Thesis

Major adverse cardiovascular events in patients with cancer treated with immune checkpoint inhibitors

submitted by

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Graz, 25.06.2025 Michael Fink m.p.

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Zusammenfassung

Hintergrund: Bei onkologischen Patient*innen, die mit Immuncheckpoint Inhibitoren (ICI) behandelt werden, besteht das Risiko, dass therapiebedingte Nebenwirkungen auftreten. Hinweise häufen sich, dass ICI-Therapien Entzündungsprozesse im Körper fördern und Atherosklerose verursachen können, die wiederum zu schwerwiegenden unerwünschten kardiovaskulären Ereignissen (MACE) führen kann.

Methoden: In dieser retrospektiven Kohortenstudie wurden erwachsene Patient*innen mit einer histologisch verifizierten Tumordiagnose eingeschlossen, die an der Medizinischen Universität Graz eine ICI-Therapie erhalten haben. Die Daten wurden durch elektronische Datenerfassung gesammelt, welche demographische Daten zu Patient*innen, Komorbiditäten, Tumormerkmale, Behandlungsmodalitäten, ICI-Therapie und Endpunkte dieser Studie enthielten. Der primäre Endpunkt der Studie war das erste Auftreten einer 4-Punkt MACE, welche als nicht tödlicher Myokardinfarkt, nicht tödlicher Insult, Hospitalisierung wegen Herzinsuffizienz oder kardiovaskulären Tod definiert wurde.

Ergebnisse: 452 Patient*innen, die an der Medizinischen Universität Graz behandelt wurden, wurden in die Studie eingeschlossen. Über einen medianen Beobachtungszeitraum von 36.9 Monaten, erlitten 47 Patienten MACE (10.4% der Studienpopulation). Von diesen, war MACE wie folgt verteilt: Insult in 19 Patienten (4.2%), Hospitalisierung für Herzinsuffizienz in 15 Patienten (3.3%), akutes Koronarsyndrom in 8 Patienten (1.8%) und kardiovaskulärer Tod in 6 Patienten (1.3%). MACE hat in 15% der Patient*innen zu permanentem ICI-Therapieabbruch und in 12.8% der Patient*innen zum Tode geführt. Das Risiko für MACE war nach ICI-Therapiebeginn erhöht verglichen mit dem Zeitraum zwischen Tumordiagnose und ICI-Therapiebeginn (transition hazard ratio nach multivariabler Anpassung für Alter, Geschlecht, Tumorstadium und Tumorart 4.43 [95%KI: 2.38-8.24, $p<0.001$]). Zu den identifizierten Risikofaktoren für MACE gehören höheres Lebensalter, ECOG≥1, höherer BMI, Vorhandensein von atherosklerotischer kardiovaskulärer Vorerkrankung und ein höherer CRP-Ausgangswert.

Conclusio: Tumorpatient*innen, die mit ICI behandelt werden, könnten ein substanziell höheres Risiko für die Entwicklung von MACE haben. Weitere Studien sind notwendig, um Patient*innen zu definieren, die von primärprophylaktischen Maßnahmen und intensivierter Überwachung profitieren könnten.

Abstract

Background: Patients with cancer treated with immune checkpoint inhibitors (ICI) are at risk of developing immune-related adverse events. Accumulating evidence shows that ICI therapy might progress inflammatory processes and cause atherosclerosis leading to major adverse cardiovascular events (MACE).

Methods: In this retrospective cohort study, consecutive adult patients with histologically confirmed cancer treated with ICI at Medical University of Graz were included. Comprehensive data was collected through electronic chart review, containing patient demographics, comorbidities, cancer characteristics, cancer treatment modalities, ICI therapy and study outcomes. The primary study endpoint was the first occurrence of 4-point MACE, defined as a composite of non-fatal myocardial infarction, non-fatal stroke, hospitalization for heart failure, or cardiovascular death.

Results: 452 consecutive patients treated with ICI at the Medical University of Graz were included. Over a median follow-up period of 36.9 months, 47 patients suffered a MACE (10.4% of the study population). Of those, the index MACE was stroke in 19 patients (4.2%), hospitalisation for heart failure in 15 patients (3.3%), ACS in 8 patients (1.8%), and cardiovascular death in 6 patients (1.3%). MACE led to permanent ICI discontinuation in 15.0% of patients and was fatal in 12.8%. Risk MACE was increased after ICI initiation compared to the timeframe between cancer diagnosis and ICI start (transition hazard ratio upon multivariable adjustment for age, sex, cancer stage and cancer type: 4.43 [95%CI: 2.38-8.24, $p<0.001$]). Identified risk factors for MACE included higher age, ECOG ≥ 1, higher BMI, presence of prior atherosclerotic cardiovascular disease, and higher baseline levels of CRP.

Conclusion: Patients with cancer treated with ICI might have a substantial risk of developing MACE, warranting future research to identify patients that might benefit from primary prophylactic interventions and surveillance strategies.

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Abbreviation index

5-FU *5-fluorouracil*

ACS *acute coronary syndrome*

ACT *adoptive cell transfer*

AMI *acute myocardial infarction*

APC *antigen presenting cell*

BMI *body mass index*

CAR-T *chimeric antigen receptor T-cell*

COPD *chronic obstructive pulmonary disease*

CRP *C reactive protein*

CTL *cytotoxic T-lymphocytes*

CTLA-4 *cytotoxic T-lymphocyte-associated antigen 4*

CV *cardiovascular*

CVAE *cardiovascular adverse events*

CVD *cardiovascular disease*

DC *dendritic cell*

ECOG *Eastern Cooperative Oncology Group performance index*

ES-SCLC *extensive-stage small cell lung cancer*

HCC *hepatocellular carcinoma*

HF *heart failure*

HNSCC *head and neck squamous cell carcinoma*

ICI *immune checkpoint inhibitors*

IHD *ischemic heart disease*

IL-2 *interleukin-2*

IrAE *immune-related adverse events*

LDH *lactate dehydrogenase*

MACE *major adverse cardiovascular event*

MHC *major histocompatibility complex*

NK *natural killer cell*

NSCLC *non-small cell lung cancer*

OVT *oncolytic virus therapy*

PD-1 *programmed cell death-1*

PD-L1 *programmed cell death ligand-1*

PD-L2 *programmed cell death ligand-2*

RCC *renal cell cancer*

SCLC *small cell lung cancer*

SHR *sub-distribution hazard ratio*

TAA *tumor-associated antigen*

TCR *T-cell receptor*

TCR-T *T-cell receptor therapy*

TH1 *T-helper 1 cell*

TH2 *T-helper 2 cell*

TH17 *T-helper 17 cell*

TIL *tumor-infiltrating lmyphocytes*

Tregs *regulatory T cells*

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Introduction

Immunotherapy has led to a major shift in the survival prospects of patients with cancer over the past years. The approval of immune checkpoint inhibitors (ICIs) began with anti-CTLA-4 therapy for melanoma in 2011, followed by PD-1 inhibitors in 2014 and subsequent combination regimens (1). Since then, these therapies have significantly enhanced overall survival in patients with melanoma and have been included to the treatment of over 17 additional cancer typer. With improved survival due to advances in cancer therapy, there is growing awareness of complications associated with treatment, particularly those affecting morbidity and mortality such as cardiovascular (CV) toxicities (2).

1.1 Major adverse cardiovascular events

Cardiovascular events represent a heterogeneous group of complications and disease manifestations, including primary vascular, cardiac, and thrombotic / thromboembolic diseases and complications.

According to Statistics Austria, cardiovascular diseases (CVD) were responsible for 34.7 % of all deaths in 2023 making it the leading cause of death (3). In clinical trials, the term "Major adverse cardiovascular events (MACE)" is increasingly used as a primary endpoint in order to encompass the most severe complications of cardiovascular diseases. However, the term is not uniformly defined in medical literature, which limits the comparability of clinical studies.

In 2008, the U.S. Food and Drug Administration (FDA), subsequently adopted by the European Medicines Agency (EMEA), proposed the use of a so-called three-point MACE outcome in all studies assessing the cardiovascular safety of antidiabetic drugs. This included acute myocardial infarction (AMI), which, according to the catalogue of common terminology criteria for adverse events (CTCAE) Version 5, is defined as a disorder characterized by gross necrosis of the myocardium due to an interruption of blood supply to the area. It also included stroke, defined as a disorder characterized by a decrease or absence of blood supply to the brain caused by obstruction (thrombosis or embolism) of an artery resulting in neurological damage and cardiovascular mortality (4). Some studies also used a four-point MACE, including hospitalization for unstable angina pectoris or revascularization procedures or a five-point MACE extending this by including heart failure (HF) (4,5).

The components of the MACE endpoints and the diagnostic codes used often vary between studies. The aim of a review by Bosco et al. was to systematically identify the most used definitions of MACE in observational studies based on administrative data. A total of 920 articles were screened, 412 underwent full-text review, and 58 studies were included in the final analysis. Only 8.6% (5 out of 58) used the standard three-point MACE definition. None of the studies used the extended four-point definition (adding unstable angina pectoris) or the five-point definition (adding both, unstable angina pectoris and heart failure). The most frequently reported combinations of MACE were: AMI and stroke (15.5%, 9/58), AMI, stroke and all-cause mortality (13.8%, 8/58) and AMI, stroke and cardiovascular death (8.6%, 5/58) (6).

1.2 Cardiovascular complications in patients with cancer

There is now a growing body of evidence that patients with cancer are also at an increased risk for cardiovascular complications, including cardiomyopathy/heart failure, coronary artery disease, stroke and pericardial disease (7,8). The increase in cardiovascular risk has been established both during active malignancy, and as long-term complication in cancer survivors.

For example, it has been shown that patients who develop or suffer from cancer have a higher risk of MI than the general population (9). Navi et al. found, that the 6-month cumulative incidence of MI was 2.0% in patients with cancer, compared to 0.7% in age-matched controls without cancer (10). In a large study by Paterson et al. with a total of 224,016 participants it was shown that a new cancer diagnosis was associated with an increased risk of fatal and non-fatal cardiovascular events, even after adjustment for baseline risk factors (8).

Several studies have shown that cancer is associated with an increased risk of stroke, stroke recurrence, stroke severity, early neurological deterioration and in-hospital death and that an estimated 12% of all stroke patients have an occult underlying malignancy (9,11–15).

However, while the association between cardiovascular disease and cancer was initially focused on breast cancer and childhood cancers, it has recently been shown that the risk of suffering MACE varies among different types of cancer. Mitchell et al. showed that the cumulative incidence of MACE was highest in patients with lung cancer, myeloma, and leukemia and lowest in patients with breast cancer, prostate cancer, and melanoma among 839,934 patients and across nine cancer types. The incidence of stroke was observed to be

significantly higher in individuals diagnosed with lung cancer, whereas HF was found to be most prevalent in those with myeloma and lung cancer (16).

The underlying mechanisms linking cardiovascular risk and cancer are not fully understood and are likely multifactorial, yet several shared patient-specific risk factors between cancer and cardiovascular disease likely affect individual risk profiles, including age, obesity, tobacco use, diabetes, hypertension and hyperlipidemia predispose to both (17). Beyond that, cancer itself is known to induce a prothrombic state and can thereby enhance the risk for thromboembolic events. Specific mechanisms that may contribute to cancer-associated hypercoagulability include direct coagulation activation by cancer cells and their micro-environment, bi-directional interactions between cancer and platelets, endothelial activation, and systemic inflammation in malignancy (9,11,18–20).

Specifically, it is hypothesized that inflammation is a driving factor in both CVD and cancer. The concept of "inflammaging" is described as a chronic sterile low-grade inflammation that occurs with advanced age in the absence of manifest infection. Inflammaging contributes to the risk of various morbidities, including CVD and cancer in the elderly through inflammatory mediators or modifiers. (9,11).

Numerous factors, such as genetic susceptibility, central obesity, increased intestinal permeability, changes in the composition of the microbiota, cellular senescence, NLRP3 inflammasome activation, oxidative stress due to dysfunctional mitochondria, immune cell dysregulation and chronic infection as inflammatory modifiers might contribute to the risk of various morbidities including CVD and cancer in an aging population (9,11).

Beyond patient and cancer-specific factors, cancer-specific therapies, can further increase the risk of MACE in patients with cancer (21).

1.3 Cardiovascular complications in patients with cancer treatment

Different types of local and systemic treatment modalities used in patients with cancer have been linked to an increased cardiovascular risk.

First, radiotherapy for cancer treatment may contribute to the risk of MACE. Radiation has been shown to increase the risk of vascular damage, cardiomyopathy, pericardial disease, and valvular disease (22). In a population-based case-control study of major coronary events (i.e., AMI, coronary revascularization, or death from ischemic heart disease) in 2,168 women undergoing radiotherapy for breast cancer it could be shown, that exposure

of the heart to ionizing radiation during radiotherapy for breast cancer is directly proportional to the increase in the rate of ischemic heart events (23).
A paper by Hermann et al provides consensus definitions for the most reported CV toxicities including cardiomyopathy/heart failure and myocarditis, vascular toxicity, hypertension, arrhythmias and QTc prolongation (24).
Vascular toxicity, defined in this paper (Hermann et al.) as the induction or aggravation of vascular disease in the setting of cancer therapy, has been described for alkylating agents, such as cyclophosphamide, "alkylating-like" platinum drugs, such as cisplatin and antimicrotubule drugs (e.g. vincristine, vinblastine, vinorelbine as well as paclitaxel, docetaxel and nab-paclitaxel). Paclitaxel has been mentioned to cause vasospasm leading to ACS (24–26). Vascular toxicity has also been described for antimetabolites (e.g. purine and pyrimidine analogues with its best-defined substance 5-fluorouracil (5-FU), as well as capecitabine and gemcitabine). Other substances which might cause vascular toxicity are proteasome inhibitors (e.g. bortezomib, carfilzomib, and ixazomib), aromatase inhibitors, angiogenesis inhibitors (e.g. thalidomide, lenalidomide, pomalidomide) and the vascular signaling pathway inhibitors anti-VEGF drugs (e.g. sunitinib, sorafenib, axitinib, pazopanib, ponatinib, bevacizumab and regorafenib) (24,27).

The use of immunotherapeutics, particularly immune checkpoint inhibitors (ICIs), which has led to a fundamental change in cancer therapy, has also been associated with CV complications, including an increased risk of myocardial injury and heart failure as well as ischemic stroke (28,29).

1.4 Cancer immunotherapy

Cancer immunotherapy has led to a dramatic shift in recent years due to groundbreaking advances in the therapeutic armamentarium and unprecedented improvements in long-term prognosis of subgroups of treated patients. Generally, there are five major types of immunotherapy: oncolytic virus therapies, cancer vaccines, cytokine therapies, adoptive cell transfer and immune checkpoint inhibitors (30,31).

1.4.1 Oncolytic virus therapy

Oncolytic virus therapy use native or engineered viruses to induce oncolytic activity and enhance the antitumor immune response (31). Those viruses have the capability to trigger

cell autolysis, cell honing, vascular supply destruction and boost other adjuvant therapies (32). A well-studied treatment is T-Vec, which is a genetically modified herpes-simplex virus with granulocyte–macrophage colony-stimulating factor. It is commonly used in therapy against metastatic melanoma (30,32).

1.4.2 Cancer vaccines

Cancer vaccines provide the antigen using tumor-specific antigen (TSA) or tumor-associated antigen (TAA) (32,33). Those antigens are then taken up by antigen-presenting cells (APC) and presented to CD4+ and CD8+ T cells (34). CD4+ cells initiate an immune response by communicating with other immune cells, and CD8+ cytotoxic T-lymphocytes (CTL) identify and eliminate tumor cells (35).

Different types of cancer vaccines have been developed that vary in the way in which the antigen is delivered. There are petide/protein-vaccines, cellular vaccines and genetic vaccines (32,36–38).

1.4.3 Cytokines

Cytokines serve as messengers to ensure an adequate immune response (30–32). They are essential for proliferation and activation of immune cells (39). Cytokines are secreted by both immune and non-immune cells in response to cellular stresses, such as infection, inflammation and tumorigenesis (30,31). In tumor therapy cytokines are required to invade the tumor regions and stimulate the immune effector cells and cytotoxic effector cells (32,39). FDA-approved IL-2 therapy has been used to treat melanoma and renal cell carcinoma for the past 20 year. IL-2 is required for T-cell activation and also activates an antitumor response (30–32,39).

1.4.4 Adoptive cell transfer

Adoptive cell transfer (ACT) utilizes autologous T cells that are expanded ex vivo, which are then reinfused into the patient to eliminate cancer cells (30,40). The most common forms to use ACT are tumor infiltrating lymphocytes (TIL), t-cell receptor therapy (TCR-T) and chimeric antigen receptor T-cell (CAR-T) therapy (39).

TILs are obtained from surgically resected tumors. Therefore, huge t-cell populations with a great specificity against the tumor can be acquired (40,41).

TCR-T use T cells with tumor-specific t-cell-receptors. Those receptors recognize TAA with high specificity (40,42). TCR however, is dependent on the presence of major histocompatibility complex (MHC) which is a huge limitation because solid tumors

possess the ability to downregulate their MHC expression and therefore evade the immune recognition (31). It must be pointed out that TILs and TCR-T are not yet available for clinical use, as they are still undergoing clinical trials (41,42).
CAR-T cells can bypass the MHC system and recognize specific antigens on the tumor cells. But CAR-T cell therapy is limited to target antigens on the cells surface (42,43). CAR-T cell therapy is already a well-established treatment for various hematologic diseases (40,44).

1.4.5 Immune checkpoint inhibitors

Cancer immunotherapy has advanced rapidly in recent years, and the most widely used immunotherapeutic agents are immune checkpoint inhibitors (ICI) (45). Although there are many antibodies in clinical development targeting different immune checkpoints, such as LAG3, TIGIT, TIM3, B7H3, CD39, CD73, adenosine A2A receptor and CD47, the most widely used therapies target cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) (45,46).

1.4.5.1 CTLA-4 pathway

Generally, two stimulatory signals are required to activate T cells (45,47,48). First, the TCR must bind to a specific antigen presented by the MHC of APCs, which serves as the major stimulatory signal (47). Second the CD28 receptor presented on T cells binds to either CD80/B7.1 or CD86/B7.2 molecules on activated APCs (47,49). Once stimulated, CD28 triggers glucose uptake and cell cycle progression in T cells by rising the expression of Bcl-X and interleukin-2 (IL-2) to reduce apoptosis and induce T cell proliferation (47,50,51).
CTLA-4 is an inhibitory receptor mainly expressed by T cells and it is upregulated upon T cell activation (45). CTLA-4 is also a CD28 homolog, and they compete for the same ligands (CD80/B7.1 or CD86/B7.2) (48). But CTLA-4 has an approximately 20-fold higher affinity than CD28, therefore it prevents the CD28 induced T cell activation by lowering the costimulatory signals (45,52).
CTLA-4 is also constitutively present on regulatory T cells (Tregs), which is important to regulate and supress immune responses (45,47,48).

1.4.5.2 PD-1/PD-L1 pathway

PD-1 is a costimulatory receptor present on activated T cells, natural killer cells (NKs), B lymphocytes, macrophages, dendritic cells (DCs), monocytes and especially on tumor-specific T cells (48,53,54). When its ligands PD-L1 or PD-L2 are bind to the receptor, T cell proliferation, differentiation and activation in the effector phase and cytokine production are inhibited (46,55). PD-L1 is expressed by macrophages, activated T cells and B cells, DCs and tumor cells as their way to escape anti-tumor responses, whereas PD-L2 is primarily expressed by DCs, monocytes and also tumor cells (48,53).

1.4.5.3 Immune checkpoint inhibitors

The discovery of these pathways and their blocking antibodies has led to a tremendous advances in cancer therapy (45,56). ICIs are currently approved for various cancer types, while PD-1 antibodies are covering the greatest number of cancer (45). In recent years, a growing proportion of patients with cancer is becoming eligible for treatment with ICIs, with currently 55% of patients with cancer estimated to be eligible for ICI therapy during the course of disease (57).

Table 1: Selection of Indications for Immune Checkpoint Inhibitors for the treatment of cancer (45)

Therapy	Approved indication
Anti-CTLA-4	
Ipilimumab	Metastatic melanoma
Anti-CTLA-4 in combination with anti-PD-1	
Ipilimumab and nivolumab	Metastatic RCC
	Metastatic colorectal cancer
	HCC
	Metastatic melanoma
	NSCLC
	Esophageal squamous cell carcinoma
Anti-PD-1	
Nivolumab	Metastatic melanoma
	Late-stage NSCLC
	Late-stage SCLC
	Metastatic RCC
	Hodgkin´s lymphoma
	HNSCC
	Urothelial carcinoma
	Metastatic colorectal cancer
	HCC

Pembrolizumab	Metastatic melanoma
	Metastatic nonsquamous NSCLC
	Metastatic squamous NSCLC
	NSCLC
	Metastatic NSCLC
	Metastatic SCLC
	Metastatic HNSCC
	Hodgkin´s lymphoma
	Primary mediastinal large B cell lymphoma
	Metastatic urothelial carcinoma
	Non-muscle invasive bladder cancer
	Solid tumors
	Metastatic gastric and gastroesophageal junction carcinoma
	Recurrent locally advanced or metastatic squamous cell carcinoma of the esophagus
	Recurrent or metastatic cervical cancer
	HCC
	Recurrent locally advanced or metastatic Merkel cell carcinoma
	Metastatic RCC
Anti-PD-L1	
Atezolizumab	Locally advanced or metastatic urothelial carcinoma
	Metastatic nonsquamous NSCLC
	Metastatic triple-negative breast cancer
	ES-SCLC
Durvalumab	NSCLC
	ES-SCLC
	Locally advanced or metastatic urothelial carcinoma
Avelumab	Metastatic Merkel cell carcinoma
	Locally advanced or metastatic urothelial carcinoma
	Locally advanced or metastatic RCC

Table abbreviation: RCC (renal cell cancer), SCLC (small cell lung cancer), NSCLC (non-small cell lung cancer), ES-SCLC (extensive-stage small cell lung cancer, HCC (hepatocellular carcinoma), HNSCC (head and neck squamous cell carcinoma)

1.5 Immune-related adverse events

Whereas ICI led to a dramatic improvement in the treatment of patients with cancer, immune-mediated adverse events might occur due to the strong and systemic inflammatory response caused by ICIs (45).

IrAEs are fairly common and, as shown by Lee et al., are estimated to occur in approximately 90% of patients treated with anti-CTLA-4 therapy and about 70% of patients treated with anti-PD-1/PD-L1 therapy (58). Those events can affect almost every organ system and can be classified into five categories: asymptomatic/mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4) and fatal (grade 5) according to the Common Terminology Criteria for Adverse Events (CTCAE) (59). The exact pathophysiologic mechanisms underlying irAEs are unclear, yet some mechanisms have been proposed to play a role in the development of irAEs such as autoreactive T cells, B cells/autoantibodies, cytokines and the host microbiome (59–62).

Autoreactive T cells: Anti-CTLA-4 and anti-PD-1/PD-L1 therapy can enhance antitumor responses through T cell proliferation and activation. However, they also destroy peripheral T cell tolerance, leading to hyperinflammation and autoimmunity by the same mechanisms (58–60) most commonly happening in organs that are highly dependent on peripheral T cell tolerance to maintain immune homeostasis, such as the skin and colon (63,64).

B cells/autoantibodies: Anti-CTLA-4 and anti-PD-1/PD-L1 therapy induce an accumulation of activated B cell subpopulations, mainly $CD21^{lo}$ B cells, and antibody-producing plasmablasts, which correlates with irAEs (58,65).

Cytokines: When cytokines, which are important for immune homeostasis, are out of balance, the result can be hyperinflammation or autoimmunity. ICIs may cause that imbalance by increasing cytokine production (58). Especially IL-17 has been associated with irAEs, such as colitis, due to its proinflammatory functions (66). On one hand low levels of IL-6 are also associated with irAEs (59,67). On the other Phillips et al. showed that high levels of IL-6 were a common finding in patients with cutaneous irAEs (68).

Microbiome: The microbiome also influences immune homeostasis (69). Organs that are constantly in contact with external influences, such as skin, lung and colon, are rich in commensal organisms which in turn may influence the organ´s susceptibility to autoimmune and inflammatory diseases. This may also explain why irAEs are most prevalent in these organs (58).

Dubin et al. could show that patients without colitis had increased fecal abundance of the Bacteroidetes phylum (70).

1.5.1 Cutaneous irAEs

Cutaneous adverse events represent the most frequent irAE. They appear more often in patients treated with anti-CTLA-4 therapy (68%) than in patients treated with anti-PD-1/PD-L1 therapy (38%) and can present as maculopapular rash, lichenoid/lichen planus-like eruption, psoriasiform dermatitis, eczematous eruption, vitiligo, alopecia and bullous pemphigoid, Stevens-Johnson syndrome (60,71,72). Maculopapular rash occurs in approximately 49-68% patients receiving anti-CTLA-4 treatment, whereas in patients receiving anti-PD-1/PD-L1 treatment it only appears in 20%. The second most common cutaneous irAE after maculopapular rash is pruritus with an overall prevalence of 13-20%, and it also has a higher incidence in patients with anti-CTLA-4 agents (25-36%) (73).

1.5.2 Gastrointestinal irAEs

The most common gastrointestinal manifestations of irAEs are diarrhea and colitis (59,60). In patients receiving anti-PD1/PD-L1 treatment the prevalence of colitis is 0.7-1.6% and of diarrhea 12.1-13.7%, therefore, lower than in patients receiving anti CTLA-4 treatment with incidences of 8-22% for colitis and 27-54% for diarrhea (59,74).

1.5.3 Endocrine irAEs

Barroso-Sousa et al. showed that endocrine diseases appeared in roughly 10% of the patients treated with ICI (75). The most common diseases are hypothyroidism, hyperthyroidism, hypophysitis and insulin-deficient diabetes (59,75). The incidence for endocrine irAEs varies depending on the usage of mono- or combination therapy (75,76). Barroso-Sousa et al. reported the highest incidences of hypothyroidism (13.2%), hyperthyroidism (8%), and hypophysitis (6.4%) in patients treated with combination therapy (75).

1.5.4 Pulmonary irAEs

Pulmonary irAEs are frequently reported, with interstitial lung disease and pneumonia being the most common findings (59,77). Nishino et al. showed that pneumonitis appeared in 2.7% for PD-1 inhibitor monotherapy, and 6.6% for combination therapy (78).

1.5.5 Neurological irAEs

The incidence of neurological irAEs is about 1% (79). Neurological irAEs are more frequent in patients treated with a combination of anti-PD-1 and anti-CTLA-4 therapy, followed by anti-PD-1 mono- and last anti-CTLA-4 monotherapy (80). In a systemic review by Marini et al., it was found that neuromuscular presentations constituted 75% of all the neurological irAEs, while central nervous system complications accounted for 25%. The most prevalent neuromuscular manifestations were myositis (32%), Guillan-Barré syndrome and other peripheral neuropathies (22%) and myasthenic syndromes (14%) (81). Whereas encephalitis (13%), cranial neuropathies (7%) and meningitis (3%) were reported with lesser frequency (81).

1.5.6 Renal irAEs

Acute kidney injury represents the most prevalent renal irAE with an estimated incidence of 1-2% in patients with monotherapy and 5% with combination therapy (82,83). Acute tubulointerstitial nephritis is the primary histological finding in those patients (82). Glomerular diseases such as minimal change disease, IgA nephropathy, focal segmental glomerulosclerosis, Goodpasture´s disease, vasculitis and immune complex-mediated glomerulonephritis were also documented (83).

1.6 ICI-related cardiovascular toxicities

In clinical trials evaluating ICI, specific cardiovascular adverse events (CVAE) are reported as relatively rare (84,85). In a meta-analysis compromising 83,315 patients, Nielsen et al. demonstrated that the incidence of CVAE, in this meta-analysis defined as “any-grade and high-grade cardiovascular adverse events and/or myocarditis” in patients treated with anti-PD1/anti-PD-L1 antibodies was 0.8% in clinical trials. The incidence of patients undergoing treatment with anti-CTLA4 antibodies was 1.07% (86).

In a retrospective study, Chen et al. sought to assess the incidence of CVAEs in a real-world setting.
The most common comorbidities were preexisting heart disease, diabetes mellitus and hypertension among the 1,047 enrolled patients. Chen et al. reported an incidence of 7.0% (87).

In a pooled analysis conducted by Naqash et al., the incidence of MACE in patients with anti-PD1/anti-PD-L1 antibodies was found to be 0.6% (88). Palassin et al. pointed out that the incidence of MACE in a real-world setting may be higher than that observed by Naqash et al., due to a number of factors: It is a common practice in clinical trials to exclude patients with coexisting medical conditions (89). The study was conducted between 2015 and 2019, whereas the first description of ICI-related myocarditis was published in 2016 by Johnson et al. (89,90). Furthermore, the relatively short follow-up period and the relatively low sample size may not be adequate (89).
Many clinical trials supporting current anticancer therapies failed to report cardiovascular events. Bonsu et al demonstrated that 51.3% of trials in cancer drug trials (from the Drugs@FDA, clinicaltrials.gov, MEDLINE, and publicly available U.S. Food and Drug Administration (FDA) drug reviews) reported no MACE during follow-up. MACE was defined as incident myocardial infarction, stroke, heart failure, coronary revascularization, atrial fibrillation, or cardiovascular death. The authors speculated that particularly in the beginning of drug trials, the primary focus was anticancer efficacy and not CVAE. This might have led to an underrepresentation of MACE (91).

In general, CVAE encompass a range of possible outcomes, including, but not limited to, myocarditis, pericarditis, arrhythmias, cardiomyopathies, vasculitis, myocardial infarction, sudden cardiac death, stroke, atherosclerosis/atherosclerotic lesions and thromboembolic events (84,85,92).
It has been estimated that T-cell proliferation and expansion play a crucial role in the development CVAE (90,92). It has also been found that T cells have a pivotal function in the progression of atherosclerosis, which can result in atherosclerotic plaque rupture and subsequent atherosclerosis-related acute vascular events (92).

1.6.1 Arterial thromboembolic events

In a systemic review by Solinas et al. the incidence of venous thromboembolic events (VTE) was found to be 2.7% amongst a total of 20,273 patients, whereas the incidence of arterial thromboembolic events (ATE) was 1.1%. The majority of those events was associated with PD-1/PD-L1-inhibitors (93).
Drobni et al. demonstrated that the use of ICI is associated with an augmented incidence of cardiovascular events. In their study, the authors defined cardiovascular events as a

composite of myocardial infarction, coronary revascularisation and ischemic stroke. The findings of Drobni et al. showed that ICI-treatment was associated with a more than 4-fold increase in the risk of a composite cardiovascular outcome, a 7-fold increase in the risk of myocardial infarction, a 3-fold increase in the risk of coronary revascularisation and a 4-fold increase in the risk of ischemic stroke (94).
In an imaging sub-study within the same cohort, Drobni et al. included 40 patients treated with ICI, longitudinally quantifying thoracic atherosclerotic plaque burden. They found 3-fold progression in the total plaque volume from 2.1% per year pre ICI treatment to 6.7% per year post ICI treatment (94).

Synoptically, discrepant rates of cardiovascular adverse events were reported in clinical trials evaluating ICI in patients with cancer, with emerging real-world data suggesting a relevant cardiovascular risk and some studies reporting a high risk of MACE in ICI treated patients. This topic is of particular relevancy based on the high clinical efficacy of ICI, with a relevant proportion of patients with sustained treatment responses, rendering MACE as important secondary cause of morbidity and mortality. Further, the potential impact on long-term cardiovascular risk in patients treated in the curative setting warrants further investigation. Currently, limited data exist on the cumulative risk of MACE, potential risk factors, and outcomes of MACE in ICI treated patients. Therefore, the aim of the present study was to quantify MACE risk and explore the clinical risk profiles for MACE among patients treated with ICI at the Medical University of Graz.

2 Methods

2.1 Study design and patient cohort

In this retrospective cohort study, adult patients with histologically confirmed cancer were included. In detail, patients treated with ICI at the Medical University of Graz between January 2015 and November 2021 included in the AUTRICHE registry were included. AUTRICHE is a registry-based study collecting data from ICI-treated patients in Austria. The study was approved by the institutional ethics committee (Medical University of Graz: No. 31-357 ex 18/19).

2.2 Study procedures and outcomes

Data on patient demographics, comorbidities, cancer characteristics, cancer treatment, ICI-therapy and study outcomes were obtained by electronic chart review. The primary study endpoint was the first occurrence of 4-point MACE, defined as a composite of non-fatal myocardial infarction, non-fatal stroke, hospitalization for heart failure, or cardiovascular death. The observation period for occurrence of MACE was defined as the time period from first day of ICI therapy until the case of an event of death, loss of follow-up, or the occurrence of MACE. Objective criteria and confirmation by an independent adjudication committee were mandatory for the verification of a MACE event.

Secondary study endpoints were (a) 3-point MACE, defined as a composite of non-fatal myocardial infarction, non-fatal stroke and cardiovascular death, (b) non-fatal MACE, defined as a composite of non-fatal myocardial infarction, non-fatal stroke, and hospitalization for heart failure, and (c) all-cause mortality, with data obtained from the official Austrian death registry and electronic medical files.

2.3 Statistical analysis

Baseline clinicopathologic characteristics and treatment specifics were summarized using absolute frequencies and percentages or median with corresponding interquartile range (IQR), as appropriate. The median follow-up time was calculated with the reverse Kaplan-Meier method. When analyzing MACE outcomes, a competing risk framework accounting for all-cause mortality as competing outcome event was used to avoid overestimation of cumulative risks in the setting of substantial underlying mortality (95). Cumulative incidences of MACE were obtained with the competing risk estimator and corresponding standard errors according to Marubini and Valsecci, applying Gray's test for between-

group comparisons (96,97). The association between ICI-exposure and MACE risk was analyzed in a time-dependent analyses, using a multi-state model, adjusting for potential confounders. Here, follow-up was initiated at the day of cancer diagnosis and patients were followed for non-fatal MACE (to avoid immortal time-bias), loss-of follow-up, or death. ICI therapy was implemented as time-dependent covariable in a Cox regression model. Further, the incidence rates for MACE between cancer diagnosis and ICI initiation compared to after ICI initiation were analyzed, calculating incidence rate ratio and 95% confidence intervals (CI). Similarly, the association between MACE after ICI-initiation and risk of all-cause mortality was analyzed in time-dependent analysis and by using a landmark analysis for visualization.

Further, modelling of the association between clinicopathologic risk factors and biomarkers with MACE risk was conducted in a proportional sub-hazard regression model according to Fine and Gray, adjusting for potential confounders in multivariable analysis (cancer type, stage, age, sex) (98). Next, candidate variables with a $p<0.01$ in univariable risk factor analysis were implemented in a joint multivariable model. Upon backwards elimination in a stepwise procedure, the ideal candidate predictors for MACE were identified, using a cut-off for variable omission of $p>0.157$, according to optimization of the Akaike iteration criterion (AIC) for nested hierarchical models (99). Models identified in the variable selection process were used to derive a pragmatic point-based risk score for future MACE risk. The discriminatory performance of the model was evaluated via internal validation, using a bootstrapping algorithm to draw 100 random samples from the study population, calculating the discriminatory index Harrel´s C and corresponding 95% CIs.

An alpha level of 0.05 was defined as threshold for statistical significance. No adjustment for multiple testing was performed due to the descriptive nature of the primary study outcome (i.e., cumulative incidence of 4-point MACE after ICI initiation), and the hypothesis-generating, exploratory nature of secondary study analyses. Statistical analyses were performed using Stata version 16.1 (StataCorp LP, Collage Station, TX, USA).

3 Results

3.1 Study cohort

Overall, 452 consecutive patients treated with immune checkpoint inhibitors (ICI) at the Medical University of Graz between 01.01.2015 and 30.11.2021 were included. The median age at ICI-initiation was 66 years (interquartile range [IQR]: 58-72) and 177 patients (39.2%) were female. The Eastern Cooperative Oncology Group (ECOG) performance status was 0 in 294 patients (65.0%), 1 in 144 (31.9%), and ECOG ≥2 in 14 (3.1%). The median revised Charlson comorbidity index (assigning points according to the original version without cancer variable) was 3 (IQR: 2-4). A prior atherosclerotic cardiovascular disease was present in 95 patients (21.0%), including coronary artery disease (n=63, 13.9%), peripheral artery disease (n=31, 6.9%), and cerebrovascular disease (n=25, 5.5%). A prior history of acute coronary syndrome (ACS) was present in 17 patients (3.8%), whereas a prior stroke history was present in 18 (4.0%). Overall, 13 patients (2.9%) had congestive heart failure at ICI initiation and 36 (8.0%) had atrial fibrillation. COPD (chronic obstructive pulmonary disease) was diagnosed in 90 patients (19.9%), arterial hypertension in 209 (46.2%), hyperlipidaemia in 75 (16.6%) and diabetes mellitus in 56 (16.6%).

Of 319 patients with available information on smoking status, 84 (26.3%) reported as current smokers, 164 (51.4%) as previous smokers (defined as smoking cessation >28 days prior to ICI initiation), and 72 (15.8%) as never-smokers. Continuous antiplatelet therapy was used in 74 patients (16.4%), whereas continuous therapeutic anticoagulation was used in 76 (16.8%).

The most frequent tumour types were non-small cell lung cancer (NSCLC; n=181, 40.0%), melanoma (n=76, 16.8%), and renal cell carcinoma (n=70, 15.5%%), followed by urothelial cancer (n=45, 10.0%), head and neck squamous cell carcinoma (n=20, 4.4%) and various other cancer types (Table 1). At ICI initiation, most patients had distant metastatic cancer (i.e., stage IV disease; n=398, 88.1%). Most patients received ICI in palliative treatment intention (n=420, 92.9%), mostly in the first line (n=209, 46.2%) or second line setting (n=144 (31.9%). The median time from cancer diagnosis to ICI therapy was 13.3 months (IQR: 3.4-38.3). The number of applied ICI treatment cycles was highly heterogeneous (median: 6, IQR: 3-13, range: 1-74). The most frequent ICI agents were nivolumab (n=219, 48.5%) and pembrolizumab (n=197, 43.6%) followed by ipilimumab-

nivolumab-combination (n=26, 5.7%), atezolizumab (n= 25, 5.5%), ipilimumab (n=7, 1.6%) and durvalumab (n=4, 0.9%). Concomitant chemotherapy was used in 43 patients during ICI therapy (9.5%) and concomitant targeted anticancer agents in 12 patients (2.7%). A subsequent line of systemic anticancer therapy after ICI was applied in 170 patients (37.6%). Details on baseline characteristics of patients included in the study cohort are provided in Table 2.

Table 2: Baseline characteristics of study cohort (n=452)

Variable	**n (% missing)**	**Median [IQR] or count (%)**
Demographics and clinical characteristics		
Age (years)	452 (0%)	66 [58-72]
Female	452 (0%)	177 (39.2%)
ECOG	452 (0%)	
-ECOG 0		294 (65.0%)
-ECOG 1		144 (31.9%)
-ECOG ≥2		14 (3.1%)
Charlson comorbidity index		9 [7-10], range: 2-15
Revised Charlson comorbidity index*		3 [2-4], range: 0-10
BMI (kg/m²)	452 (0%)	24.7 (21.4-28.1)
Smoking history	319 (29.4%)	
- Current smoking		84 (26.3%)
- Former smoker		164 (51.4%)
- Never smoker		71 (22.3%)
Comorbidities	452 (0%)	
- Prior atherosclerotic cardiovascular disease**		95 (21.0%)
- Prior coronary artery disease		63 (13.9%)
- Prior cerebrovascular disease		25 (5.5%)
- Prior peripheral artery disease		31 (6.9%)
- Prior ACS		17 (3.8%)
- Prior stroke		18 (4.0%)
- Prior COPD		90 (19.9%)
- Chronic heart failure		13 (2.9%)
- Atrial fibrillation		36 (8.0%)
- Arterial hypertension		209 (46.2%)
- Diabetes mellitus		56 (12.4%)
- Hyperlipidaemia		75 (16.6%)
Prior antiplatelet therapy	452 (0%)	74 (16.4%)

Prior therapeutic anticoagulation	452 (0%)	76 (16.8%)
Cancer characteristics		
Tumour type	452 (0%)	
-Non-small cell lung cancer		181 (40.0%)
-Melanoma		76 (16.8%)
-Renal-cell carcinoma		70 (15.5%)
-Bladder / urothelial		45 (10.0%)
-HNSCC		20 (4.4%)
-Colorectal		11 (2.4%)
-Breast		10 (2.2%)
-Gastroesophageal		10 (2.2%)
-Other***		29 (6.4%)
Stage	452 (0%)	
-I		2 (0.4%)
-II		5 (1.1%)
-III		42 (9.3%)
-IV		398 (88.1%)
-unknown		5 (1.1%)
Therapy specifics		
Treatment setting	452 (0%)	
-Palliative		420 (92.9%)
- 1st line		209 (46.2%)
- 2nd line		144 (31.9%)
- 3rd line		48 (10.6%)
- 4th line		19 (4.2%)
-Adjuvant		19 (4.2%)
-Other****		13 (2.9%)
Immune checkpoint inhibitor agent	452 (0%)	
-Nivolumab		219 (48.5%)
-Pembrolizumab		197 (43.6%)
-Ipilimumab + Nivolumab		26 (5.7%)
-Atezolizumab		25 (5.5%)
-Ipilimumab		7 (1.6%)
-Durvalumab		4 (0.9%)
Therapy cycles	452 (0%)	6 [3-13]; range: 1-74
Time from diagnosis to immunotherapy (months)	452 (0%)	13.3 (3.4-38.3)
Concomitant therapy during immune checkpoint inhibitor	452 (0%)	
-Chemotherapy		43 (9.5%)
-Targeted Therapy		12 (2.7%)
-Radiotherapy		3 (0.7%)

Subsequent systemic therapy	452 (0%)	170 (37.6%)
Additional subsequent ICI therapy*****	452 (0%)	4 (0.9%)

Table legend: *Revised Charlson comorbidity index without points assigned for cancer variable. ** Prior atherosclerotic cardiovascular disease include prior coronary artery disease, peripheral artery disease, and cerebrovascular disease. ***Others include cancer of unknown primary (n=8), small cell lung cancer (n=4), prostate cancer (n=3), anal cancer (n=2), mesothelioma (n=2), thyroid cancer (n=2), biliary tract cancer (n=1), osteosarcoma (n=1), chondrosarcoma (n=1), malignant peripheral nerve sheath tumour (n=1), small intestine cancer (n=1), soft tissue sarcoma (n=1), squamous cell carcinoma of the skin (n=1), and thymic carcinoma (n=1). ****Including maintenance setting and neoadjuvant therapy. *****Including pembrolizumab (n=2) and nivolumab (n=2) monotherapy.

3.2 Follow-up and survival

Over a median follow-up period of 36.9 months (IQR: 20.5-54.9), 272 patients died (59.65% of the study population). The Kaplan-Meier survival estimate at 1 year was 58.5% (95%CI: 53.8-63.1), at 2 years 43.4% (38.5-48.2), at 3 years 35.6% (95%CI: 30.7-40.6), at 4 years 30.6% (95%CI: 25.5-35.8), and after 5 years 30.0% (95%CI: 24.9-35.2). Figure 4 displays the Kaplan-Meier survival function of the study cohort. The cause of death was retrievable in 269 patients. In those, the adjudicated cause of death was cancer progression in 202 patients (77.3%), cancer-related causes other than progression in 23 patients (8.6%), other causes in 36 (13.4%), cardiovascular causes in 6 (2.2%) and cancer treatment-related in 2 patients (0.7%), including one patient with pneumonia and acute kidney injury and one patient with treatment related Steven-Johnson-Syndrome, Sepsis and multi-organ failure.

Figure 1: Kaplan-Meier survivor function of study cohort

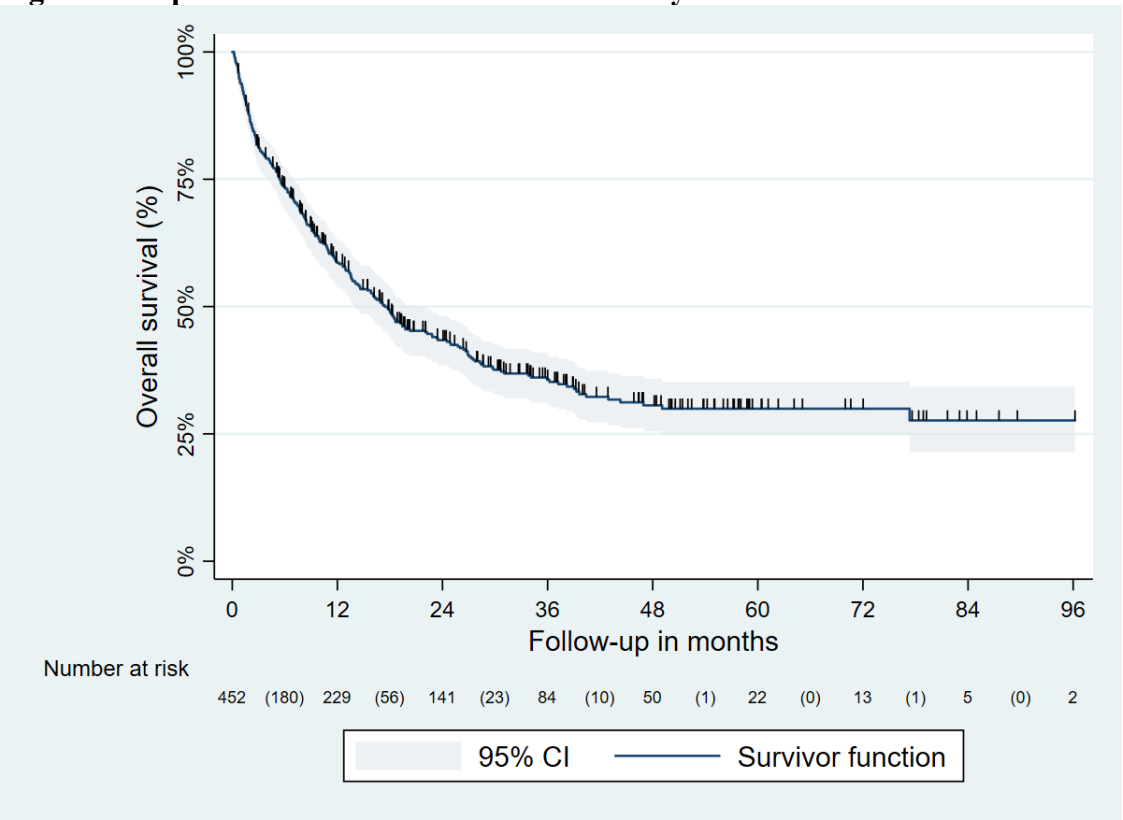

Figure legend: Survivor function of the full study cohort after initiation of ICI. Ticks indicate censoring. Risk-table indicates number at risk at each timepoints and number of failure events for risk timeframe in brackets. Abbreviation: CI: confidence interval.

3.3 Risk of major adverse cardiovascular events after ICI initiation

Over a median follow up of 36.9 months (IQR: 20.5-54.9), we observed a major adverse cardiovascular event (MACE) in 47 patients (10.4% of the study population). Of those, the index MACE was stroke in 19 patients (4.2%), hospitalisation for heart failure in 15 patients (3.3%), ACS in 8 patients (1.8%), and cardiovascular death in 6 patients (1.3%). The median time from ICI initiation to the occurrence of MACE was 5.7 months (IQR: 2.3-18.3). The majority of MACE occurred during the active ICI treatment timeframe (n=32, 68% of MACE), whereas 17 MACE (32%) occurred ≥3 months after the last ICI cycle.

The cumulative incidence in competing risk analysis of 4-point MACE (i.e., composite of ACS, stroke, hospitalisation for heart failure, or cardiovascular death) over the complete follow-up duration was 14.3% (95%CI: 9.7-19.7). The corresponding competing risk cumulative incidence estimates at 6 months were 5.4% (95%CI: 3.5-7.7), at 1 year 7.6% (95%CI: 5.3-10.3), at 2 years 9.0% (95%CI: 6.5-12.0), at 3 years 10.6% (95%CI: 7.8-

13.9), at 4 years 11.6% (95%CI: 8.6-15.2) and at 5 years 12.8% (95%CI: 9.1-16.2). The cumulative incidence of 4-point MACE is visualized in Figure 5.

Figure 2: Cumulative incidence of 4-point MACE after ICI initiation

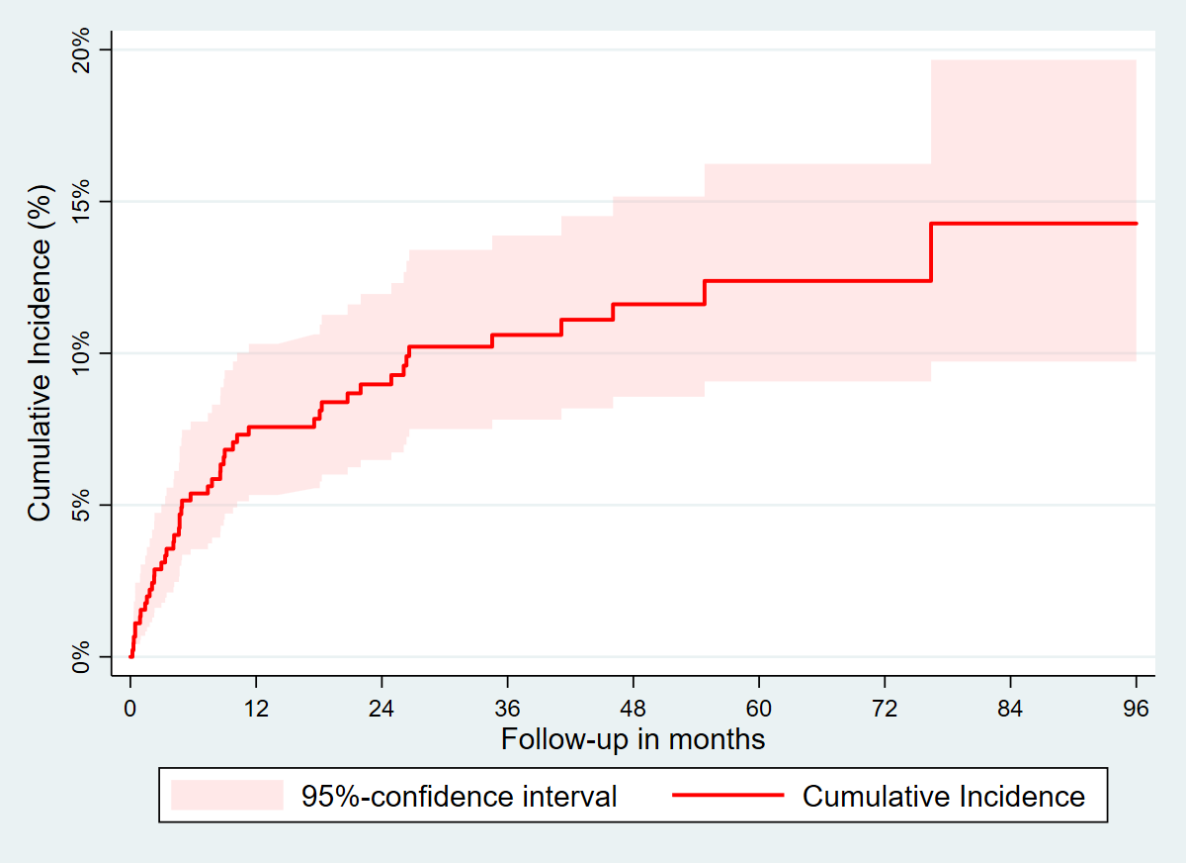

Figure legend: Cumulative incidence function and 95%-confidence interval obtained in competing risk analysis, accounting for all-cause mortality as competing outcome event. 4-point MACE represents a composite outcome of ACS, stroke, hospitalisation for heart failure, or cardiovascular death, whichever came first.

In a sensitivity analysis, we analysed the cumulative incidence of 3-point MACE after ICI initiation, defined as a composite outcome of ACS, stroke, or cardiovascular death. The cumulative incidence of 3-point MACE over the complete duration of follow-up was 10.7% (95%CI: 6.5-16.1). The corresponding cumulative incidence at 6 month was 3.8% (95%CI: 2.3-5.9), at 1 year 5.3% (95%CI: 3.4-7.6), at 2 years 6.1% (95%CI: 4.1-8.7), at 3 years 7.4% (95%CI: 5.1-10.3), at 4 years 8.0% (95%CI: 5.5-11.0), and at 5 years 8.8% (95%CI: 5.9-12.2). The cumulative incidence of 3-point MACE is visualized in Figure 6.

Figure 3: Cumulative incidence of 3-point MACE after ICI initiation

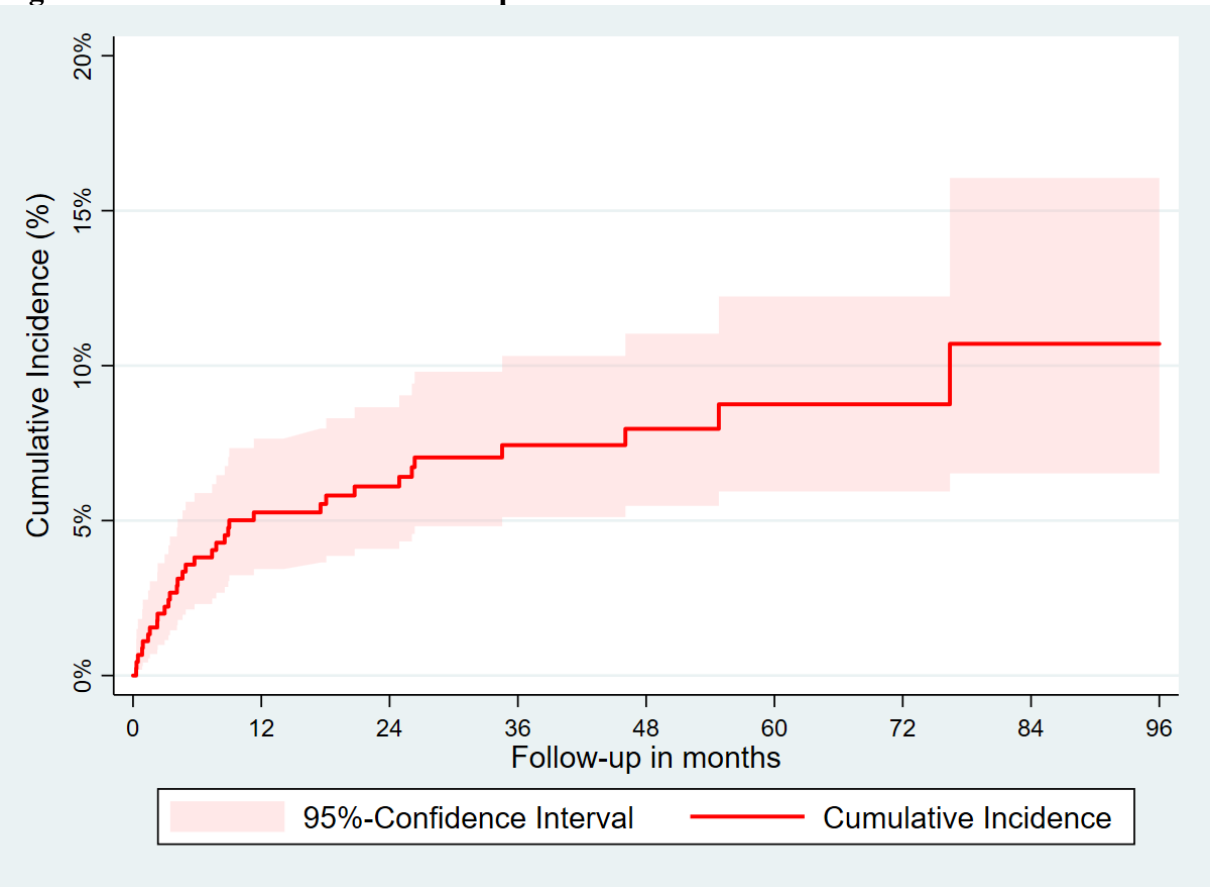

Figure legend: Cumulative incidence function and 95%-confidence interval obtained in competing risk analysis, accounting for all-cause mortality as competing outcome event. 3-point MACE represents a composite outcome of ACS, stroke, or cardiovascular death, whichever came first

3.4 ICI as time-dependent risk fator for MACE

Upon analysing the time-dependent effect of ICI therapy on the risk of MACE in the study cohort, a multi-state model was used, analysing non-fatal MACE to avoid immortal-time bias. Patients were followed from the time of cancer diagnosis until death, loss of follow-up, or non-fatal MACE, using ICI-therapy as time-dependent covariable in a Cox proportional hazards model. In total, 56 non-fatal MACE were observed. Of those, 39 events occurred after ICI initiation and 16 events were diagnosed between cancer diagnosis and ICI initiation, including ACS in 5 patients, stroke in 7 patients, and hospitalisation for heart failure in 4 patients. The transition hazard ratio (THR) for MACE after initiation of ICI compared to the timeframe from cancer diagnosis to ICI therapy was 4.65 (95%CI: 2.53-8.52, p<0.001). This association prevailed upon multivariable adjustment for age, sex, cancer stage and cancer type (adjusted THR: 4.43 [95%CI: 2.38-8.24, p<0.001]).

The incidence rate of non-fatal MACE between cancer diagnosis and ICI start was 1.1 events / 100 patient years (PY) compared to 5.5 events / 100PY after ICI initiation. The incidence rate ratio for non-fatal MACE for the time period after ICI initiation compared to the timeframe between cancer diagnosis and ICI initiation was 4.59 (95%CI: 2.45-8.61, p<0.001).

3.5 Outcomes of MACE

The occurrence of MACE led to permanent discontinuation of ICI therapy in 7 patients (15% of all MACE). MACE leading to treatment discontinuation included stroke in 4 patients, heart failure in 3 patients, and heart-failure with new-onset cardiac arrythmia in one patient. Overall, 6 patients had a fatal cardiovascular event after ICI-initiation (case-fatality rate: 12.8%).

The association of the occurrence of MACE after ICI initiation with all-cause mortality was analysed in a time-dependent analysis. The THR for death after the occurrence of MACE was 2.49 (95%CI: 1.65-3.75, p<0.001). This association prevailed upon multivariable adjustment for age, sex, cancer stage and cancer type (THR: 2.53 [95%CI: 1.66-3.85, p<0.001]). The median overall survival after MACE was 1.91 months (95%CI: 0.85-4.53), compared to 18.6 months (95%CI: 15.8 vs 24.87) in those without MACE (Mantel-Byar p<0.001). In Figure 7, a landmark analysis of overall survival according to the occurrence of MACE within 3 months after ICI initiation is visualized.

Figure 4: Landmark analysis of overall survival according to MACE-diagnosis within 3 months of ICI therapy.

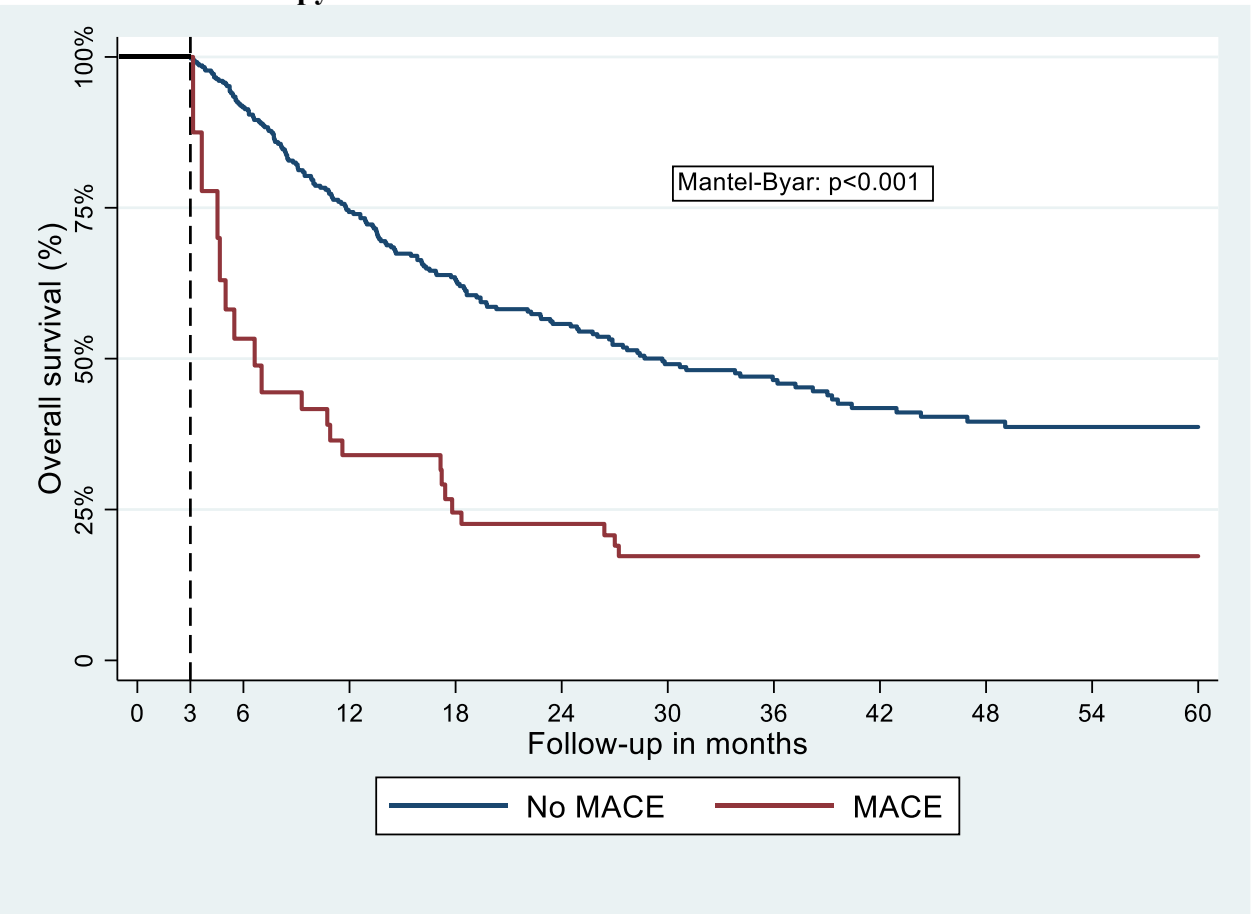

Figure legend: Overall survival estimates obtained for patients alive after 3 months of ICI therapy according to the occurrence of MACE until the set landmark at 3 months.
Abbreviation: MACE: major adverse cardiovascular event.

3.6 Risk factors for MACE

The association of baseline clinicopathologic and treatment specifics with future risk of 4-point MACE was analysed in competing risk regression, accounting for all-cause death as competing outcome event. Higher age was associated with increased MACE-risk (sub-distribution hazard ratio [SHR] per 10-year increase: 1.67 [95%CI: 1.18-2.65], SHR for age ≥60 vs others: 2.27 [95%CI: 1.06-4.88]), whereas sex was not associated with MACE (SHR for male vs female: 1.14 [95%CI: 0.63-2.07]). Higher MACE risk was observed for decreased performance status (SHR for ECOG≥1 vs 0: 1.99 [95%CI: 1.12-3.51]), higher BMI (SHR per point increase: 1.09 [95%CI: 1.03-1.15]), and higher revised Charlson Comorbidity Index (SHR per point increase: 1.21 [95%CI: 1.06-1.38]). The SHR for MACE for patients with prior atherosclerotic cardiovascular disease was 2.22 (95%CI: 1.23-4.02). No association with MACE was observed for prior antiplatelet therapy (SHR:

1.61 [95%CI: 0.83-3.17]) or therapeutic anticoagulation (SHR: 1.72 [95%CI: 0.89-3.30]) at study baseline. Smoking status was not significantly associated with future MACE risk (SHR for current smokers vs other: 1.22 [95%CI: 0.59-2.54]; SHR for ≥20 packyears vs others: 1.29 [95%CI: 0.63-2.64]).

The SHR for MACE within specific cancer types was 1.73 (95%CI: 0.97-3.07) for NSCLC, 0.61 (95%CI; 0.26-1.44) for melanoma, 1.17 (95%CI: 0.55-2.49) for renal cell carcinoma, and 1.21 (95%CI: 0.48-3.08) for urothelial cancers. No association was observed between cancer stage and MACE risk (SHR for stage IV vs others: 0.96 [95%CI: 0.41-2.27]). The cumulative incidence of MACE was 18.7% (95%CI: 11.5-26.5) for NSCLC, 11.8% (95%CI: 3.7-24.8) for melanoma, 17.1% (95%CI: 6.9-21.3) for renal cell carcinoma, and 13.3% (4.7-26.4) for urothelial cancer.

Different therapeutic strategies were not significantly associated with MACE risk, with an SHR for pembrolizumab therapy of 1.15 (95%CI: 0.65-2.03), for nivolumab of 0.70 (95%CI: 0.39-1.26), for ipilimumab of 0.82 (95%CI: 0.26-2.64), and for concomitant chemotherapy of 0.90 (95%CI: 0.32-2.51). The SHR for MACE per ICI-cycle increase was 1.00 (95%CI: 0.98-1.02).

No significant difference in MACE risk was observed according to levels of baseline biomarkers including haemoglobin (SHR per unit increase: 1.09 [95%CI: 0.95-1.24]), platelet count (SHR per unit increase: 1.00 [95%CI: 1.00-1.00]), leucocyte count (SHR per unit increase: 1.02 (95%CI: 0.97-1.07), lactate dehydrogenase (SHR per 100 units increase: 1.01 [95%CI: 0.93-1.11]), or albumin (SHR: 0.85 [95%CI: 0.56-1.29]). Baseline levels of C-reactive protein were associated with a higher risk of MACE (SHR for levels ≥5mg/dl: 2.30 [95%CI: 1.04-5.11]). Comprehensive results of risk factor analyses are presented in Table 3.

Table 3: Risk factors for MACE after ICI-initiation.

Variable	**SHR for MACE (95%CI)**
Male sex	1.14 (0.63-2.07), p=0.660
Age (per 10-year increase)	1.67 (1.18-2.65), p=0.004
Age (≥60 vs below)	2.27 (1.06-4.88), p=0.035
Smoking	
- Current vs others	1.22 (0.59-2.54), p=0.591
- Current or former vs never	0.98 (0.44-2.16), p=0.955
- PY: ≥20 vs others	1.29 (0.63-2.64), p=0.480
ECOG (≥1 vs 0)	1.99 (1.12-3.51), p=0.018

BMI (per point increase)	1.09 (1.03-1.15), p=0.002
Charlson Comorbidity Index (per point increase)*	1.21 (1.06-1.38), p=0.005
Prior atherosclerotic cardiovascular disease	2.22 (1.23-4.02), p=0.008
Arterial hypertension	2.16 (1.19-3.91), p=0.011
Hyperlipidaemia	1.63 (0.83-3.19), p=0.155
Diabetes mellitus	1.71 (0.80-3.65), p=0.163
Antiplatelet therapy at baseline	1.61 (0.83-3.17), p=0.158
Therapeutic anticoagulation at baseline	1.72 (0.89-3.30), p=0.105
Cancer type	
- NSCLC vs others	1.73 (0.97-3.07), p=0.059
- Melanoma vs others	0.61 (0.26-1.44), p=0.265
- Renal vs others	1.17 (0.55-2.49), p=0.678
- Urothelial vs others	1.21 (0.48-3.08), p=0.690
- HNSCC vs others	n/e (no events in HNSCC)
Stage IV vs I-III	0.96 (0.41-2.27), p=0.931
Treatment	
- Pembrolizumab vs other	1.15 (0.65-2.03), p=0.629
- Nivolumab vs others	0.70 (0.39-1.26), p=0.235
- Ipilimumab vs others	0.82 (0.26-2.64), p=0.746
- Concomitant chemotherapy vs others	0.90 (0.32-2.51), p=0.839
- Treatment cycles (per cycle increase)	1.00 (0.98-1.02), p=0.891
- Treatment cycles (≥6 cycles vs others)	1.28 (0.70-2.34), p=0.423
Biomarkers	
- Haemoglobin (per unit increase)	1.09 (0.95-1.24), p=0.207
- Haemoglobin <10 g/dL	0.71 (0.25-1.99), p=0.512
- Platelet count (per unit increase)	1.00 (1.00-1.00, p=0.152
- Platelet count ≥300x10⁹/L	0.80 (0.44-1.48), p=0.482
- Leucocyte count (per unit increase)	1.02 (0.97-1.07), p=0.374
- Leucocyte count >12x10⁹/L	0.72 (0.28-1.84), p==.489
- CRP (per double)	1.12 (0.99-1.27), p=0.082
- CRP (≥5mg/dl)	2.30 (1.04-5.11), p=0.041
- LDH (per 100 unit increase)	1.01 (0.93-1.11), p=0.754
- Albumin (per unit increase)	0.85 (0.56-1.29), p=0.456

Table legend: SHR for 4-point MACE obtained in competing risk regression, accounting for all-cause death as competing outcome event. *Modified Charlson Comorbidity Index without points assigned for cancer variable. Abbreviations: BMI: body mass index; CRP: C reactive protein; ECOG: Eastern Cooperative Oncology Group performance index. HNSCC: head and neck squamous cell carcinoma; LDH: lactate dehydrogenase; MACE: major adverse cardiovascular event; NSCLC: non-small cell lung cancer; SHR: sub-distribution hazard ratio.

3.7 Risk score for MACE

Variables from univariable analysis were included in a joint multivariable model. Upon backwards selection of variables, omitting candidate variables in a stepwise process until a threshold of $p<0.157$ to optimize performance according to the Akaike iteration criterion, a pragmatic point-based risk score was derived. Patients were assigned +1 point for age ≥60 years, ECOG ≥1, a prior diagnosis of atherosclerotic cardiovascular disease, and CRP levels ≥5mg/dl (i.e., above the upper limit of the norm). Upon stratifying patients according to their assigned points, the cumulative incidence of MACE in patients with 0 points (n=33, 7.3%) was 3.1% /95%CI: 2.3-13.7), with 1 point (n=116, 25.7%) 7.3% (95%CI: 3.4-13.2), with 2 points (n=164, 36.3%) 11.6% (95%CI: 4.9-21.4), with 3 points (n=105, 23.2%) 23.2% (95%CI: 12.7-35.4), and with 4 points (n=34, 7.5%) 35.4% (95%CI: 18.4-52.8). The SHR for MACE per point increase was 1.80 (95%CI: 1.33-2.43, $p<0.001$).

Internal validation via bootstrapping was performed, evaluating the discriminatory performance of the risk score for MACE risk within 100 random samples drawn from the study population. The bootstrap adjusted concordance index (Harrel´s C) was 0.67 (95%CI: 0.59-0.75). In Figure 8, the competing risk cumulative incidence functions of MACE with patients stratified according to points assigned in the risk score is visualized.

Figure 5: Cumulative incidence of MACE according to point-based risk score.

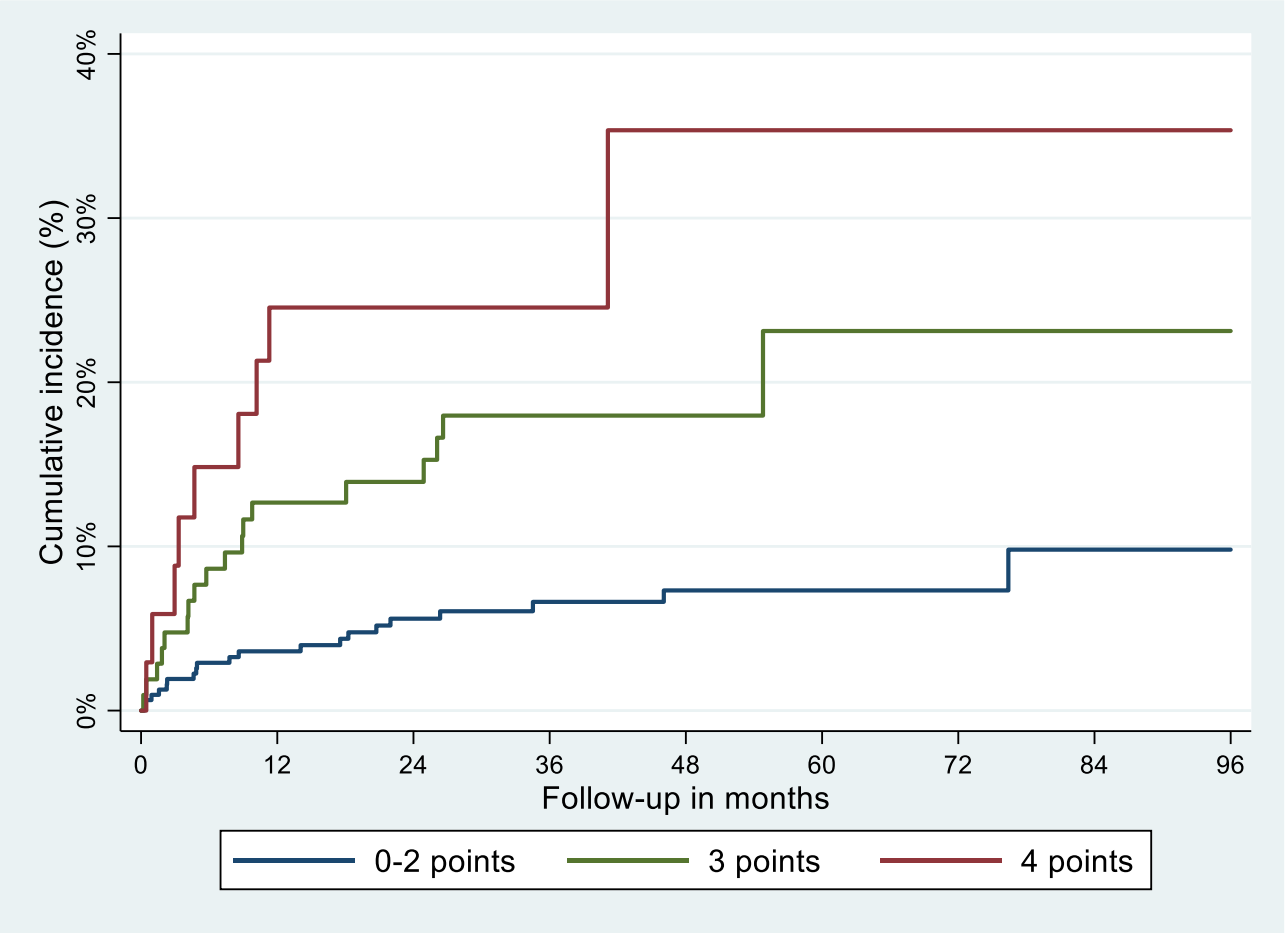

Figure legend: Cumulative incidence functions obtained in competing risk analysis, accounting for all-cause mortality as competing outcome event. Patients are stratified according to points assigned in a pragmatic point-based risk score, assigning +1 point to patients ≥60 years of age, ECOG ≥1, a prior diagnosis of atherosclerotic cardiovascular disease, and CRP levels ≥5mg/dl.

4 Discussion

In this single center retrospective cohort study, a clinically relevant risk of MACE was observed in patients treated with ICI. MACE risk was increased after ICI compared to the timeframe between cancer diagnosis and ICI start. Detrimental clinical outcomes were observed among patients developing a MACE, including treatment discontinuation, CV death and a strong association with risk of all-cause mortality. Based on risk factor analyses, risk of MACE seems to be strongly mediated by underlying patient-, cancer and treatment related factors. Further, a simple, pragmatic point-based risk score efficiently stratifying MACE risk might be used to identify high risk patients.

We showed that MACE occurred in 47 (10.4%) of 452 patients with a median time to event of 5.7 months (IQR: 2.3-18.3). Of those, the index MACE was stroke in 19 patients (4.2%), hospitalisation for heart failure in 15 patients (3.3%), ACS in 8 patients (1.8%), and cardiovascular death in 6 patients (1.3%). Recent studies with the same MACE endpoints tended to have lower incidences. In a retrospective study, Chan et al. showed that amongst the 4171 patients, MACE appeared in 116 patients (2.8%). Of those, the index MACE was stroke in 55 patients (1.3%), HF in 15 patients (0.4%), ACS in 34 patients (0.8%), and cardiovascular mortality in 18 patients (0.4%) (100).

In a recent retrospective pooled analysis of MACE by Naqash et al., they found fewer incidences of MACE (88). In 6,925 patients receiving ICI treatment, MACE appeared in only 40 (0.6%) patients. Of those MACE was categorized in myocarditis 18 (0.26%), ACS 8 (0.12%), dysrhythmias 6 (0.09%), cardiomyopathy 5 (0.07%), pericardial disorders 2 (0.03%) and Cardiac arrest 1 (0.01%) (88).

In a study with 2,842 patients, Drobni et al. reported an incidence of 4.2% with the endpoints being myocardial infarction, coronary revascularization and ischemic stroke (94). However, in a nationwide Danish study, the one-year absolute risk of MACE in patients treated with PD1 inhibitor with lung cancer was 9.7% and 6.6% in patients with melanoma; in patients treated with CTLA-4 inhibitor and melanoma 7.5%. The combined endpoints were arrhythmia, myocarditis, pericarditis, heart failure and cardiovascular death (101). Laenens et al. demonstrated similar incidences as we did in their cohort analysis. They included 672 patients and MACE occurred 69 times (10.3%). Of those, stroke/transient ischemic attack was present 15 (2.2%), HF 48 (7.1%) and ACS 15 (2.2%) times (102). Between study differences in MACE risk might be explained by differences in

underlying patient populations, reporting and documentation practices of cardiovascular adverse events, and variations in outcome definitions.

It is of significant importance to find out if there is a direct link between immunotherapy and an increased risk of MACE, or whether it is the cancer patient factor itself, with its possible comorbidities, that carries an increased risk of MACE.
ICIs block the inhibitory mechanisms of the patient's immune system, thereby triggering an immune response against tumour cells (45). However, by activating the immune system, immunological and inflammatory processes can become persistently intensified, leading to irAEs that can affect any organ (58).

Although ICI-associated myocarditis is rare (reported in less than 1% of patients), it has a very high mortality rate (1,103). The condition is well described, and several approaches have been proposed to explain its pathogenesis. However, the mechanisms of cardiotoxicity have not yet been fully investigated. It is assumed that inflammation, mediated by CD4+ T cells, plays the main role (104). There is evidence that PD-1 plays an important role in the prevention of myocarditis. Wang et al. were able to show that mice with a genetic predisposition to autoimmune diseases developed fatal myocarditis when lacking PD-1. In contrast, those with the same predisposition but normal PD-1 did not (105). In a similar study, Love et al. discovered that removing CTLA-4 on T cells also induced severe myocarditis in mice (106). However, when IL-12 was absent, the growth of CD8+ T cells was prevented and the risk of myocarditis decreased. In addition, PD-L1, which is present in human heart muscle, contributes to protection against immune-mediated cardiac damage and inflammation (107). Another possible mechanism of ICI-associated myocarditis is clonal expansion of T cells. Because the tumor and heart muscle have a similar or identical antigen and immune checkpoint inhibitors (ICI) can enhance the activity of T cells targeting that antigen, potentially leading to the development of autoimmune myocarditis (90).

Atherosclerotic events are notably more common, and accumulating evidence suggests that immune checkpoint inhibitors (ICIs) may worsen underlying inflammatory conditions (108). Atherosclerosis is widely recognized as a chronic inflammatory disease, driven by complex interactions between various immune cells through the release of inflammatory mediators within the arterial wall (109,110). Immune checkpoints play a key role in

regulating these interactions and their blockade with monoclonal antibodies may significantly influence the course and progression of atherosclerosis (111).

In simple terms, activated M1 macrophages initiate and sustain inflammation, whereas activated M2 macrophages reduce inflammation. M1 macrophages, on one hand, induce the augmentation of intracellular lipids, formation of foam cells and secret proinflammatory cytokines like IL-1β, IL-6 and TNF-α. On the other hand, M2 macrophages are polarized in association with IL-4 and IL-13 and secret anti-inflammatory cytokines like IL-10 and cause collagen formation and lipid clearance. An imbalance between those pro- and anti-inflammatory factors is an important factor in the development, progression and rupture of atherosclerotic plaques (112,113).

Atherosclerosis is hypothesised to be a mainly T cell driven disease. T cells that play a role in its pathophysiology are CD8+ cytotoxic T cells and CD4+ lymphocytes such as T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17) and T regulatory cells (Treg) (114,115). Th1 cells represent the predominant cell type observed in plaques and are associated with plaque progression. Th1 cells are considered to produce the proinflammatory cytokines TNFα and IFN-γ (116,117). IFN-γ triggers cell recruitment of macrophages and T cells and enhances cytokine secretion and plaque formation (115). Th2 cells suggested to be atheroprotective via downregulation of the production of IFN-γ (115) . However, King et al. could show that the loss of IL-4, which is also a Th2 cytokine, in mice led to fewer plaque formation (118). Therefore, the role for Th2 cells in atherosclerosis still remains unclear and needs further studies (115). Treg cells play an atheroprotective role by secreting IL-10 and TGF-β (114,115,119). IL-10 reduces Th1 differentiation, thus leading to fewer recruitment of macrophages and T cells (120). TGF-β also reduces recruitment of macrophages and T cells, whilst increasing plaque stability (115,121).
Gotsman et al. could show that deficiency of PD-1/2 in their mouse model increased atherosclerosis. They also demonstrated that there was higher secretion of IFN-γ, leading to a proatherogenic state (122).
Poels et al. showed that mice treated with either CTLA-4 or PD-1 antibodies had more plaque inflammation especially due to increased CD8+ T cells. They speculated that those increased CD8+ T cells provoked death of macrophages leading to more

necrotic core formation. The expanded necrotic core formation could also be seen in their mouse model (123).

Ewing et al. investigated the CTLA-4 pathway. Hypercholesterolemic ApoE3 Leiden mice were given abatacept, a CTLA-4Ig fusion protein that inhibits CD28-CD80/86 co-stimulatory T cell activation. The researchers found that the development of atherosclerosis was reduced by 78.1% in mice receiving abatacept. They also showed decreased IFN-γ and increased IL-10 levels. Vascular lesion size was 66.7% larger in mice receiving CTLA-4 blocking antibodies compared to isotype-treated controls (124).

In humans with coronary artery disease or ACS Lee et al. and Li et al. showed that they had lower expression of PD-1 or PD-L1 suggesting that higher expression of PD-1 or PD-L1 plays a protective role in development and progression of atherosclerosis (125,126). Fernandez et al. demonstrated that exhausted T cells in atherosclerotic plaques expressing PD-1 exist in humans. The researchers speculated that treatment with ICI in patients with cardiovascular diseases might trigger T cell activation in preexisting plaques leading to aggravation of atherosclerosis (127). Synoptically, these mechanistic studies indicate that ICI treatment might have a proatherogenic effect thus leading to MACE. Our study confirmed that ICI-treated patients are at higher risk of developing MACE. Prior to ICI start, proper evaluation and identifying risk factors might help to filter patients who have a particularly high risk of MACE. Developing a simple point-based risk score might also help to easily categorize patients in subgroups of low-, intermediate- or high-risk patients for MACE which might be useful for clinical practice to identify patients that might be candidates for more intensive clinical monitoring or the development of risk-stratified cardiovascular surveillance and prevention strategies among ICI-treated patients in the future.

Importantly, several study-inherent limitations must be acknowledged upon interpreting our findings. First, the data in this study was collected retrospectively from existing medical files, meaning there could be missing information. Furthermore, some variables were unable to get hold on due to the unavailability of health care data from outside medical providers, which might result in an over- or underrepresentation of MACE in our study. We were not able to define and measure a longitudinal parameter or biomarker. Lastly, we lacked an external cohort to validate the prognostic accuracy of the established risk score.

5 Conclusion

The findings of this study indicate that patients treated with ICI are at increased risk of MACE. Clinicians must be aware of potentially severe cardiovascular irAEs associated with ICI. Further clinical studies are required to demonstrate that cardiovascular irAEs other than myocarditis are causally associated with ICI. In the future, studies to define biomarkers or clinical parameters to better predict MACE risk might be highly useful. According to existing guidelines, all treated patients should undergo a comprehensive cardiovascular risk assessment and optimization of preventive therapies as indicated, and close clinical monitoring of potential cardiovascular adverse events should be conducted (128).

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