SUPPLEMENTAL MATERIAL

This appendix has been provided by the authors to give readers additional information about their work

Supplement to: **Association between Immune Checkpoint Inhibitors with Cardiovascular Events and Atherosclerotic Plaque.**

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Supplemental Methods

Document I/A. Key words used for each of the adjudicated clinical events

For cardiovascular events, we did an individual chart review of each chart for any event of interest, at any time. These potential events were identified using a key word search. These key word searches included any of the following terms: myocardial infarction, MI, type 2 MI, type 1 MI, heart attack, ischemia, demand ischemia, positive troponin, ACS, acute coronary syndrome, PCI, percutaneous coronary intervention, angiogram, coronary angiogram, coronary, stent, CABG, coronary artery bypass surgery, coronary revascularization, revascularization, STEMI, NSTEMI, CVA, cerebrovascular accident, stroke, TIA, and transient ischemic attack. Data from the individual charts where a key word was found were then used to populate a CRF and all CRF's were then adjudicated using our standard definitions (below) by an investigator blinded to all other variables.

Document I/B. Definitions used for each of the adjudicated clinical events

Myocardial infarction (MI)	• The clinical presentation consistent with diagnosis of myocardial ischemia and infarction. • Detection of a rise and/or fall of cardiac biomarker values (preferably cardiac troponin) with at least one value above the 99th percentile upper reference limit and with at least one of the following: ♦ Symptoms of ischemia ♦ New or presumed new significant ST-segment-T wave (ST-T) changes or new left bundle branch block (LBBB). ♦ Development of pathological Q waves in the ECG. ♦ Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality. ♦ Identification of an intracoronary thrombus by angiography. • **ECG manifestations of acute myocardial ischemia (in absence of left ventricular hypertrophy (LVH) and left bundle branch block (LBBB)):** ♦ **ST elevation** New ST elevation at the J point in two contiguous leads with the cut-points: ≥ 0.1 mV in all leads other than leads V2-V3 where the following cut-points apply: ≥ 0.2 mV in men ≥ 40 years (≥ 0.25 mV in men < 40 years) or ≥ 0.15 mV in women. ♦ **ST depression and T-wave changes** New horizontal or down-sloping ST depression ≥ 0.05 mV in two contiguous leads and/or new T inversion ≥ 0.1 mV in two contiguous leads with prominent R wave or R/S ratio > 1. • **ECG changes associated with prior myocardial infarction** ♦ Pathological Q-waves, as defined above

	♦ R-wave ≥ 0.04 seconds in V1-V2 and R/S ≥ 1 with a concordant positive T-wave in the absence of a conduction defect. • **Criteria for prior myocardial infarction** Any one of the following criteria meets the diagnosis for prior MI: ♦ Pathological Q waves with or without symptoms in the absence of non-ischemic causes ♦ Imaging evidence of a region of loss of viable myocardium that is thinned and fails to contract, in the absence of a non-ischemic cause. ♦ Pathological findings of a prior myocardial infarction.
Coronary revascularization: • Percutaneous coronary intervention (PCI)	• Placement of an angioplasty guide wire, balloon, or other device (e.g., stent, atherectomy catheter, brachytherapy delivery device, or thrombectomy catheter) into a native coronary artery or coronary artery bypass graft for mechanical coronary revascularization.
• Coronary artery bypass graft surgery (CABG)	• Surgical procedure to improve blood flow to the heart by using a healthy blood vessel (vein or artery) to bypass a blocked portion of one or more coronary arteries.
Ischemic stroke	• An acute episode of focal or global neurological dysfunction caused by brain, spinal cord, or retinal vascular injury because of infarction. **Cerebral infarction** may be documented by the following: ♦ brain imaging or, ♦ persistence of symptoms beyond 24 hours or ♦ death within 24 hours. **Stroke classification:** • **Ischemic stroke—**An acute episode of focal cerebral, spinal, or retinal dysfunction caused by infarction of central nervous system tissue. In case, the hemorrhage may be a consequence of ischemic stroke, the stroke is an ischemic stroke with hemorrhagic transformation and not a hemorrhagic stroke.

Document II. Computed tomography analysis methods.

Computed Tomography Image Acquisition: We included all patients with melanoma with three evaluable serial contrast-enhanced thoracic computed tomography scans, at > three months before immune checkpoint inhibitor (scan 0), ≤ three month prior to starting immune checkpoint inhibitor (scan 1), and the most recent available (scan 2). Computed tomography images were acquired per standard departmental protocol for cancer screening. All scans had excellent image quality. Corrupted or incomplete datasets as well as scans with slice thickness > three mm were excluded from the analysis.

Computed Tomography Image Analysis: Aortic plaque measurements were performed on contrast-enhanced chest computed tomography in the descending thoracic aorta. The ascending aorta as well as the aortic arch were spared due to motion artifacts on non-gated images. Image analysis was performed using a dedicated and validated analysis software (QAngio CT, version 3.1.4.2, Medis Medical Imaging Systems, Leiden, the Netherlands) by a radiologist with 7 years of experience in computed tomography. All analyses were performed blinded to date of study, date of start of immune checkpoint inhibitor and all other study variables. Aortic segments for evaluation were established manually. Segment length and level were kept identical for all three time points to minimize variability. Segmentation of inner and outer vessel boundaries was performed in a semi-automated fashion with manual adjustments. Plaque volume was calculated automatically (defined as vessel volume – lumen volume). Voxels with an attenuation of ≥130 Hounsfield Units (HU) were assigned to the calcified plaque volume portion. Plaque volumes with attenuation less than 130 HU was considered non-calcified plaque. This volumetric plaque assessment technique has demonstrated excellent intra- and inter-observer, as well as interscan reproducibility (Ref 20-22). Relative plaque volume measures were assessed as percent of total segment volume. Plaque change was calculated as the difference in plaque volume measured on

two consecutive scans (i.e., scan 2 – scan 1 and scan 1 – scan 0). Annualized plaque progression rate was computed as plaque change per year given in absolute and relative rates (mm^3 and %).

Supplemental Tables

Table I. Baseline laboratory variables of patients treated with immune checkpoint inhibitors and controls.							
		Cases (n=2842)		**Controls (n=2842)**			***P* Value**
Baseline laboratory parameters – mean. (SD)							
	Data available (n)			***Data available (n)***			
Hemoglobin (g/dL)	2599	11.9	(2.0)	2687	13.1	(1.7)	<0.001
White blood count (Thousand/uL)	2599	8.1	(9.0)	2659	7.4	(4.5)	0.001
Glomerular filtration rate (mL/min/1.73m^2)	1035	68.2	(23.9)	422	72.2	(30.4)	0.008
Total cholesterol (mg/dL)	896	176.2	(46.5)	1568	180.7	(45.7)	0.021
Low density lipoprotein (mg/dL)	784	100.0	(36.9)	1183	100.0	(34.4)	0.96
High density lipoprotein (mg/dL)	810	50.0	(20.0)	1448	52.2	(17.9)	0.006

Table II. Univariable Cox proportional hazard model results of the composite cardiovascular outcome (myocardial infarction, revascularization, ischemic stroke)

	Hazard Ratio	95 % Confidence Interval		Wald test *P* Value
Demographic variables				
Male sex	1.41	1.12	1.77	0.003
Age	1.04	1.03	1.05	<0.001
Body mass index	1.01	0.99	1.03	0.55
Systolic blood pressure	1.02	1.01	1.02	<0.001
Cardiovascular risk factors				
Hypertension	1.81	1.43	2.29	<0.001
Diabetes mellitus	1.75	1.35	2.27	<0.001
Smoking current or prior	1.29	0.94	1.77	0.11
Hyperlipidemia	1.59	1.27	2.00	<0.001
History of any cardiovascular event	3.07	2.38	3.97	<0.001
Chronic kidney disease	1.48	1.08	2.04	0.016
Medications and prior potentially cardiotoxic therapies				
Angiotensin converting enzyme inhibitor or angiotensin II receptor blocker	1.63	1.27	2.08	<0.001
Aspirin	2.06	1.62	2.62	<0.001
Statin therapy	1.61	1.26	2.05	<0.001
Prior radiation therapy	1.73	1.29	2.32	<0.001
Fluorouracil	1.22	0.80	1.86	0.37
Anthracyclines	0.51	0.27	0.96	0.038
Immune checkpoint inhibitors	4.68	3.52	6.23	<0.001
Immune checkpoint inhibitor type				
Monotherapy				
Programmed death-ligand-1	1.25	0.83	1.89	0.29
Cytotoxic-T-Lymphocyte associated protein 4	0.77	0.42	1.39	0.38
Programmed death-protein 1	1.25	0.83	1.89	0.29
Combination Therapy				
Cytotoxic-T-Lymphocyte associated protein 4/Programmed death protein 1	0.77	0.42	1.39	0.38
Cancer types				
Non-small cell lung cancer	1.66	1.31	2.11	<0.001
Melanoma	0.80	0.62	1.03	0.08
Head and neck cancer	0.88	0.63	1.25	0.48
Renal and genitourinary cancer	0.94	0.60	1.49	0.80
Breast cancer	0.49	0.24	0.98	0.044
Gastrointestinal cancer	0.92	0.54	1.57	0.76
Gynecologic cancer	0.99	0.58	1.70	0.98

Table II. Continue. Univariable Cox proportional hazard model results of the composite cardiovascular outcome (myocardial infarction, revascularization, ischemic stroke)				
Lymphoma	0.77	0.39	1.49	0.43
Hepatobiliary cancer	0.47	0.18	1.26	0.13
Pancreatic cancer	1.39	0.52	3.72	0.52
Other type of cancer	1.24	0.79	1.95	0.35

Table III. The number of patients with an event and number of events, the rate per 100-person years from our cohort of 2842 cases and the hazard ratio for cardiovascular events. Cardiovascular events are compared for the one-year period pre-immune checkpoint inhibitor and one-year period post-immune checkpoint.

	Pre-treatment		**Post-treatment**		**Hazard Ratio* (95% CI)**	***P* Value**
End-point, n (%)	**No. of patients with event %**	**Rate per 100 person-yr**	**No. of patients with event %**	**Rate per 100 person-yr**		
Cardiovascular events	43 (1.51%)	1.52	83 (2.92%)	5.55	3.59 (2.47-5.21)	<0.001
End-point, n (%)	**No. of events. %**	**Rate per 100 person-yr**	**No. of event. %**	**Rate per 100 person-yr**		
Myocardial infarction	16 (0.56%)	0.58	37 (1.30%)	2.38	3.01 (1.21-6.27)	<0.001
Coronary revascularization	13 (0.46%)	0.46	22 (0.77%)	1.41	2.48 (1.07-5.34)	<0.001
Ischemic stroke	21 (0.74%)	0.74	35 (1.23%)	2.25	2.20 (1.15-4.42)	<0.001

*** Cox proportional hazard model**

Table IV. The number of patients with an event and number of events, the rate per 100-person years from our cohort of 2842 cases and the hazard ratio for cardiovascular events. The table is restricted to those who had events in the two-year period pre-and post-starting an ICI after excluding patients who died within 60 days of the event.

	Pre-treatment		**Post-treatment**		**Hazard Ratio* (95% CI)**	***P* Value**
End-point, n (%)	**No. of patients with events %**	**Rate per 100 person-yr**	**No. of patients with events %**	**Rate per 100 person-yr**		
Cardiovascular events	66 (2.34%)	1.18	102 (3.61%)	4.74	4.15 (3.01-5.73)	<0.001
End-point, n (%)	**No. of events. %**	**Rate per 100 person-yr**	**No. of events. %**	**Rate per 100 person-yr**		
Myocardial infarction	27 (0.95%)	0.48	51 (1.80%)	2.41	4.24 (2.46-7.71)	<0.001
Coronary revascularization	25 (0.88%)	0.44	33 (1.17%)	1.56	2.89 (1.26-5.89)	<0.001
Ischemic stroke	26 (0.92%)	0.46	36 (1.27%)	1.70	1.73 (1.31-5.37)	<0.001

*** Cox proportional hazard model**

Table V. Clinical characteristics of the subjects with melanoma included in the computed tomography study.	
Characteristics	**N=40**
Demographic	
Age– yr. mean. (SD)	67±11
Male - %	55%
Race or ethnic group – no. (%)	
White race	100%
Non-Hispanic ethnicity	84.6%
Immune checkpoint inhibitor – %	
Immune checkpoint inhibitor monotherapy	87.5%
Immune checkpoint inhibitor combination therapy	12.5%
Number of cycles, no. (IQR)	8.5 (4.5, 23.5)
Presence of immune related adverse event of any degree	45%
Immune related adverse event grade 1-2	17.5%
Immune related adverse event grade 3-4	27.5%
Corticosteroid therapy	57.5%

Table VI. Comparison between baseline and follow-up characteristics of the patients with melanoma included in the computed tomography study.		
Characteristics	**At scan 0**	**At scan 1**
Cardiovascular risk factors – no. (%)		
Hypertension	47.5%	52.5%
Diabetes	7.5%	10.0%
Never smoker	52.5%	55.0%
Systolic blood pressure, mmHg. (SD)	133±17.1	135±17.1
Cardiovascular diagnoses – no. (%)		
History of myocardial infarction	7.5%	10.0%
History of coronary revascularization	10.0%	12.5%
History of ischemic stroke	0	0
Cardiovascular medications – no (%)		
Aspirin	17.5%	27.5%
Statin	42.5%	42.5%

Table VII. Total and non-calcified plaque volume at each of the three imaging time-points.				
	N=40	Scan 0	Scan 1	Scan 2
Median (IQR), mm^3	Plaque volume, mm^3	1438 (703, 2690)	1567 (703, 2676)	2183 (923, 4150)
	Non-calcified plaque volume, mm^3	1285 (643, 2193)	1130 (592, 1986)	1725 (733, 3584)

Supplemental Figures and Figure Legends

Figure I. Schematic of the case-crossover study design. Patient A represents a person who starts an immune checkpoint inhibitor and is hospitalized for an acute vascular event during the 2-year control interval (light-shaded areas) prior to exposure. Patient B represents a person who starts an immune checkpoint inhibitor and who has an atherosclerotic event during the risk interval, within 2-years after starting an immune checkpoint inhibitor (dark-shaded areas). The case-crossover study assessed the relative incidence of cardiovascular events during the risk interval as compared with the control interval. Note that the figure is not to scale.

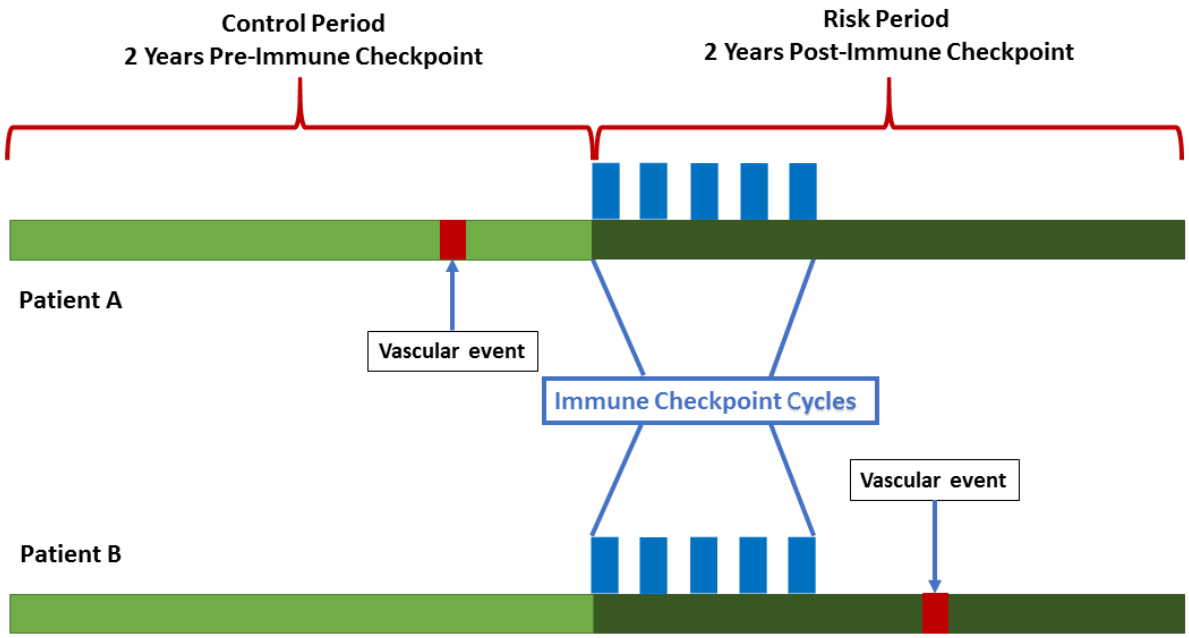


Figure II. Schematic of the patient selection for the imaging. We analyzed all patients with melanoma treated with an immune checkpoint inhibitor with the following inclusion criteria: 1. They had undergone three thoracic contrast-enhanced computed tomography studies as part of their routine clinical care for cancer staging. 2. The first computed tomography was >3 months prior to the immune checkpoint inhibitor initiation, the second ≤3 months prior to starting, and a third at least one year after starting an immune checkpoint inhibitor (Figure III in the Supplement), 3. Subjects had atherosclerosis on the first computed tomography study, and 4. Images were of adequate quality for quantification of plaque. The final sample size meeting all the inclusion criteria was 40 subjects. The study population was enriched by the inclusion of only patients with atherosclerotic plaque on the baseline study. When more than one study was available for the baseline study, the oldest study was analyzed. When more than one study was available for the follow-up study then the most recent study was analyzed.

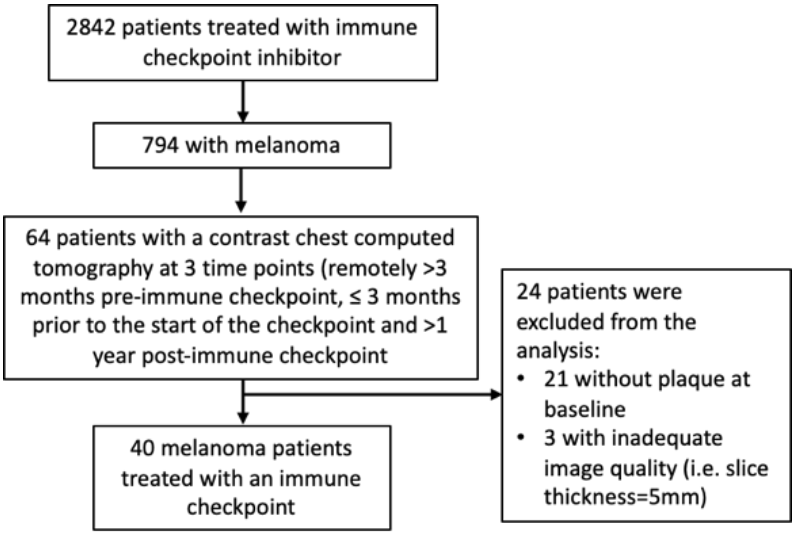
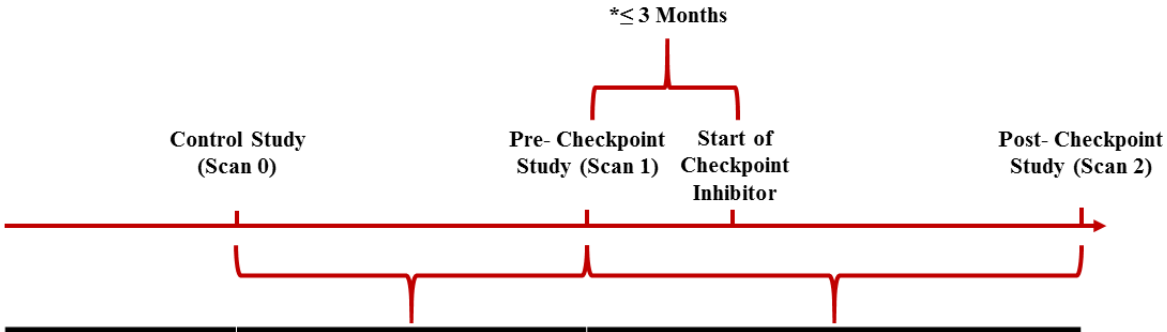


Figure III. Schematic of the timing of the imaging studies. The interval from the first thoracic computed tomography study to the pre-immune checkpoint inhibitor study was a median of 490 days (range 149 - 1325 days) and the interval from the pre-immune checkpoint inhibitor study to the post-immune checkpoint inhibitor study was 1171 days (range 519 - 1486 days). The median interval from the pre-immune checkpoint inhibitor imaging study to the start of immune checkpoint inhibitor was 16.5 days (range 10.5 - 31.5). A subject may have had more than one computed tomography study in the period from the first study to the pre-immune checkpoint inhibitor study. If so, the oldest study was chosen. Similarly, a subject may have had several imaging studies after starting an immune checkpoint inhibitor. If so, the most recent computed tomography study was chosen. The total plaque volume was quantified at each time-point chosen and then adjusted for the varying time intervals between the scans to create an annualized rate of plaque volume change. The yearly change in plaque volume from between the two intervals (pre-immune checkpoint inhibitor to post-immune checkpoint inhibitor) was compared. Additional measures of interest included the non-calcified plaque volume.



	Change in Aortic Plaque Pre-Immune Checkpoint	Change in Aortic Plaque Post-Immune Checkpoint
	Interval from Control CT Study to Pre-Checkpoint Inhibitor CT	Interval from Pre-Checkpoint Inhibitor CT Study to Post-Checkpoint Inhibitor CT
Chest Computed Tomography (n=40)	490 Days (149, 1325)	1171 Days (519, 1486)

*Median interval from scan 1 to start of checkpoint inhibitor was 16.5 days (Interquartile range, 10.5 - 31.5)

Figure IV. Forest plot of the hazard ratios for the composite cardiovascular outcome stratified by sub-groups within the matched cohort study. We report the *P* value for the interaction term from the Cox proportional model and the *P* value from the heterogeneity chi-squared test. Significant interaction was observed in body mass index, hypertension and statin use and immune checkpoint inhibitor therapy. However, the hazard ratios for each subgroup only differed in subgroup analysis for hypertension.