be paradigm bending to use imaging as an initial pharmacodynamic marker of drug activity, given the expense of immunotherapy treatments, such an approach might be considered.

Thus, ICOS-based immune imaging could provide clinicians with an important parameter of effectiveness of immunotherapy. Rather than developing numerous imaging agents custom designed to predict the outcomes of each drug, an approach that measures T-cell response is apt to be more universal. Of course, superiority over conventional imaging including FDG PET would have to be proven, but this should not be hard. This could justify the sustainable commercial development of this or similar lower molecular weight ICOS-targeted agent. While there are improvements to be made, ICOS PET imaging is a good start in the right direction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Mayer AT, Gambhir SS. The immunoimaging toolbox. J Nucl Med 2018;59: 1174–82.
2. Bensch F, van der Veen EL, Lub-de Hooge MN, Jorritsma-Smit A, Boellaard R, Kok IC, et al. (89)Zr-atezolizumab imaging as a non-invasive approach to assess clinical response to PD-L1 blockade in cancer. Nat Med 2018;24:1852–8.
3. Pandit-Taskar N, Postow M, Hellmann M, Harding J, Barker C, O'Donoghue J, et al. First-in-human imaging with (89)Zr-Df-IAB22M2C anti-CD8 minibody in patients with solid malignancies: preliminary pharmacokinetics, biodistribution, and lesion targeting. J Nucl Med 2020;61:512–9.
4. Yi JS, Ready N, Healy P, Dumbauld C, Osborne R, Berry M, et al. Immune activation in early-stage non-small cell lung cancer patients receiving neoadjuvant chemotherapy plus ipilimumab. Clin Cancer Res 2017;23:7474–82.
5. Alam IS, Mayer AT, Sagiv-Barfi I, Wang K, Vermesh O, Czerwinski DK, et al. Imaging activated T cells predicts response to cancer vaccines. J Clin Invest 2018; 128:2569–80.
6. Xiao Z, Mayer AT, Nobashi TW, Gambhir SS. ICOS is an indicator of T-cell-mediated response to cancer immunotherapy. Can Res 2020;80:3023–32.
7. Rudd CE, Schneider H. Unifying concepts in CD28, ICOS and CTLA4 co-receptor signalling. Nat Rev Immunol 2003;3:544–56.
8. Nishino M, Hatabu H, Hodi FS. Imaging of cancer immunotherapy: current approaches and future directions. Radiology 2019;290:9–22.
9. van der Veen EL, Bensch F, Glaudemans AWJM, Lub-de Hooge MN, de Vries EGE. Molecular imaging to enlighten cancer immunotherapies and underlying involved processes. Cancer Treat Rev 2018;70:232–44.