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# Cardiac MRI study of adverse events in patients treated with immune checkpoint inhibitors: a prospective cohort study of cardiac adverse events

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## ABSTRACT

Immune checkpoint inhibitors (ICIs) revolutionized cancer therapy, yet require management of immune-related adverse events (irAEs). Fulminant myocarditis is a rare irAE, but lower-severity cardiac events are being reported more frequently, leading to an unmet need for irAE prevention, early diagnosis, and treatment, especially for long-life-expectancy patients. We recruited 57 patients, stratified according to therapy regime (monotherapy (30%) or combination (33%) cohort) or history of cardiac disease or presence of at least two cardiovascular risk factors other than prior or active smoking (cardiovascular cohort (37%)). We performed a complete cardiological assessment with clinical visit, 12-lead ECG, multiparametric cardiac MRI as well as peripheral blood mononuclear cell immunophenotyping, prior to ICI initiation and around 2 months later. ICI treatment was associated with a significant left ventricular ejection function (LVEF) reduction pre-ICI versus post-ICI treatment (60.1±8% to 58.1±8%, p=0.002, paired t-test) and more than 3% LVEF loss in a substantial proportion of patients (18; 32%). These patients also showed significantly higher T2 values (p=0.037, unpaired t-test), putative sign of cardiac edema. The loss of cardiac function did not differ among patients with different tumor types, therapy regimes or history of cardiac disease. Immunophenotyping analyses showed a reduction of programmed cell death protein 1 staining on both $CD4^+$ and $CD8^+$ T cells, and an upregulation of HLA-DR on $CD8^+$ T cells. Using a very sensitive and comprehensive approach in patients unselected for cardiac history, we found a subclinical but significant LVEF decrease. These findings may inform ongoing discussions on optimal management of cardiac irAEs in patients undergoing ICI treatment and warrant further evaluation.

## INTRODUCTION

Impressive and durable clinical responses to immune checkpoint inhibitor (ICI) immunotherapy led to the regulatory approval of ICIs blocking programmed cell death protein 1 (PD-1) or its ligand, programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte antigen 4 (CTLA-4) in a variety of solid and hematological malignancies.[1] Clinicians thus have to effectively manage a novel spectrum of toxicities, a set of autoimmune-like/inflammatory side effects, termed 'immune-related adverse events' (irAEs), affecting potentially every tissue or organ. Based on current knowledge, ICI-induced myocarditis involves only around 1% of ICI-treated patients with cancer, but it is fatal in 50% of those cases, and most likely occurs within 6 weeks of treatment initiation.[2] This creates an urgent clinical need to better understand cardiac irAE pathophysiology, and to generate suitable management, especially now that ICIs have begun to be used even as adjuvant therapy in cancer-free patients with potential for a long life expectancy. Furthermore, different combination strategies of ICIs with other immunomodulating agents or different anticancer therapies such as chemotherapy, antiangiogenic agents, tyrosine-kinase inhibitors (TKIs), and more recently antibody-drug conjugates, are currently under clinical investigation or have already entered routine clinical practice,[3 4] potentially inducing additional, combinatorial organ damage.

Several reports suggest that beyond the symptomatic or fatal form of ICI-induced myocarditis,[5 6] there may exist "low-grade" subclinical myocardial disease, the long-term complications of which are not currently known.[7]

In previous preclinical findings, we showed that anti-PD-1 treatment of mice with no prior immunological or cardiac defects led to significant T cell infiltration and inflammation in the myocardium, causing a small but significant reduction in left heart ventricle systolic function.[8] These mechanistic findings, which have been independently confirmed,[9] hint that ICI might be triggering a wider range of immunologically-driven myocardial dysfunction manifestations, of which fulminant myocarditis may represent just the "tip of the iceberg".[2]

To address the scarcity of data in this field, we set up a prospective cohort study performing a clinical and radiological cardiac assessment, using cardiac MRI (cMRI), currently the most reliable and accurate tool available for the monitoring of cardiac function and detection of tissue changes,[10] coupled with immunophenotyping of peripheral blood immune cells, in patients undergoing ICI therapy as monotherapy or in combination with chemotherapy, TKIs or other immunomodulatory agents (eg, a second ICI).

## METHODS

### Study design and participants

We prospectively recruited patients undergoing ICI therapy at the Humanitas Cancer Center from May 2021 to October 2024. Patients were assigned to three groups: (1) ICI therapy given as monotherapy (monotherapy cohort), (2) ICI therapy given in combination with chemotherapy, TKI or other immunomodulating agents (combination cohort), (3) history of cardiac disease or presence of at least two cardiological risk factors other than prior or active smoking (cardiovascular cohort). Patients were not eligible for the study if they were previously exposed to any immunomodulating agent, if the combination agent had a well-recognized cardiotoxic potential (eg, anthracycline), if they were diagnosed with acute or chronic myocarditis (any time prior to enrollment) or if they had experienced acute (in the last 3 months) cardiac events. Participants underwent complete cardiological assessment including clinical visit, 12-lead ECG and multiparametric cMRI at two time points: prior initiation of the ICI therapy, and around 8 weeks later (±1 week). Peripheral blood samples for immunophenotyping were collected directly before each cMRI.

For correlation analyses, clinical benefit from ICI therapy was defined as disease control (complete response or partial response or stable disease (SD) according to RECIST criteria) lasting≥6 months or≥4 months, as first line or from the second line onwards, respectively, for patients treated in a metastatic setting. For patients receiving ICI therapy as adjuvant treatment, the benefit corresponded to a disease-free survival (DFS)≥12 months.

### Cardiac magnetic resonance protocol

cMRI scans were performed using a 1.5 Tesla whole-body scanner (MAGNETOM Aera; Siemens Healthcare, Erlangen, Germany). A standardized procedure was used, including two-chamber, three-chamber and four-chamber views; short-axis cine images covering both ventricles; basal, mid, and apical short-axis T1, using modified Looked Locker Inversion recovery sequences and T2 mapping, using T2-prepared single-shot steady-state free-precession (SSFP) sequences with different T2 prep times. For the definition of normal native T1 and T2 mapping values, a local reference range of gender-adjusted and age-adjusted reference was used and derived from a cohort of 100 healthy subjects.

As recommended, phantom-based quality control checks were performed periodically to ensure that the status and stability of the cMRI system had not changed significantly between the establishment of normative values and clinical scanning.[11]

Cine images were acquired using breath-hold SSFP sequences with retrospective ECG-gating in end-expiration breath-holds.

### Peripheral blood cell immunophenotyping via multiparameter flow cytometry

(See online supplemental methods).

### Statistical analysis

Statistical analysis was performed in GraphPad Prism. Data sets were tested for normal distribution prior to applying parametric or non-parametric post-tests.

Multiparameter flow cytometry data were analyzed using a pairwise Wilcoxon signed-rank method with FDR correction, to examine the differences among paired groups, after normalizing for confounding factors (batch effect, tumor type, previous chemo/radio therapy; see online supplemental methods).

## RESULTS

### Patients' characteristics

Between May 2021 and October 2024, 57 patients with different solid tumors who underwent ICI treatment at our institution were recruited in the study. 17 (30%) patients were assigned to the monotherapy cohort, 19 (33%) to the combination cohort, and 21 (37%) to the cardiovascular cohort, respectively. Most patients (49; 86%) were treated with ICI for locally advanced/metastatic disease (33 as first-line therapy, 16 from second-line on), while 8 (14%) as adjuvant therapy. The range of tumor types included was wide, the most represented being clear cell renal carcinoma (20; 35%), melanoma (15; 26%), colorectal cancer (4; 7%) and non-small cell lung cancer (3; 5%). Most patients received an anti-PD-1 agent (46; 81%), while in the combination cohort ICI plus a TKI (19; 33%) was the most frequent combinatorial strategy administered; within patients receiving ICI plus other immunomodulating agents, anti-PD-1 + anti-CTLA-4 was the most common regimen (4; 7%).

The patients' cardiovascular risk profile was heterogeneous; smoking (prior or active) and hypertension were the most prevalent risk factors (both 39; 68%), followed

**Table 1** Patient baseline characteristics (n=57)

| | |
|---|---|
| Age median (range) | 62 (29–85) |
| Sex | N (%) |
| Male | 40 (70) |
| Female | 17 (30) |
| Tumor type | N (%) |
| Kidney | 20 (35) |
| Melanoma | 15 (26) |
| Colorectal | 4 (7) |
| Lung | 3 (5) |
| Urothelial | 3 (5) |
| Duodenum | 3 (5) |
| Pancreas | 2 (4) |
| Skin squamous cell | 2 (4) |
| Stomach | 2 (4) |
| Head and Neck | 2 (4) |
| Mesothelioma | 1 (1) |
| Treatment setting | N (%) |
| Adjuvant | 8 (14) |
| First line | 33 (58) |
| Subsequent lines | 16 (28) |
| Immune target | N (%) |
| PD-1 | 46 (81) |
| PD-L1 | 4 (7) |
| PD-1/CTLA-4 | 4 (7) |
| PD-L1/PD-L2 | 2 (4) |
| PD-1/IL-2 | 1 (1) |
| Combination therapy | N (%) |
| TKI | 19 (33) |
| Immunomodulating agent | 5 (9) |
| Chemotherapy | 3 (5) |
| Cardiovascular characteristics | N (%) |
| BMI medium (range) | 25 (18–36) |
| Smoking | 39 (68) |
| Hypertension | 39 (68) |
| Dyslipidemia | 22 (39) |
| Chronic kidney disease moderate-to-severe | 19 (33) |
| Diabetes | 7 (12) |
| Cardiovascular therapy | N (%) |
| ACEi/ARB | 28 (49) |
| Beta-blockers | 20 (35) |
| Ca++ antagonist | 14 (25) |
| Statins/ezetimibe | 12 (21) |
| K sparing | 4 (7) |
| Cohort | N (%) |
| Monotherapy | 17 (30) |
| Combination | 19 (33) |
| Cardiovascular | 21 (37) |
| Ischemic cardiomyopathy | 6 (29) |
| Hypertensive cardiomyopathy | 3 (14) |
| Dilated cardiomyopathy | 5 (24) |
| >2 cardiovascular risk factors | 10 (48) |

ARB, Angiotensin 2 Receptor Blockers; BMI, body mass index; CTLA-4, cytotoxic T-lymphocyte antigen 4; IL-2, interleukin 2; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TKI, tyrosine-kinase inhibitor.

by dyslipidemia (22; 39%). Among 21 patients enrolled in the cardiovascular cohort, 6 had chronic ischemic cardiomyopathy, 3 hypertensive cardiomyopathy, and 5 dilated cardiomyopathy. Baseline characteristics of participants in the study are summarized in table 1, while specific clinic characteristics of patients recorded in the cardiovascular cohort are detailed in online supplemental table 1.

## Efficacy and AEs

With a median follow-up time of 25.3 months (range 2–41.3), we could assess clinical benefit in 51 (89%) patients. Median overall survival of the entire population was not reached, while at 2 years it was 58.8%. Median progression-free survival (mPFS) of the entire population was 28.8 months, specifically mPFS for first-line patients was 38.1 months, while mPFS for patients treated in subsequent lines was 4.2 months.

Among patients treated for advanced/metastatic disease as first line (28; 49%), disease control rate (DCR) at 6 months was 89%, overall response rate (ORR) 57%, with a median duration of response (mDoR) of 23 months; while among patients treated in subsequent lines (16; 28%), DCR at 4 months was 50%, ORR 12%, with a mDoR of 16 months. Median DFS of patients receiving ICI therapy in the adjuvant setting was 39.1 months, with all of them (7; 12%) being relapse-free at 12 months.

Nine (24%) patients experienced non-cardiovascular irAEs during treatment, namely grade 2 alanine aminotransferase/aspartate aminotransferase increased (3; 33%), grade 1–2 rash maculopapular (2; 22%), grade 2 hypo/hyperthyroidism (4; 44%), grade 2 lipase/amylase increase (2; 22%) and grade 2 colitis (1; 11%).

Two major adverse cardiovascular events were noted during the study's follow-up. In the first case, a symptomatic grade 3 myocarditis occurred in a patient (2.7%), included in the monotherapy cohort, after the second cycle of a first-line anti-PD-1 treatment for metastatic melanoma. After a 3 month follow-up, the patient was asymptomatic for angina and a new cMRI confirmed stable signs of fibrosis. The patient continued active oncological surveillance with radiological confirmation of SD at the most recent disease evaluation.

A second patient, with a diagnosis of metastatic colorectal cancer, recruited in the cardiovascular cohort due to anamnestic presence of two risk factors other

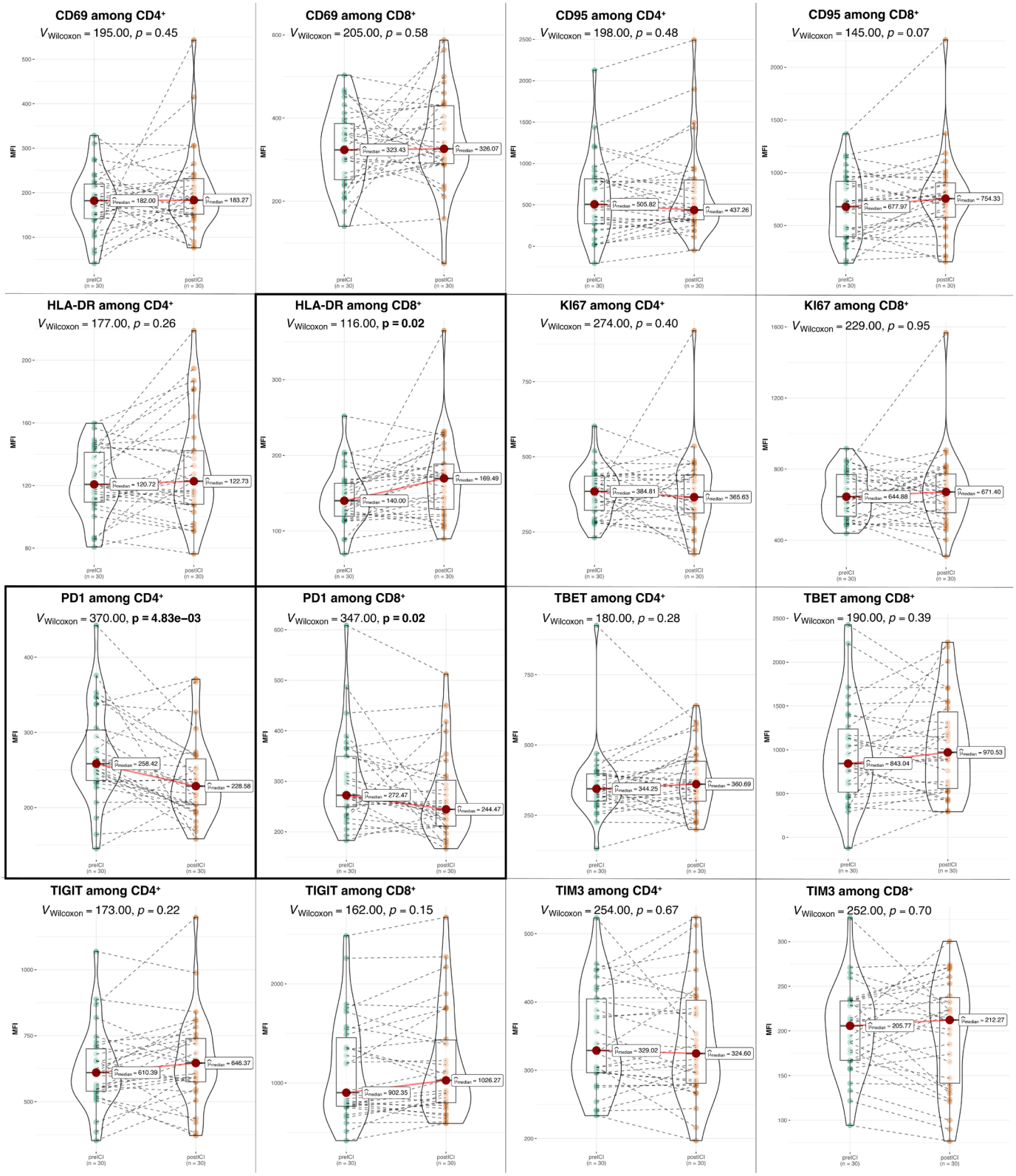


**Figure 1** ICI treatment activates T cells across the entire patient cohort. Box-violin plot showing the mean fluorescence intensity (MFI) of different surface and intracellular markers on T cell subsets of patients with cancer pre-ICI and post-ICI treatment. Bold frames highlight significant changes in the MFI of PD1 and HLA-DR-expressing cells among $CD4^+$ and/or $CD8^+$ T cells. Wilcoxon signed-rank test (p=0.02; p=0.005; p=0.02). ICI, immune checkpoint inhibitor; PD1, programmed cell death protein 1.

than prior or active smoking (hypertension and dyslipidemia), experienced a grade 3 acute myocardial infarction after 2 months of third-line anti-PD-1 plus chemotherapy (received as part of a phase I clinical trial).

### Immune phenotyping

Immunophenotyping of the peripheral blood mononuclear cells collected before and 2 months after ICI therapy initiation, available at both time points for the first 30 patients, showed that both $CD8^+$ and $CD4^+$ T cells displayed a reduction of PD-1 staining at the second time point, likely linked to the occupancy of the PD-1/PD-L1 axis in the patients treated with anti-PD-1[12] (figure 1; p=0.02 and p=0.005, respectively, Wilcoxon signed-rank test, with significance retained after FDR correction; full statistical analysis in online supplemental table 2); T cell activation marker HLA-DR[13] was upregulated on $CD8^+$ T cells (figure 1, p=0.02; as above; gating online supplemental figure 1).

### Cardiac MRI results reveal a loss of systolic function across the entire cohort

At baseline cMRI, the mean left ventricular ejection function (LVEF) of the entire population was 60.1% (±8); when stratifying patients into the three cohorts, LVEF was not significantly lower in the cardiovascular cohort as compared with the monotherapy and combination cohort (56.0±10 vs 62.5±7 and 62.6±5 respectively, p=0.056, one-way analysis of variance (ANOVA)). The difference was significant when comparing the cardiovascular cohort to the combined mono and combination therapy cohorts (p=0.013, parametric t-test). Mean global T1 and T2 mapping values were within normal limits in the entire population (1017±34 and 48.4±3 respectively) and no differences were observed across the three cohorts of patients (online supplemental table 3).

At an average of 66 days after the beginning of treatment, all patients underwent a follow-up cMRI. The cMRI analysis showed a significant LVEF reduction pre-ICI versus post-ICI treatment in the entire population (table 2), indicating a small but statistically significant loss of cardiac function in patients who underwent ICI treatment (figure 2A; p=0.002, paired t-test). 18 out of 57 (32%) patients showed a loss of more than 3 percentage points of EF[13 14]; these patients also had significantly increased T2 cMRI values, a potential indicator of on-treatment onset of cardiac edema (figure 2B, p=0.037, unpaired t-test).

We also identified a significant increase in the left ventricle maximum thickness over time (p=0.036, one-way ANOVA), which was not associated with a concomitant increase in left ventricle mass (p=0.161, one-way ANOVA), rendering it unlikely to be of clinical relevance (table 2).

No statistically significant differences in right and left ventricle EF, nor decrease in end-diastolic volume were observed between the subcohorts of patients (table 2). The loss of systolic function did not differ among tumor

**Table 2** Cardiac MRI data at follow-up

| Cardiac MRI follow-up | All (57) baseline | All (57) 2-month follow-up | P value | Delta monotherapy (17) | Delta combo therapy (19) | Delta cardiovascular (CV) (21) | P value | Delta mono+combo (36) | P value CV versus (mono+combo) |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | | Mean (SD) | Mean (SD) | Mean (SD) | | Mean (SD) | |
| Index LVEDV | 73.7±15 | 69.1±14 | **0.027** | −2.7±14 | −6.4±11 | −4.6±20 | 0.908 | −4.6±12 | 0.984 |
| LV ejection fraction | 60.1±8 | 58.1±8 | **0.002** | −1.3±5 | −3.8±5 | −0.9±5 | 0.197 | −2.6±5 | 0.212 |
| Max thickness | 9.0±3 | 9.5±2 | **0.036** | 0.9±1 | 0.6±1 | −0.1±2 | **0.048** | 0.7±1 | **0.049** |
| Index mass | 54.4±12 | 53±12 | 0.161 | −1.5±8 | −1.3±8 | −1.6±7 | 0.763 | −1.4±8 | 0.923 |
| Index RVEDV | 70.9±17 | 70.0±15 | 0.631 | −2±9 | −5.4±14 | 3.8±12 | 0.088 | −3.7±12 | **0.029** |
| RV ejection fraction | 59.8±8 | 58.5±7 | 0.168 | 0.3±7 | −3.2±5 | 0.6±8 | 0.193 | −1.5±6 | 0.631 |
| LA area | 20.2±7 | 20.9±11 | 0.530 | 1.3±4 | −2.7±4 | 3.5±13 | **0.011** | −0.8±4 | 0.187 |
| Global T1 mapping | 1017±34 | 1010±37 | 0.232 | −11±45 | −5.1±30 | −3.4±41 | 0.991 | −7.8±38 | 0.687 |
| Global T2 mapping | 48.4±3 | 48.3±3 | 0.663 | −0.2±3 | −0.3±3 | 0±2 | 0.909 | −0.2±3 | 0.742 |

One-way ANOVA was used to compare the three cohorts; a parametric t-test was used to compare the CV cohort to the combined non-CV cohorts.
Bold values are statistically significant (<0.05)
LA, left atrial; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; RV, right ventricular; RVEDV, right ventricular end-diastolic volume.

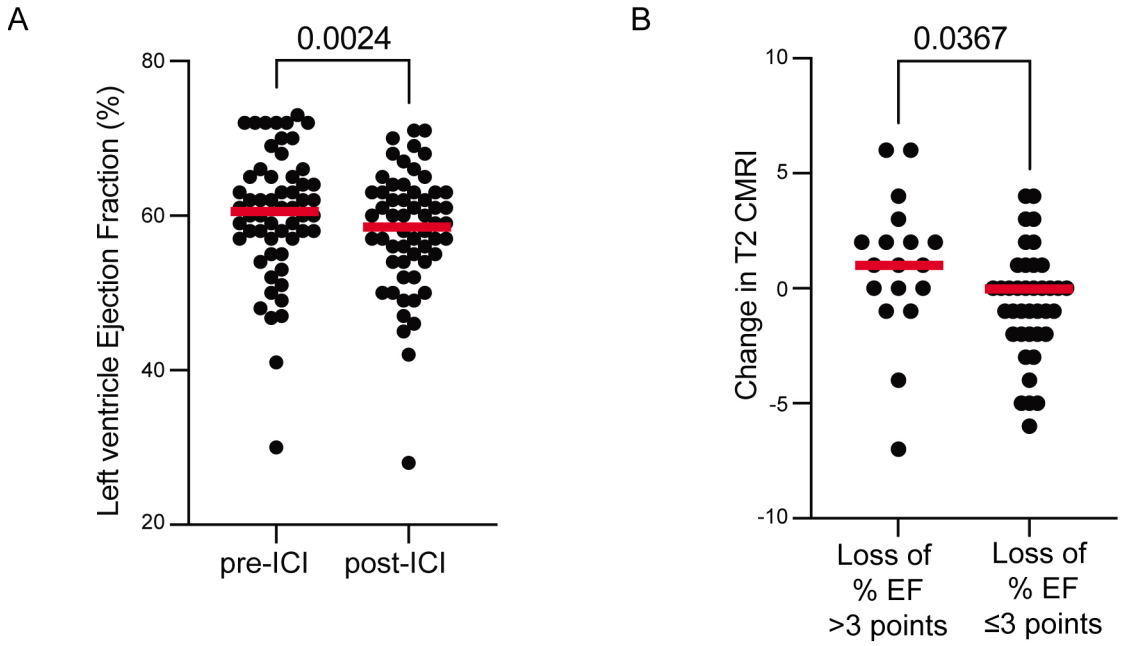


**Figure 2** cMRI shows loss of systolic function across the entire patient cohort A. Scatter dot plot showing change in left ventricle ejection fraction (%) pre-ICI versus post-ICI treatment. Paired t-test (p=0.0024). (B) Scatter dot plot showing change in T2 cMRI values post-ICI versus pre-ICI treatment; patients split on the basis of their loss of ejection fraction of more than versus less than or equal to 3%. Unpaired t-test (p=0.0367). cMRI, cardiac MRI; EF, ejection fraction; ICI, immune checkpoint inhibitor.

types nor correlated with specific pre-ICI anticancer treatments (online supplemental figure 2A,B). In an exploratory analysis, no difference was observed in terms of PFS between patients who had a loss of more than 3 percentage points of EF and those who did not (p=0.537); similarly, no PFS difference was observed among the three patients' cohorts (p=0.197) (online supplemental figure 2C,D). Only two among patients who experienced non-cardiological irAEs had a loss of more than 3 percentage points of EF, thus we cannot infer any correlation between the occurrence of non-cardiovascular irAEs and ICI-induced cardiac damage.

## DISCUSSION

We present data from patients enrolled in a prospective study conducted in a high-volume cancer center, aimed at characterizing early cardiovascular events secondary to ICI therapy which may evolve into full-blown myocarditis. Given the low incidence of cardiological irAEs, in current clinical practice, most recent oncological international guidelines do not explicitly recommend cardiological assessments before or during ICI therapy in the absence of cardiac symptoms (see both American Society of Clinical Oncology[15] and European Society of Medical Oncology[16] guidelines). The European Society of Cardiology (ESC) guidelines on cardio-oncology do clearly endorse a baseline assessment with ECG and troponin assay for all patients and an additional echocardiography evaluation for high-risk patients.[17] Unfortunately, outside of clinical trials, this management is not routinely adopted; this may be occurring due to the costs of additional monitoring, coupled with the relatively low frequency of cardiac fulminant events. Yet the high number of patients exposed to immunotherapy, both as single agent, in combination with other anticancer agents, as well as in the neo/adjuvant context for highly-prevalent solid tumors, drives a need for further evaluation. Our starting hypothesis was that a prospective evaluation could identify groups of patients at higher risk of development of cardiovascular events. For this purpose, while recruiting an all-comer cohort, we identified patients with known cardiovascular risk (highlighted in the ESC guidelines),[17] as well as combination therapy patients, whose enhanced pharmacological burden could be driving a higher toxicity risk.[4 5]

Previous longitudinal studies, retrospective case series, and meta-analyses describe low occurrence of symptomatic cardiac irAEs.[4 18–21] Accordingly, in the present study we observed a low incidence of symptomatic severe cardiovascular events: an acute myocarditis, deemed immune-related and treated accordingly, and an episode of acute infarction.

By applying a prospective cardiac function assessment with cMRI in a cohort of ICI-treated patients, not selected for suspected cardiac symptoms, our study was able to show more than 3% EF loss in a substantial proportion of patients (14; 38%). It is important to highlight that, while this difference is small and will almost certainly be below the detection threshold of echocardiography, the standard-of-care cardiac monitoring tool, the loss of systolic function is both statistically significant and above the threshold considered meaningful for cMRI by

radiologists.[13 14] The few prospective studies published so far have found different and very low degrees of loss of heart function; some of these variations may be attributed to cohort variance, but also differences in the methods of cardiac monitoring (eg, echocardiography vs cMRI).[22] The novel contribution of our study is the examination of an "all-comers" prospective cohort, enabling an unbiased estimation of the incidence of overall cardiac dysfunction, combined with the use of cMRI, the most reliable means of examining cardiac abnormalities, pretreatment and on-treatment. Despite being very limited in severity, this loss of cardiac function, coupled with the large percentage of patients presenting it (32%) and the increased use of ICI, calls for more accurate cardiological monitoring, a fact undermined by the current discrepancy between oncological and cardiological guidelines. This is especially valid for patients with ICI indication for adjuvant therapy, who are thus cancer-free, potentially having long life expectancy.

The low-grade EF loss observed in our entire cohort may or may not share the same underlying immune-driven pathogenesis mechanisms with the rare fulminant ICI myocarditis cases. It is worth noting that T cell-mediated mechanisms are implicated not only in fulminant ICI myocarditis,[23] but also in cardiac diseases such as heart failure, irrespective of whether this involves reduced or preserved EF.[24] A common mechanism underlying these conditions is immune activation of T cells, which we observed across our cohort, and which is the expected outcome of treatment with ICI; ICI aim to activate all T cells, hoping to include those specific for tumor-associated antigens. Further study is required to dissect any mechanistic differences between fulminant and low-grade cardiac AEs.

In retrospective reports, cardiovascular events linked to ICI treatment have been found more frequently in patients with a previous history of cardiovascular disease.[25] We observed variations in cardiac structure and function (both left and right chambers) in the combination cohort compared with the others. These findings are not statistically significant in this analysis, calling for increased patient enrollment. Further studies will clarify any basis for recommending additional follow-up for groups with higher risk of cardiotoxicity, whether due to prior risk factors or due to combination therapy.

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**Contributors** AL, CP, MF, GC, MS, and MK designed the initial study, MK sourced funding. AL, AD, PP, ADM, MCT, MP, GF, AS, MS handled all oncological aspects of the study, CP, CD'A, AM, GC handled all cardiological aspects of the study, LM, FC, SF, MF, handled all radiological aspects of the study, CC, MC, EL, MK handled all immunological aspects of the study, SS and LG handled statistical analysis. AL, CP, CC, MS, GC, and MK wrote the manuscript, all authors critically reviewed and approved the manuscript. GC, MS, and MK are the shared guarantors of the study. GC, MS and MK are joint last authors.

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## REFERENCES

1 Sharma P, Goswami S, Raychaudhuri D, *et al*. Immune checkpoint therapy-current perspectives and future directions. *Cell* 2023;186:1652–69.
2 Zaha VG, Meijers WC, Moslehi J. Cardio-Immuno-Oncology. *Circulation* 2020;141:87–9.
3 Gandhi L, Rodríguez-Abreu D, Gadgeel S, *et al*. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* 2018;378:2078–92.
4 Rini BI, Moslehi JJ, Bonaca M, *et al*. Prospective Cardiovascular Surveillance of Immune Checkpoint Inhibitor-Based Combination

Therapy in Patients With Advanced Renal Cell Cancer: Data From the Phase III JAVELIN Renal 101 Trial. *J Clin Oncol* 2022;40:1929–38.
5 Johnson DB, Balko JM, Compton ML, *et al*. Fulminant Myocarditis with Combination Immune Checkpoint Blockade. *N Engl J Med* 2016;375:1749–55.
6 Norwood TG, Westbrook BC, Johnson DB, *et al*. Smoldering myocarditis following immune checkpoint blockade. *J Immunother Cancer* 2017;5:91.
7 Palaskas NL, Segura A, Lelenwa L, *et al*. Immune checkpoint inhibitor myocarditis: elucidating the spectrum of disease through endomyocardial biopsy. *Eur J Heart Fail* 2021;23:1725–35.
8 Martini E, Kunderfranco P, Peano C, *et al*. Single-Cell Sequencing of Mouse Heart Immune Infiltrate in Pressure Overload-Driven Heart Failure Reveals Extent of Immune Activation. *Circulation* 2019;140:2089–107.
9 Michel L, Helfrich I, Hendgen-Cotta UB, *et al*. Targeting early stages of cardiotoxicity from anti-PD1 immune checkpoint inhibitor therapy. *Eur Heart J* 2022;43:316–29.
10 Francone M, Figliozzi S, Monti L, *et al*. Multiparametric cardiac magnetic resonance unveiling the mechanisms and early manifestations of anticancer drug cardiotoxicity. *Eur Radiol* 2023;33:8439–41.
11 Messroghli DR, Moon JC, Ferreira VM, *et al*. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). *J Cardiovasc Magn Reson* 2017;19:75.
12 Huang AC, Postow MA, Orlowski RJ, *et al*. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature New Biol* 2017;545:60–5.
13 Bellenger NG, Davies LC, Francis JM, *et al*. Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson* 2000;2:271–8.
14 Grothues F, Smith GC, Moon JCC, *et al*. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol* 2002;90:29–34.
15 Schneider BJ, Naidoo J, Santomasso BD, *et al*. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: ASCO Guideline Update. *J Clin Oncol* 2021;39:4073–126.
16 Curigliano G, Lenihan D, Fradley M, *et al*. Management of cardiac disease in cancer patients throughout oncological treatment: ESMO consensus recommendations. *Ann Oncol* 2020;31:171–90.
17 Lyon AR, López-Fernández T, Couch LS, *et al*. 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). *Eur Heart J Cardiovasc Imaging* 2022;23:e333–465.
18 D'Souza M, Nielsen D, Svane IM, *et al*. The risk of cardiac events in patients receiving immune checkpoint inhibitors: a nationwide Danish study. *Eur Heart J* 2021;42:1621–31.
19 Waheed N, Fradley MG, DeRemer DL, *et al*. Newly diagnosed cardiovascular disease in patients treated with immune checkpoint inhibitors: a retrospective analysis of patients at an academic tertiary care center. *Cardiooncology* 2021;7:10.
20 Dolladille C, Akroun J, Morice P-M, *et al*. Cardiovascular immunotoxicities associated with immune checkpoint inhibitors: a safety meta-analysis. *Eur Heart J* 2021;42:4964–77.
21 Drobni ZD, Alvi RM, Taron J, *et al*. Association Between Immune Checkpoint Inhibitors With Cardiovascular Events and Atherosclerotic Plaque. *Circulation* 2020;142:2299–311.
22 Faron A, Isaak A, Mesropyan N, *et al*. Cardiac MRI Depicts Immune Checkpoint Inhibitor-induced Myocarditis: A Prospective Study. *Radiology* 2021;301:602–9.
23 Axelrod ML, Meijers WC, Screever EM, *et al*. T cells specific for α-myosin drive immunotherapy-related myocarditis. *Nature New Biol* 2022;611:818–26.
24 Alcaide P, Kallikourdis M, Emig R, *et al*. Myocardial Inflammation in Heart Failure With Reduced and Preserved Ejection Fraction. *Circ Res* 2024;134:1752–66.
25 Laenens D, Yu Y, Santens B, *et al*. Incidence of Cardiovascular Events in Patients Treated With Immune Checkpoint Inhibitors. *J Clin Oncol* 2022;40:3430–8.