JACC: CARDIOONCOLOGY VOL. 2, NO. 4, 2020

EDITORIAL COMMENT

Blind Spot

^{18}F-FDG PET Fails to Reveal Atherosclerosis Aggravated by Cancer Immunotherapy*

Klaus Ley, MD,[a,b] Payel Roy, PhD[a]

Immune checkpoint inhibitors (ICIs) are a class of monoclonal antibody (mAb)-based anticancer drugs that reinforce T cell–mediated immune responses by blocking co-inhibitory T cell receptors ("check points"), thereby overcoming tumor-induced immune evasion. The Food and Drug Administration-approved ICIs—ipilimumab (anti-CTLA-4 [anti-cytotoxic T lymphocyte-associated antigen 4] mAb); nivolumab and pembrolizumab (anti-PD-1 [anti-programmed cell death protein 1] mAbs); and atezolizumab, avelumab, and durvalumab (anti-PD-L1 [anti-programmed cell death protein ligand 1] mAbs)—have been shown to be efficacious in the treatment of several malignancies. In this issue of *JACC: CardioOncology*, Poels et al. (1) investigated whether ICIs increase atherosclerosis. This hypothesis is based on the premise that, by overriding critical inhibitory regulations, ICIs lead to excessive inflammation, cytokine release, and immune cell infiltration affecting several organs, consequently resulting in immune-related adverse effects (2). Cardiovascular toxicities associated with ICIs, initially underestimated due to a lack of systematic monitoring and heterogenous clinical presentations, have now gained attention due to their fulminant progression and fatal consequences (3). In a retrospective 8-center international registry study, Mahmood et al. (4), reported 1.14% estimated prevalence of ICI-induced myocarditis with a median onset time of 34 days in patients with a mean age of 65 ± 13 years (4). Over a median follow-up of 102 days, 16 of 35 myocarditis cases experienced a major adverse cardiovascular event. A review of Vigibase, the World Health Organization's database of individual case safety reports, revealed 46% fatality in 101 cases of ICI-induced severe myocarditis in patients with malignancies, mostly melanoma and lung cancer (5). In a multicenter case study series, Heinzerling et al. (6) documented autoimmune myocarditis, cardiomyopathy, heart failure, myocardial fibrosis, and cardiac arrest in 8 cases of ICI-related cardiotoxicity in melanoma patients.

Unexpectedly, plaque inflammation in pembrolizumab and nivolumab or ipilimumab-treated stage IV melanoma patients using 2-deoxy-2-[fluorine-18]fluoro- D-glucose (^{18}F-FDG) positron emission tomography (PET) integrated with computed tomography showed no difference in the aortic, carotid, spinal, or splenic signal. ^{18}F-FDG uptake mostly measures myeloid cell content, because their glucose consumption results in visible deoxyglucose uptake. To address the mechanism, the authors applied ^{18}F-FDG PET to *Apoe*$^{-/-}$ mice treated with anti-CTLA-4 and anti-PD-1 mAbs for 4 weeks. They report no signal difference in bone marrow and a slight signal increase in the spleen, but this was not mirrored by accumulation of Ly6C^{+} inflammatory monocytes, which increased slightly in the bone marrow but not in the spleen. However, the authors found a small but consistent and significant increase in CD3^{+} T cells, effector (CD44^{+}) CD4^{+} T cells, and FoxP3^{+} T regulatory cells. The increase in effector CD4 T cells was mirrored by a concomitant decrease in naïve CD4 T cells. Histological analysis of CD3^{+} cells in non-hematopoietic organs revealed marked T cell infiltration in the lungs and heart but not in the colon.

*Editorials published in *JACC: CardioOncology* reflect the views of the authors and do not necessarily represent the views of *JACC: CardioOncology* or the American College of Cardiology.

From the [a]Laboratory of Inflammation Biology, La Jolla Institute for Immunology, La Jolla, California, USA; and the [b]Department of Bioengineering, University of California-San Diego, La Jolla, California, USA.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: CardioOncology* author instructions page.

ISSN 2666-0873 https://doi.org/10.1016/j.jaccao.2020.08.008

Next, the authors focused on aortic arch and root lesions in mice. The aortic arch showed no difference in plaque area, but mice receiving ICIs showed more pathologic intimal thickening according to the Virmani classification. Hematoxylin and eosin staining revealed more necrotic core in the mice treated with anti-CTLA-4 and anti-PD-1, more leukocyte ($CD45^+$), T cell ($CD3+$) and CD8 T cell infiltration, more apoptotic cells measured by TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining, and more immunofluorescence signal for the adhesion molecules ICAM (intercellular adhesion molecule)-1 and VCAM (vascular cell adhesion molecule)-1. Interestingly, Mac-3 staining, a macrophage marker, was decreased. Similar data were obtained in the aortic root.

This study is important because it begins to unravel the mechanism by which ICI treatment can increase atherosclerosis. It is clinically important that there is no signal in ^{18}F-FDG PET, a commonly used imaging modality that sees myeloid cells but not T cells. FDG PET was used as a readout in the negative GLACIER (Study to Evaluate the Safety, Tolerability, and Activity of Intravenous MLDL1278A in Patients on Standard-of-Care Therapy for Stable Atherosclerotic Cardiovascular Disease) atherosclerosis vaccination trial (7). The present study shows that ^{18}F-FDG-PET is not a suitable readout for ICI-induced plaque inflammation. The authors largely reproduced this in mice.

Aortas and lymphoid organs from mice are easily accessible and can be analyzed by flow cytometry after tissue harvesting and dissociation. Mice treated with anti-CTLA-4 and anti-PD-1 show not only proinflammatory changes (increased $CD3^+$, $CD4^+$, $CD8^+$ effector T cells), but also more anti-inflammatory $FoxP3^+$ regulatory T cells. The net effect is not more plaque, but rather a less favorable, more complex plaque phenotype with more pathologic intimal thickening, more necrotic core (known to be associated with plaque instability), and more adhesion molecules.

A major limitation of this study is the stark difference in the pathology between patients and mice. First, on the one hand, the melanoma patients did not have a history of cardiovascular disease, and only half of them had known cardiovascular risk factors. The *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mouse models, on the other hand, did not have any malignancy but had existing atherosclerosis. Second, the length of ICI treatment was significantly longer in mice (4 to 5 weeks of a ~2-year life span) than in humans (6 weeks of ~70-year life span). Third, the authors did not measure the levels of atherosclerosis-associated inflammatory markers such as high sensitivity C-reactive protein, serum matrix metalloproteinases, and cytokines.

Future studies are likely to provide deeper insights into the immune cell changes induced by ICI therapy. Multiparametric analysis of the infiltrated T cells by mass cytometry or single-cell RNA sequencing is likely to reveal more phenotypic heterogeneity of intraplaque activated immune cells. 5′ single-cell RNA sequencing can also be used to reconstruct the T cell receptor α and β chains, thus determining the clonality of the infiltrating T cells. In mice and humans, some CD4 T cells are specific for epitopes in apolipoprotein B, a major atherosclerosis antigen (8,9), which can be assessed by tetramers or restimulation assays. A proposed mechanism for ICI-mediated autoimmune myocarditis in cancer patients is the clonal expansion of cross-reactive T cells that recognize a common antigen shared by the tumor and the cardiac myocytes (10), but this hypothesis remains to be tested rigorously. Determining the antigen specificity, functional properties and clonal identity of the infiltrating T cells will help unravel the molecular pathogenesis of ICI-induced aggravation of subclinical atherosclerosis.

For now, this study shows that ^{18}F-FDG-PET is not a suitable method to follow plaque inflammation in response to ICI. This means that better imaging techniques or other biomarkers are needed to monitor the response to ICI treatment, including possible exacerbated atherosclerosis.

AUTHOR DISCLOSURES

Dr. Ley is co-founder of Atherovax. Dr. Roy has reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr. Klaus Ley, Laboratory of Inflammation Biology, La Jolla Institute for Immunology, 9420 Athena Circle Drive, La Jolla, California 92037. E-mail: klaus@lji.org. Twitter: @ljiresearch.

REFERENCES

1. Poels K, van Leent MMT, Boutros C, et al. Immune checkpoint inhibitor therapy aggravates T cell-driven plaque inflammation in atherosclerosis. J Am Coll Cardiol CardioOnc 2020;2:599–610.

2. Martins F, Sofiya L, Sykiotis GP, et al. Adverse effects of immune-checkpoint inhibitors: epidemiology, management and surveillance. Nat Rev Clin Oncol 2019;16:563–80.

3. Khunger A, Battel L, Wadhawan A, More A, Kapoor A, Agrawal N. New insights into mechanisms of immune checkpoint inhibitor-induced cardiovascular toxicity. Curr Oncol Rep 2020;22:65.

4. Mahmood SS, Fradley MG, Cohen JV, et al. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Coll Cardiol 2018;71:1755–64.

5. Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis. Lancet 2018;391:933.

6. Heinzerling L, Ott PA, Hodi FS, et al. Cardiotoxicity associated with CTLA4 and PD1 blocking immunotherapy. J Immunother Cancer 2016;4:50.

7. Lehrer-Graiwer J, Singh P, Abdelbaky A, et al. FDG-PET imaging for oxidized LDL in stable atherosclerotic disease: a phase II study of safety, tolerability, and anti-inflammatory activity. J Am Coll Cardiol Img 2015;8:493–4.

8. Kimura T, Kobiyama K, Winkels H, et al. Regulatory CD4(+) T cells recognize major histocompatibility complex class II molecule-restricted peptide epitopes of apolipoprotein B. Circulation 2018;138:1130–43.

9. Wolf D, Gerhardt T, Winkels H, et al. Pathogenic autoimmunity in atherosclerosis evolves from initially protective ApoB-reactive CD4+ T-regulatory cells. Circulation 2020;142:1279–93.

10. Johnson DB, Balko JM, Compton ML, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med 2016;375:1749–55.

KEY WORDS atherosclerosis, autoimmunity, FDG-PET, immune checkpoint inhibitor