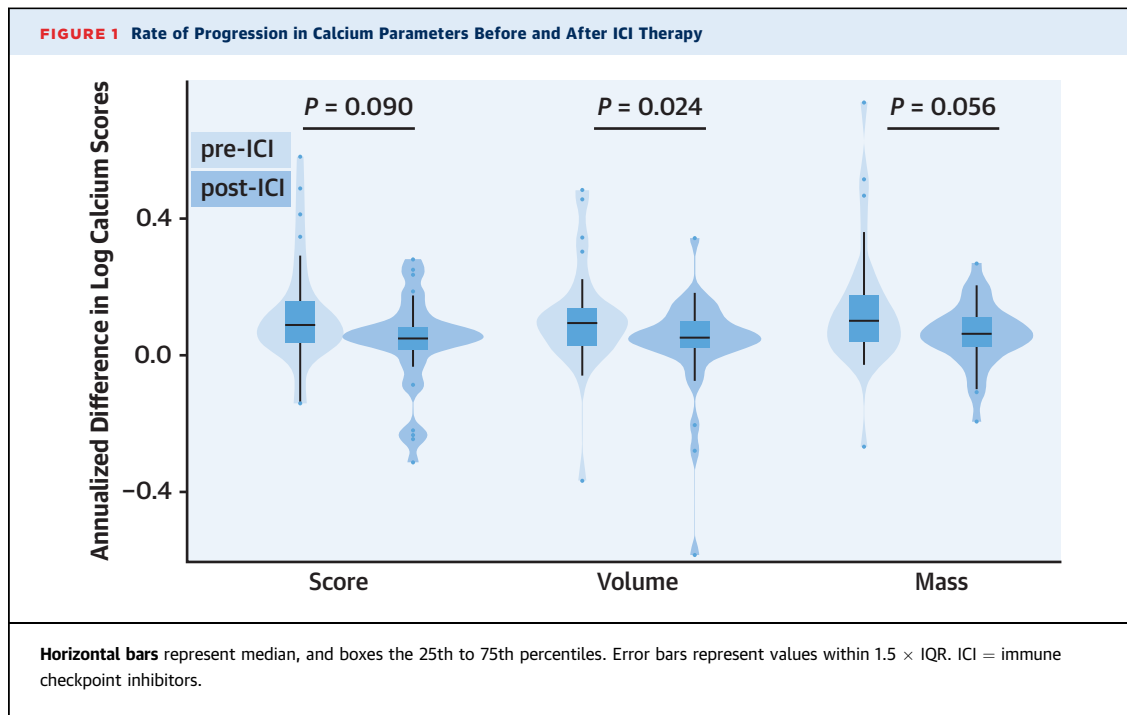RESEARCH LETTER

Immune Checkpoint Inhibitors' Effects on Calcified Aortic Plaques in Melanoma Survivors

A Retrospective Cohort Study

A recent study by Drobni et al[1] showed that aortic plaque volumes by contrast-enhanced computed tomography (CT) increased faster after immune checkpoint inhibitors (ICIs) compared with before, suggesting an association of ICIs with propagation of atherosclerosis. Atherosclerotic calcification is an index of plaque stability and thought to increase mechanical stress bearing, resulting in more stable plaques.[2,3] To our knowledge, no prior study has characterized the effects of ICIs on vascular calcification, specifically. Herein, we assess vascular calcification in melanoma patients with long-term survival (>2 years) following ICI treatment. After approval by the institutional review board of Vanderbilt University, we performed a retrospective cohort study of 839 patients with advanced melanoma who initiated ICIs between 2010 and 2020 at Vanderbilt University Hospital. We identified 35 patients with aortic calcifications on chest and abdominal CTs before ICI initiation, and compared the change in calcium measures before and after therapy. All CTs were part of staging positron emission tomography CTs, were noncontrast, were from a single center, and had a slice thickness of <3.75 mm. We analyzed quantifiable measures of atherosclerotic calcification including calcium score (Agatston units [AU]), volume (mm^3), and mass (mg)[4] from the aortic root to the iliac bifurcation utilizing OsiriX calcium scoring software (OsiriX Imaging). Threshold for calcium detection was 130 HU with an area of >1 mm^3 as described by Agatston.[4] Intrareader and inter-reader reproducibility showed excellent agreement in 20 scans (intra-reader kappa 0.99 [95% CI: 0.98-0.99; $P <$ 0.001] and inter-reader kappa 0.99 [95% CI: 0.98-0.99; $P <$ 0.001]. Main outcome measures were annualized change in aortic calcium score, volume, and mass. Logarithmic transformation of calcium indices (base 10) was used to achieve normality as the

data were skewed toward higher calcium levels. Annual progression rates were calculated as: Pre_ICI rate = ([$\log_{10}$baseline_CT$_{calcium\_measure}$] $-$ [$\log_{10}$pre_ICI_CT$_{calcium\_measure}$])/y and Post_ICI rate = ([$\log_{10}$post_ICI_CT$_{calcium\_measure}$] $-$ [$\log_{10}$baseline_CT$_{calcium\_measure}$])/y. Rates were compared with Wilcoxon signed rank test. Statistical analysis was performed using Stata v16.1 software (StataCorp). Median (Q1-Q3) age was 66 (56-74) years, 63% were male, and 86% had metastatic melanoma. Median of systolic and diastolic blood pressures were 132 (Q1-Q3: 125-142) mm Hg and 83 (Q1-Q3: 72-92) mm Hg, respectively. Median therapy duration was 436 (Q1-Q3: 342-732) days, and 66% of patients received pembrolizumab, 20% nivolumab and ipilimumab, 9% nivolumab, and 6% atezolizumab. Hypertension was present in 86% of the cohort, 51% of whom used statins, 46% were smokers, 26% had coronary artery disease, 26% were diabetic, 17% had a history of myocardial infarction or stroke, and 6% had peripheral vascular disease. Median for CT timepoints were as follows: pre-ICI CT 288 (Q1-Q3: 133-30) days before; baseline CT 11 (Q1-Q3: 28-0) days before; post-ICI CT 743 (703-805) days after ICI initiation. Median days between baseline and pre-ICI CT were 302 (Q1-Q3: 250-418) days, and baseline and post-ICI CT were 735 (Q1-Q3: 560-857) days. **Figure 1** shows annualized changes in calcium score, volume, and mass between consecutive CTs. The annual growth rate in calcified plaque volume was significantly higher pre-ICI compared with the post-ICI period (0.10 $_{log}$mm^3/y vs 0.05 $_{log}$mm^3/y; $P = 0.024$). Changes in mass and score were slightly higher pre-ICI but did not reach statistical significance (0.10 vs 0.06 $_{log}$mg/y; $P = 0.056$, and 0.09 vs 0.05 $_{log}$AU/y; $P = 0.090$, respectively). In subgroup analysis, there was a statistically significant decrease in calcium volume and mass change in patients who received combination ICIs (n = 7) (0.11 vs 0.04 $_{log}$mm^3/y; $P = 0.016$, and 0.13 vs 0.05 $_{log}$mg/y; $P = 0.047$, respectively), but not in those who received anti-PD-1 or anti-PDL1 alone. A similar significant decrease after ICI was seen in volume and mass change rate in those who received steroids (n = 11) for immune-related adverse effects (0.09 vs 0.05 $_{log}$mm^3/y; $P = 0.003$, and 0.08 vs 0.04 $_{log}$mg/y; $P = 0.042$, respectively), but not in those who did not. Patients who were on statins (n = 18) had increased

FIGURE 1 Rate of Progression in Calcium Parameters Before and After ICI Therapy

Horizontal bars represent median, and boxes the 25th to 75th percentiles. Error bars represent values within $1.5 \times$ IQR. ICI = immune checkpoint inhibitors.

rates of progression in calcium score, volume, and mass before ICI compared with post-ICI (0.09 vs 0.04 $_{log}$AU/y; $P = 0.021$, 0.09 vs 0.05 $_{log}$mm^3/y; ($P = 0.034$, 0.10 vs 0.05 $_{log}$mg/y; $P = 0.018$, respectively); whereas patients not on statins had similar trends without statistical significance. There were no statistically significant changes in those who received mitogen-activated protein kinase inhibitor (n = 5); however, all rates were lower post-ICI.

In summary, in this cohort of melanoma patients, the growth rate of calcified volume in aortic plaques was reduced post-ICI. Atherosclerotic plaques may become more inflamed with enhanced calcium clearance by osteoclast-like macrophages after ICIs, leading to a relative increase in noncalcified vs calcified plaque volume.[5] This composition change could promote a more unstable plaque phenotype leading to increased cardiovascular events as observed after ICI therapy.[2,3] Recent evidence demonstrates calcification of coronary plaques is inversely related to cardiovascular events. In the periphery, carotid plaque inflammation is inversely correlated with calcification,[2] and decreased calcification in aortic plaques increases the stroke risk.[3] Our study shows the growth rate in calcified aortic plaque volume decreases, whereas mass does not change after ICI therapy. Our findings complement prior work,[1] suggesting plaque composition changes toward a more vulnerable plaque that could be more prone to rupture after ICI therapy, providing a potential explanation for the increased risk of cardiovascular events. Our subgroup analyses suggest that those who received combination ICI therapy and steroids for immune-related adverse effects had more significant decreases in the growth rate of plaque calcification measures. This may be due to additive T-cell activation with combination therapy and a stronger invigoration of the immune system in those who developed immune toxicities requiring steroids. We also observed a stronger effect of ICIs on calcium measures in those receiving statins, which could reflect a positive interaction in those with higher cholesterol levels, or this could be due to immune-modulatory effects of statins. Our study is limited by its retrospective nature, small sample size, lack of information on total plaque volume, and potential for residual confounding. Nevertheless, we demonstrate that ICI use is associated with decreased growth rate of calcified plaque volume, which could potentially lead to a more vulnerable plaque composition. Future mechanistic studies will help to understand the biological basis of these changes in plaque composition.

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