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ORIGINAL RESEARCH

# Clinical presentation of cardiac symptoms following treatment with tumor-infiltrating lymphocytes: diagnostic challenges and lessons learned

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**Background:** Treatment with tumor-infiltrating lymphocytes (TILs) is rapidly evolving for patients with solid tumors. Following metastasectomy, TILs (autologous, intratumoral CD4+ and CD8+ T cells with the potential to recognize tumor-associated antigens) are isolated and non-specifically expanded *ex vivo* in the presence of interleukin-2 (IL-2). Subsequently, the TILs are adoptively transferred to the patients after a preconditioning non-myeloablative, lymphodepleting chemotherapy regimen, followed by administration of high-dose (HD) IL-2. Here, we provide an overview of known cardiac risks associated with TIL treatment and report on seven patients presenting with cardiac symptoms, all with different clinical course and diagnostic findings during treatment with lymphodepleting chemotherapy, TIL, and HD IL-2, and propose a set of clinical recommendations for diagnosis and management of these symptoms.

**Patients and methods:** This single-center, retrospective study included selected patients who experienced TIL treatment-related cardiac symptoms at the Netherlands Cancer Institute. In addition, 12 patients were included who received TIL in the clinical trial setting without experiencing cardiac symptoms, from whom complete cardiac biomarker follow-up during treatment was available [creatine kinase (CK), CK-myocardial band, troponin T and N-terminal pro-B-type natriuretic peptide].

**Results:** Within our TIL patient population, seven illustrative cases were chosen from the patients who developed symptoms suspected of severe cardiotoxicity: myocarditis, myocardial infarction, peri-myocarditis, atrial fibrillation, acute dyspnea, and two cases of heart failure. An overview of their clinical course, diagnostics carried out, and management of the symptoms is provided.

**Conclusions:** In the absence of evidence-based guidelines for the treatment of TIL therapy-associated cardiotoxicity, we provided an overview of literature, case descriptions, and recommendations for diagnosis and management to help physicians in daily practice, as the number of patients qualifying for TIL treatment is rapidly increasing.

**Key words:** cardiotoxicity, tumor-infiltrating lymphocytes, interleukin-2, melanoma, non-small-cell lung cancer, guidelines

## INTRODUCTION

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TILs) is a rapidly evolving treatment field for patients with solid tumors, which has recently shown promising results in patients with melanoma and non-small-cell lung cancer (NSCLC).[1,2] In TIL treatment, autologous, intratumoral CD4+ and CD8+ T cells with the potential to recognize tumor-associated antigens are used as a personalized treatment modality. Following metastasectomy, TILs are isolated and non-specifically expanded *ex vivo* in the presence of interleukin-2 (IL-2). These expanded TILs are then adoptively transferred to patients after a preconditioning non-myeloablative, lymphodepleting chemotherapy regimen, followed by administration of high-dose (HD) IL-2. The preconditioning chemotherapy regimen and HD IL-2 are known to have the potential to cause cardiac adverse events, while the risk of TILs driving cardiotoxicity is hypothetical.[3,4] Here, we provide an overview of known cardiac risks associated with TIL

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treatment and report on seven patients presenting with cardiac symptoms, all with different clinical courses and diagnostic findings, during treatment with lymphodepleting chemotherapy, TIL, and HD IL-2 at the Netherlands Cancer Institute (NKI, Amsterdam, The Netherlands), and propose a set of clinical recommendations for diagnosis and management of these symptoms.

### Chemotherapy

The preconditioning chemotherapy regimen typically consists of a combination of cyclophosphamide, an alkylating agent, and fludarabine, a purine analog. The reduction of immunosuppressive cell populations and endogenous T cells before the cell infusion is thought to enhance the efficacy of TIL by creating a favorable immune environment, promoting their *in vivo* expansion.[5] However, next to the well-known hematological side-effects, including leukocytopenia and thrombocytopenia, both agents have also been associated with cardiotoxicity.

Cyclophosphamide-induced cardiac toxicity has an incidence of 7%-33% and is thought to occur as a result of oxidative stress in combination with direct damage to the capillary endothelium, leading to extravasation of erythrocytes, proteins, and toxic metabolites, further damaging the myocardium and capillaries.[6-8] This in turn leads to interstitial hemorrhage, micro-thrombi formation, and edema, which can result in a spectrum of clinical manifestations, typically within 48 h of administration. These manifestations include acute heart failure, arrhythmias, refractory hypotension, (hemorrhagic) myocarditis, pericarditis, or death.[6,9,10] Risk factors include a higher total dose of cyclophosphamide administered, advanced age, pre-existing risk factors for ischemic heart disease, and history of radiotherapy to the mediastinum or left chest wall.[6] Thus far, one case of fatal cardiac failure following cyclophosphamide administration before TIL infusion has been reported.[11]

In addition to the direct cardiotoxic effects of cyclophosphamide, the concomitant hyperhydration given to patients to prevent hemorrhagic cystitis may cause left- or right-sided congestive heart failure.[12]

Cardiotoxicity caused by single-agent fludarabine is rare; however, there is evidence that the combination of fludarabine with other chemotherapeutics (i.e. melphalan) can result in cardiac dysfunction. In patients with hematological malignancies both fatal and non-fatal congestive heart failure,[13,14] acute severe left ventricular failure,[15,16] hypotension and chest pain,[17] and myocarditis[18] have been observed. None of the patients in these studies had prior cardiac dysfunction.

Of note, most information is based on experiences in prestem cell transplantation settings for patients with hematological malignancies, who typically have different prior treatments, disease dynamics, and chemotherapy doses in their pre-transplant regimen. Thus far, there is limited knowledge about the potential cardiotoxicities of cyclophosphamide and fludarabine in the ACT treatment setting for solid tumors.

### TIL

Cardiotoxicity has been observed in TIL trials; however, these studies do not distinguish if this was related to chemotherapy, TIL, or IL-2. While there is currently limited information about the potential of TIL to induce cardiotoxicity, there are three hypothetical categories in which adoptively transferred T cells can induce cardiotoxicity: (i) 'on-target, on-tumor' effects causing cytokine release syndrome (CRS); (ii) 'on-target, off-tumor' effects resulting in direct cardiac injury because of shared antigens between the tumor and cardiac tissue, recognized by the T-cell product; and (iii) 'off-target, off-tumor' effects due to unexpected cross-reactivity of TILs with cardiac epitopes.[19] So far, none of these toxicities have been demonstrated to be caused by TIL infusion.

CRS is the result of *in vivo* antigen recognition, leading to T-cell activation, proliferation, and concurrent massive cytokine release.[20] It is characterized by three core symptoms (fever, hypotension, and/or hypoxia), often accompanied by constitutional symptoms, and, depending on the severity, dyspnea, coagulopathy, and organ dysfunction.[21,22] In patients treated with chimeric antigen receptor (CAR) T cells, another form of ACT, CRS has been identified as the risk factor for cardiovascular complications, including heart failure, myocardial infarction (MI), arrhythmias, cardiac arrest, and capillary leak syndrome (CLS).[23-25] The cytokine IL-6, a key mediator of CRS, is thought to play an important role in the pathophysiology of ACT-associated cardiotoxicity. IL-6 promotes oxidative stress, resulting in cardiomyocyte apoptosis, mitochondrial dysfunction, and cardiac hypertrophy via activation of the gp130/STAT3 pathway.[24,26] Furthermore, elevated IL-6 levels have been associated with atrial fibrillation and fatal ventricular arrhythmias.[27] Moreover, other pro-inflammatory cytokines, including interferon-$\gamma$, IL-1$\beta$, IL-2RA, and tumor necrosis factor-$\alpha$, can have negative inotropic and cytotoxic effects on cardiomyocytes, which can result in decreased myocardial contractility, hypotension, and cardiomyopathy.[28] Of note, these cytokines are also elevated in inflammation from other causes (i.e. sepsis). CAR-T-cell-treated patients who developed cardiotoxicities were typically older, and more likely to have a history of coronary artery disease or hyperlipidemia, or to use cardiac medication.[25] This is supported by Fradley and colleagues, who observed a markedly higher prevalence of hypertension and hyperlipidemia in melanoma patients developing cardiotoxicity after TIL treatment.[29]

'On-target, off-tumor' toxicity has been described in patients treated with TIL. In melanoma patients, rash or vitiligo, uveitis, and hearing loss have been observed due to shared antigens between melanoma cells and melanocytes.[1,30-33] Likewise, it cannot be fully excluded that T cells in the infusion product could potentially recognize cardiac epitopes, leading to cardiotoxicity.

Finally, it is possible that T cells in the infusion product cross-react with epitopes expressed on cardiac cells. This has previously been observed in patients treated with T-cell

receptor (TCR) T-cell products targeting MAGE-A3, resulting in unexpected fatal toxicities due to cross-reactivity with the cardiac protein titin.[34]

### Interleukin-2

IL-2 is a cytokine mainly produced by activated CD4+ T cells in secondary lymphoid structures and serves as a growth factor for T lymphocytes.[35,36] It activates and promotes the proliferation of effector CD8+ T cells, natural killer (NK) cells, and immunosuppressive CD4+ regulatory T cells (Tregs).[35,36] Despite its pleiotropic functions, HD recombinant IL-2 (aldesleukin) became one of the first approved immunotherapies for metastatic melanoma and renal cell carcinoma because of its potent ability to stimulate cytotoxic T cells and NK cells.[37,38] However, its use is now limited because of the severe toxicities. Despite this, in current TIL treatment protocols, HD IL-2 is used both *ex vivo* during the cell production, as well as *in vivo*. HD IL-2 administration following TIL infusion has been shown to enhance T-cell persistence and expansion *in vivo* and is therefore a key component of commonly used ACT protocols.[4,39]

Administration of IL-2, either as monotherapy or as part of TIL treatment, is associated with an array of toxicities. The most profound effects of HD IL-2 treatment are CLS due to the binding of recombinant IL-2 to the high-affinity IL-2R$\alpha\beta\gamma$ receptor expressed on endothelial cells, and toxicities resulting from immune cell activation and subsequent release of pro-inflammatory cytokines.[40] Clinical presentations include fever and chills, edema, hypotension, gastrointestinal symptoms, skin rash, and hematologic toxicities, which are typically dose-related and peak 4-6 h after administration.[40] The majority of these toxicities are reversible upon IL-2 discontinuation and can either be prevented using premedication, or effectively managed using supportive measures.[40]

A particularly challenging subgroup of IL-2-related side-effects are cardiotoxicities, as they can vary widely in presentation and severity, often making them difficult to diagnose. Documented effects on the cardiac system include hypotension, arrhythmias, angina pectoris, acute MI, and myocarditis.[37,40,41] However, real-world data on the presentation and management of these symptoms are scarce.

## PATIENTS AND METHODS

### Patients and clinical data

This single-center, retrospective study included patients treated with TIL at the NKI experiencing cardiac symptoms. Additionally, 12 patients were included who received TIL in the clinical trial setting without experiencing cardiac symptoms, from whom complete cardiac biomarker follow-up during treatment was available. The following patient and tumor characteristics were obtained: primary tumor type, age at time of treatment, sex, sites of metastases, prior treatments received, and relevant cardiac medical history. Furthermore, information on cardiac tests during screening, cardiac enzymes, cardiac symptoms, diagnostic tests carried out, symptom management, and follow-up was collected.

The study was conducted in accordance with the Declaration of Helsinki and all patients gave written informed consent.

### TIL treatment protocol

TIL treatment protocols consist of 2 days of cyclophosphamide (60 mg/kg), 5 days of fludarabine (25 mg/m$^2$) and the TIL infusion, followed by intravenous HD IL-2 (600 000 IU/kg/dose) every 8 h, with the first dose ∼4 h after the TIL infusion, until the maximum dose number is given or stopping criteria are met.[1,41] Patients were hospitalized from the day before the chemotherapy until recovery of symptoms. Blood draws took place daily until the HD IL-2 period, and thereafter at least every other day until discharge from the hospital. During chemotherapy, creatine kinase (CK), CK-myocardial band (MBm), troponin T (TropT), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were determined before and after TIL infusion, daily during the HD IL-2 period, and at least every other day until discharge.

### Laboratory results

CK, CK-MBm, TropT, and NT-ProBNP were measured on Roche Cobas Pro systems, and CK activity measurement was standardized against the International Federation of Clinical Chemistry (IFCC) reference method. At the NKI, the upper limit of normal for the laboratory results mentioned in this manuscript vary based on sex (CK and CK-MBm), or sex and age (NT-ProBNP). Reference values can be found in Supplementary Table 1, available at https://doi.org/10.1016/j.esmoop.2024.102383.

### Statistical analysis

Figures were made using GraphPad Prism version 9.4.1 and Biorender. For the 12 asymptomatic patients, the mean and standard deviations were calculated and shown in the graphs.

## RESULTS

Within our TIL patient population consisting of 108 patients with melanoma and 12 patients with NSCLC, 7 illustrative cases of patients who developed symptoms suspected of severe cardiotoxicity were selected. With the exception of case 7, none of the selected patients had a history of thoracic radiotherapy. Here, we give an overview of their clinical course, carried-out diagnostics, and management of symptoms (Table 1, Figure 1). An overview of potential cardiac symptoms per stage of TIL treatment can be found in Figure 2.

### Case 1. Myocarditis

A 73-year-old male presented with *NRAS*-mutated, metastatic melanoma progressive on first-line nivolumab, without

**Table 1. Patient characteristics and overview of clinical course**

| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 |
|---|---|---|---|---|---|---|---|
| Sex | M | M | F | F | F | F | M |
| Age | 73 | 65 | 50 | 66 | 54 | 67 | 55 |
| Primary tumor | Melanoma | Melanoma | Melanoma | Melanoma | Melanoma | NSCLC | NSCLC |
| Metastatic sites | Adrenal gland, lung, subcutaneous, lymph nodes | Brain, lung, muscle, subcutaneous | Lung, bone, lymph nodes, subcutaneous | Lymph nodes, subcutaneous | Lung, muscle, subcutaneous | Adrenal gland, brain | Adrenal gland, bone, lymph nodes |
| Cardiac history | — | NSTEMI with PTCA of LAD, RCA and AL; pulmonary embolism | — | — | Small PFO | Hypertension; atrial tachycardia; atrial extrasystoles | — |
| PreTx ECG | No abnormalities | Reduced R-wave anteroseptal leads; AP class 1 | No abnormalities | Right bundle branch block | No abnormalities | Atrial extrasystoles | No abnormalities |
| PreTx EF | 67% | 62% | 63% | 67% | 51% | 75% | 61% |
| Chemotherapy | No cardiac toxicities | No cardiac toxicities | No cardiac toxicities | Pulmonary congestion with hypoxia, edema, and tachycardia | No cardiac toxicities | Pulmonary congestion with dyspnea and edema | Low oxygen saturation, edema |
| TIL infusion | Irregular heartbeat shortly after infusion | No cardiac toxicities | No cardiac toxicities | Dyspnea and tachypnea | No cardiac toxicities | Dyspnea and chest discomfort | Low oxygen saturation |
| IL-2 | 3x; fever, chills, CLS, hypoxia | 3x; fever, chills, hypoxia | 2x; fever, chills, CLS, dyspnea | 1x; fever, chills, dyspnea | 1x; fever, chills | 1x; fever, acute dyspnea, CLS | 3x; fever, dyspnea, low saturation |
| Cardiac symptoms | Chest pain, hypoxia, hypotension | Chest pain | Hypotension with low urine output not responding to fluid challenge; chest pain | Respiratory failure, fluid retention, tachycardia | Rapid, irregular heartbeat; dyspnea | Hypertension, tachycardia | Tachycardia, edema |
| ICU admission | Yes (3 days) | Yes (3 days) | Yes (1 day) | Yes (5 and 8 days) | No | No | Yes (2 days) |
| Diagnostics | ECG (non-specific STT changes); lab (TropT and NT-proBNP ↑, kidney function ↓); TTE (EF 45%-50% + wall motion disturbances) | ECG (1 mm ST-elevation in anteroseptal/ anterior leads); lab (TropT ↑) | ECG (low voltage); lab (TropT and NT-proBNP ↑); TTE (small pericardial effusion without hemodynamic compromise) | ECG (micro-voltages); TTE (small pericardial effusion without hemodynamic compromise); lab (TropT and NT-proBNP ↑) | ECG (atrial fibrillation) | Chest X-ray (pulmonary congestion); ECG (no signs of acute ischemia); lab (TropT and NT-Pro-BNP ↑) | Chest X-ray (pre-existing pleura effusion); ECG (tachycardia -> interventricular conduction disorder, incomplete RBBB, left anterior fascicular block); lab (TropT and NT-Pro-BNP ↑); TTE (left ventricular function ↓, EF 50%) |
| Differential diagnosis | Acute coronary syndrome; type 2 myocardial infarction; peri-myocarditis | Acute coronary syndrome; type 1 myocardial infarction; myocarditis | Peri-myocarditis; myocarditis | Heart failure; peri-myocarditis; capillary leak syndrome; sepsis | Atrial fibrillation | CLS | Heart failure due to myocarditis or ischemia; CLS |
| Management | Hemodynamic monitoring; beta-blocker, furosemide | Hemodynamic monitoring | Hemodynamic monitoring | Non-invasive ventilation; ACE inhibitor, beta-blocker, aldosterone antagonist and furosemide | Beta-blocker | Non-invasive ventilation | Hemodynamic monitoring; intravenous diuretics and aldosterone receptor antagonist |
| Final diagnosis | Cardiac MRI: myocarditis | MRI heart: myocardial infarction | Suspected peri-myocarditis | Decompensatio cordis + nephrotic syndrome due to pauci-immune glomerulonephritis | Atrial fibrillation | CLS | Heart failure |
| Follow-up | Fully recovered | Fully recovered | Fully recovered | Cardiac function recovered, progressive edema due to nephrotic syndrome | Fully recovered | Fully recovered | Slow recovery, rapid disease progression |

ACE, angiotensin-converting enzyme; AL, anterolateral; AP, angina pectoris; CLS, capillary leak syndrome; ECG, electrocardiogram; EF, ejection fraction; F, female; ICU, intensive care unit; IL-2, interleukin-2; LAD, left anterior descending artery; M, man; MRI, magnetic resonance imaging; NSCLC, non-small-cell lung cancer; NSTEMI, non-ST-elevation myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PFO, patent foramen ovale; PTCA, percutaneous transluminal coronary angioplasty; RBBB, right bundle branch block; RCA, right coronary artery; TIL, tumor-infiltrating lymphocyte; TropT, troponin T; TTE, transthoracic echocardiography; US, ultrasound.

history of cardiac disease. Pre-TIL-treatment cardiac screening showed normal electrocardiogram (ECG) and left-ventricular ejection fraction (LVEF). No significant toxicities were observed during lymphodepleting chemotherapy. Shortly after TIL infusion, an irregular heartbeat was noticed during regular checkups. In the absence of ECG abnormalities, the first HD IL-2 was administered. Patient developed fever, chills, and temporary hypoxia, for which broad-spectrum antibiotics,

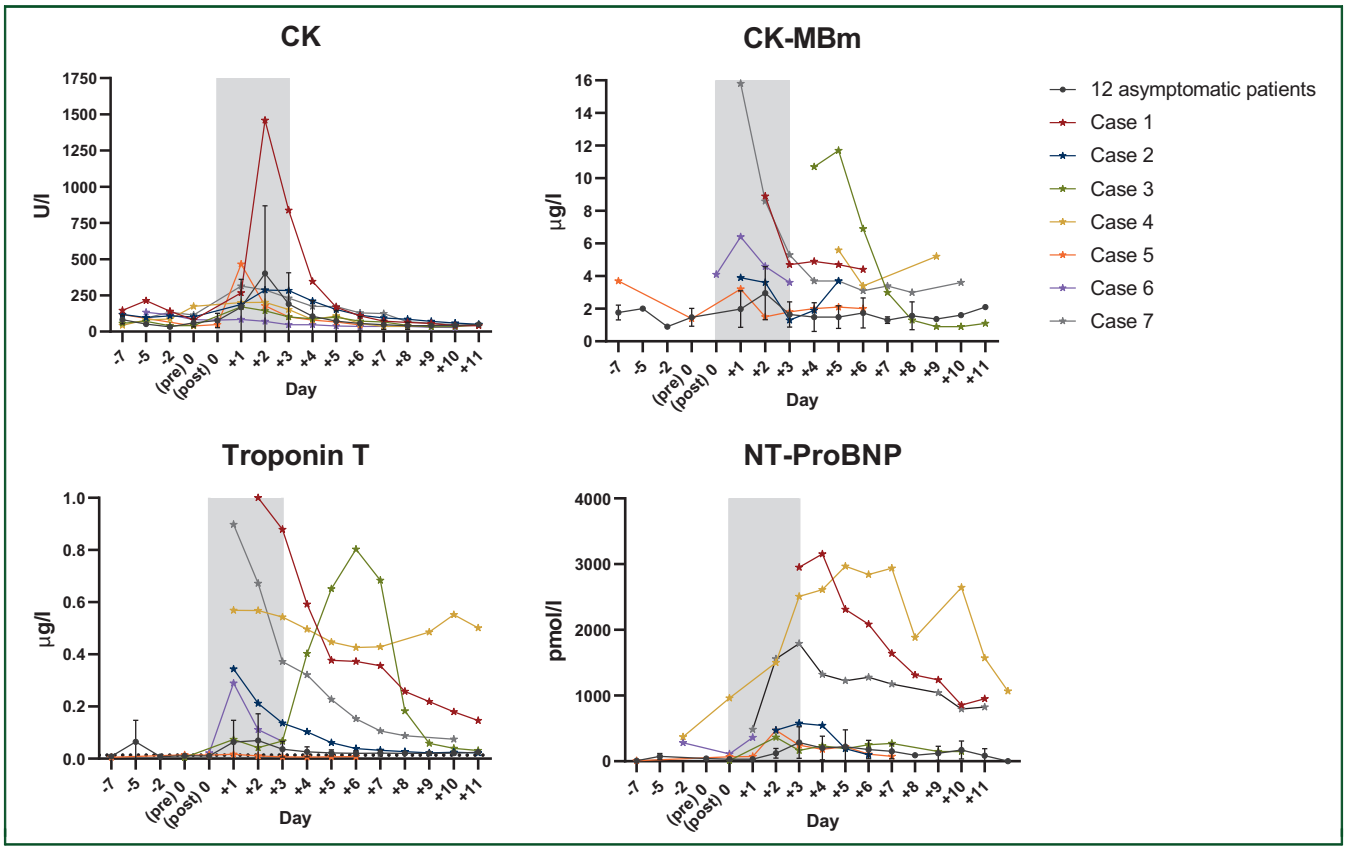


**Figure 1. Cardiac biomarkers.** Graphs show the course of cardiac biomarkers (CK, CK-MBm, Troponin T, and NT-proBNP) per case, as well as the mean and standard deviations for the 12 asymptomatic patients as reference. The upper limit of normal of TropT is indicated by the dotted line. Day 0 is the day of the TIL infusion. The gray box indicates the maximal period during which patients could have received IL-2, though the individual duration of treatment varies per patient.
CK, creatine kinase; CK-MBm, creatine kinase-myocardial band; IL-2, interleukin-2; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TIL, tumor-infiltrating lymphocyte.

pethidine, and oxygen were given, respectively. On day +1, non-specific STT changes appeared on ECG, becoming more pronounced on day +2 after the third IL-2 dose, with the patient experiencing short periods of chest pain, and CLS with hypoxia, hypotension, and decreased kidney function. High-sensitive TropT levels and NT-proBNP were elevated. The patient was admitted to the intensive care unit (ICU) for hemodynamic monitoring. Transthoracic echocardiography (TTE) showed a decreased LVEF (45%-50%) and wall motion disturbances with hypokinetic anterolateral segments. Differential diagnoses comprised acute coronary syndrome, type 2 MI, and peri-myocarditis. A close wait-and-see approach with continuous monitoring was chosen; symptoms subsided and ST-segments normalized. As the patient was pancytopenic, no anti-coagulant or antiplatelet medication was given. Over the next days, a low-dose beta-blocker and furosemide were started. With this, cardiac biomarker levels dropped, and LVEF recovered; CK-MBm levels were never markedly elevated. After 2 days, the patient was discharged to the ward and steadily recuperated. A cardiac magnetic resonance imaging (CMR) 19 days after TIL infusion, showing edema in basal anterior segments and a slightly decreased EF (51%), confirmed myocarditis (Figure 3). During outpatient clinic follow-up, the patient recovered completely (EF 60%).

### *Case 2. Myocardial infarction*

A 65-year-old man presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, and a history of coronary artery disease: non-ST-elevation MI (NSTEMI) with a percutaneous transluminal coronary angioplasty (PTCA) of the left anterior descending artery (LAD), right coronary artery (RCA) and anterolateral (AL), and a recent pulmonary embolism. Pre-treatment ECG showed reduced R-waves in anteroseptal leads; EF on TTE was normal. The patient was asymptomatic (AP class 1). No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. After the first IL-2 dose, the patient developed fever, chills, and temporary hypoxia, for which broad-spectrum antibiotics, pethidine, and oxygen were given, respectively. The following day, the patient developed chest pain 2.5 h after the third HD IL-2. ECG showed 1 mm ST-elevation in the anteroseptal/anterior leads (Figure 3). TropT was elevated, while CK-MBm remained normal. The patient was admitted to the ICU for hemodynamic monitoring. Differential diagnoses of acute coronary syndrome, type 1 MI, and myocarditis due to TIL or IL-2 were considered. As the patient was thrombocytopenic [24*$10^9$/l (150-400*$10^9$/l)], no antiplatelet therapy was added to the low-molecular-weight heparin

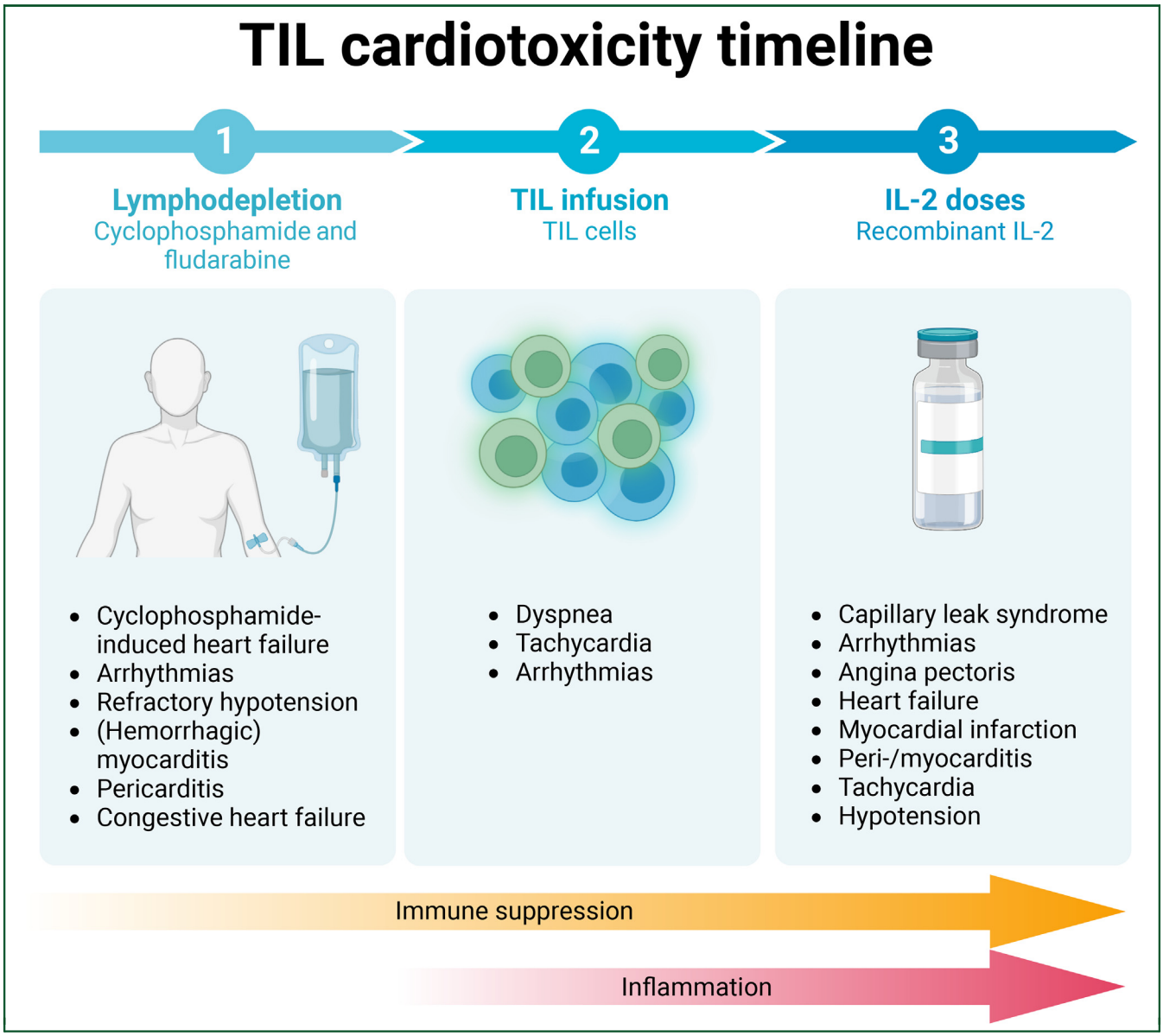


**Figure 2. TIL cardiotoxicity timeline.** This timeline shows the cardiac toxicities that can be expected based on the treatment phase the patient is in (lymphodepletion, TIL infusion, or IL-2 administrations).
IL-2, interleukin-2; TIL, tumor-infiltrating lymphocyte.

dosed for pulmonary embolism. At the time of ICU admittance, chest pain had subsided and cardiac biomarkers normalized over the next days. After 2 days, the patient was discharged to the ward where he steadily recuperated. At follow-up, TTE showed normal ventricular function without wall motion abnormalities. CMR 29 days after onset of symptoms showed minimal late gadolinium enhancement anteroseptal, confirming MI (Figure 3) with normal ventricular function (EF 60%). During outpatient clinic follow-up, the patient recovered completely.

### *Case 3. Peri-myocarditis*

A 50-year-old woman presented with progressive, *NRAS*-mutated, metastatic melanoma, with a recently diagnosed immune-related insulin-dependent diabetes mellitus after two cycles of pembrolizumab. Pre-treatment ECG and LVEF were normal. No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. After the second HD IL-2, the patient developed CLS, with hypotension and low urine output unresponsive to fluid challenge; therefore, IL-2 was discontinued. On day +4, ECG showed low voltage (Figure 3), and TropT and NT-proBNP were increasingly elevated. On day +5, the patient developed chest pain worsening with coughing and movement of the left shoulder. TTE showed small pericardial effusion without signs of hemodynamic compromise. Perimyocarditis was suspected either induced by cyclophosphamide or HD IL-2. The patient was admitted to the ICU for continuous hemodynamic monitoring. She remained stable, and was discharged to the ward after 24 h. Her pain subsided and TropT levels and NT-proBNP decreased spontaneously.

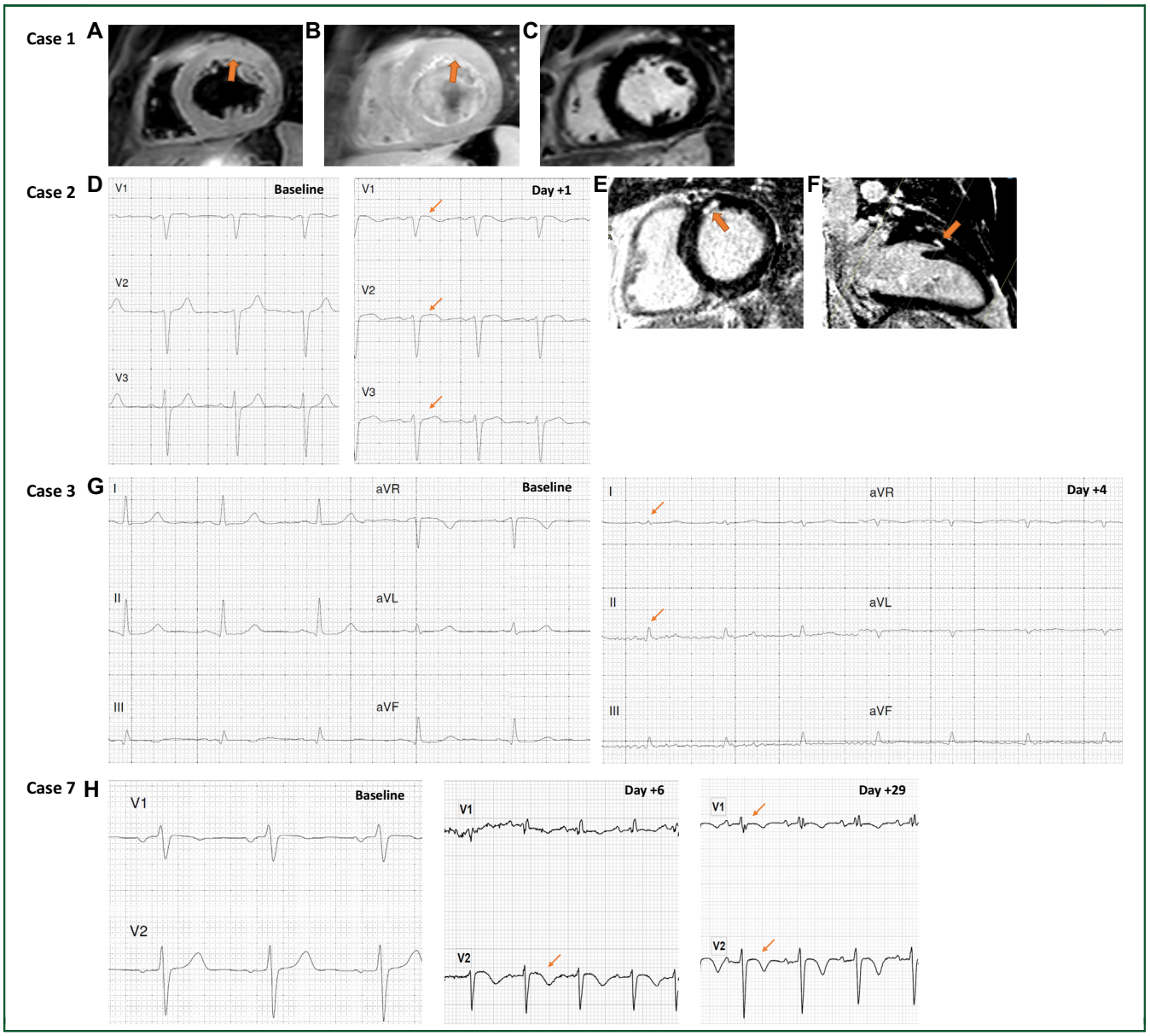


**Figure 3. Cardiac imaging.** Cardiac MRI of case 1, 19 days after TIL infusion: (A) MRI short-axis T2 SPAIR image—edema in the anterolateral wall of the most basal slice is shown by the orange arrow; (B) MRI short-axis T1 SPIR post-contrast (gadolinium) image—early enhancement is shown in the anterolateral wall of the basal slice by the orange arrow, indicating inflammation; (C) 2D short-axis late gadolinium (viability) image—no late enhancement is seen, indicating that no fibrosis is present, and thus no infarction. Baseline and on-treatment day +1 ECG from case 2 showing new ST-elevation in the anteroseptal/anterior leads indicative of myocardial infarction (D); (E & F) cardiac MRI late gadolinium image—late enhancement (LGE) subendocardial in the anteroseptal wall, which is typically for infarction (E is short axis, F shows the long axis). Baseline and on-treatment day +4 ECG from case 3 showing micro-voltages suspect of peri-myocarditis (G). Baseline and two on/post treatment ECGs from case 7 showing convex ST-segment in the precordial (septal) chest leads and an interventricular conduction disorder, incomplete right bundle branch block, and left anterior fascicular block on day +6, after which the T-wave in the anterior wall and septal precordial leads progressed to a negative T-segment on day +29 (H). 2D, two-dimensional; ECG, electrocardiogram; MRI, magnetic resonance imaging.

### *Case 4. Heart and kidney failure*

A 66-year-old woman presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, without cardiac history. Pre-treatment ECG showed a right bundle branch block; LVEF was normal. On day −2/−1 of the treatment protocol, the patient developed pulmonary congestion with hypoxia, edema, and tachycardia, without evident chest pain. ECG showed microvoltages; TTE demonstrated minimal pericardial effusion, without hemodynamic compromise. NT-proBNP and TropT were markedly elevated. Several hours after TIL infusion, the patient became increasingly dyspneic with tachypnea, which partly resolved throughout the night. After discussing with the patient, the first IL-2 dose was administered. Unfortunately, the patient's condition subsequently deteriorated with respiratory failure, for which ICU admission for non-invasive ventilation was necessary. Differential diagnosis comprised decompensated heart failure, perimyocarditis, CLS, or sepsis. Repeated TTE showed normal left and right ventricular function, and minimal pericardial effusion. TropT

and NT-proBNP remained high. Treatment with an angiotensin-converting enzyme inhibitor, beta-blocker, aldosterone antagonist, and furosemide were initiated. Recovery was slow. During follow-up cardiac function remained stable; however, peripheral edema prevailed and she suffered progressive renal failure. A diagnosis of nephrotic syndrome due to pauci-immune glomerulonephritis was confirmed.

### *Case 5. Atrial fibrillation*

A 54-year-old woman presented with *BRAF* V600E-mutated, metastatic melanoma progressive on first-line nivolumab, without history of cardiac disease. Pre-treatment ECG was normal; transthoracic echocardiogram showed an LVEF of 51% and a small patent foramen ovale (PFO). No significant toxicities were observed during the lymphodepleting chemotherapy or TIL infusion. On a regular checkup after the first HD IL-2, fever and a rapid, irregular heartbeat were observed. ECG showed atrial fibrillation with a ventricular response rate of 150 beats per minute (BPM, Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2024.102383). She was treated with a cardio-specific beta-blocker, followed by a decline in heart rate to 96 BPM. Psychical examination and ECG the next morning confirmed sinus rhythm. She experienced slight dyspnea, persisting after conversion to sinus rhythm. No further IL-2 was administered. Patient recuperated clinically and was discharged. Follow-up showed normal cardiac function and sinus rhythm.

### *Case 6. Acute dyspnea*

A 67-year-old woman presented with metastatic NSCLC progressive after treatment with atezolizumab/bevacizumab/paclitaxel/carboplatin, with a history of hypertension, and palpitations due to atrial tachycardia and atrial extrasystoles, for which she received treatment with a calcium channel blocker (amlodipine) and beta-blocker (metoprolol). Previous cardiac ultrasound demonstrated mild left ventricular hypertrophy with normal cardiac function. Pre-treatment ECG showed sinus rhythm with atrial extrasystoles; LVEF was 75%. On day −3, the third day of chemotherapy, she was mildly dyspneic. Physical examination demonstrated a tachypnea of 24/min, oxygen saturation of 86%, mild systolic murmur, and peripheral edema with a high positive fluid balance. Chest X-ray showed signs of pulmonary congestion. Treatment with furosemide was successful. Directly after TIL infusion, the patient experienced dyspnea and chest discomfort. ECG showed no signs of acute ischemia. TropT and NT-Pro-BNP were both elevated. Symptoms subsided spontaneously, and the first IL-2 infusion was administered 4 h later than originally scheduled. Several hours later, the patient experienced acute-onset severe dyspnea. By physical examination, hypoxia (82%), a respiratory rate of 44/min, bilateral crackles, hypertension, tachycardia, and fever were observed. Via a non-rebreather mask, 15 l of oxygen was administered, restoring oxygen saturation to 95%. Most symptoms spontaneously resolved within 1 h. ECG showed no signs of ischemia, and cardiac ultrasound demonstrated normal cardiac function. TropT and NT-proBNP were repeated and remained significantly elevated. Symptoms were attributed to CLS, with elevated cardiac markers secondary to possible subclinical ischemia, tachycardia, and inflammation. IL-2 was discontinued. Residual pulmonary edema resolved gradually within the following days. The patient experienced no specific cardiac symptoms during outpatient follow-up.

### *Case 7. Heart failure due to myocarditis or ischemia*

A 55-year-old male presented with metastatic NSCLC with a history of radiotherapy (1x 8Gy) on the dorsal side of costa 6 on his left side, progressive after pembrolizumab/adagrasib and carboplatin/pemetrexed, without known cardiac history. Pre-treatment resting- and stress-ECG were normal; EF was 61%. On day −2, low oxygen saturation and edema were noted for which low-dose diuretics were administered. Shortly after TIL infusion and the first two IL-2 doses, low oxygen saturation (83%) was observed without other accompanying symptoms. Oxygen was supplied with satisfactory result (97%). After the third IL-2 dose, the patient experienced progressive dyspnea, low oxygen saturation (77%), and tachycardia without chest pain, accompanied with mild general edema and low-grade fever. On day +3, the patient remained dyspneic on exertion, tachycardia persisted, and oxygen supplementation was required. Chest X-ray showed a pre-existing pleural effusion without further signs of congestion. ECG showed sinus tachycardia. Cardiac markers were markedly elevated. Symptoms were attributed to CLS, with subclinical cardiac ischemia or myocarditis as differential diagnosis for elevated cardiac markers. A close wait-and-see strategy was followed. Antibiotics were started for persistent febrile neutropenia. Follow-up ECG showed a slightly convex ST-segment in the precordial (septal) chest leads. The following days, the patient remained stable but suffered persistent fluid retention and remained oxygen supplementation dependent. On treatment day +7 TropT levels decreased, but NT-proBNP levels remained high. A differential diagnosis of CLS or heart failure due to myocarditis or subclinical ischemia was reconsidered. ECG demonstrated an interventricular conduction disorder, incomplete right bundle branch block, and left anterior fascicular block. Bedside cardiac ultrasound showed a mildly reduced EF of 50% and a possible hypokinetic ventricular septum. The patient was admitted to the ICU for continuous hemodynamic monitoring and treatment for severe fluid retention with the suspicion of myocarditis or cardiac ischemia. He remained hemodynamically stable without progression of conduction abnormalities. The patient was successfully treated with intravenous diuretics and an aldosterone receptor antagonist. Repeated cardiac ultrasound confirmed globally normal LVEF with a mid-septal hypokinetic segment. On repeated ECGs, the T-wave in the anterior wall and septal precordial leads progressed to a negative T-segment (Figure 3), possibly related

to myocarditis or subclinical ischemia. The patient slowly recuperated and was discharged from the hospital. During outpatient clinic follow-up, a CMR was considered to complete the diagnostic work-up, but due to progression of the NSCLC and physical discomfort this was no longer possible.

## DISCUSSION

Treatment using TILs has shown impressive results in melanoma and NSCLC patients, likely leading to the first approved TIL products within the next few years. With the impending approval and the increasing number of clinical trials investigating TILs, the number of patients eligible for TIL treatment is rapidly increasing. This also means that rare toxicities, such as severe cardiotoxicity, will become more prevalent. In a recent comprehensive evaluation of 43 melanoma patients treated with TIL at the Moffitt Cancer Center, an overall cardiovascular toxicity rate of 41.9% was observed, with 14 patients (33%) experiencing hypotension requiring intravenous fluids and vasopressors, 6 (14%) experiencing atrial fibrillation, and 1 patient (2%) with primary TropT elevation.[29] This is much higher than the combined reported incidence of 4% in the meta-analysis by Dafni and colleagues,[4] possibly because cardiovascular toxicities are currently not systematically reported in most trials. Information on TIL treatment-induced cardiotoxicity is limited and data regarding their optimal management are lacking. Therefore, we have provided an overview of the current literature on cardiotoxicity associated with TIL therapy and have presented seven patients treated with TIL at the NKI for their metastatic melanoma or NSCLC who developed cardiotoxicity. Here, we will propose clinical recommendations for diagnosis and management of these symptoms (Figure 4).

### *General*

Based on our experience, as described in the cases, completing the diagnosis of toxicities or following the standard guidelines for treatment is often challenging because of the patients' clinical conditions. Patients are often pancytopenic from the lymphodepleting chemotherapy, and may require close monitoring as a result of the toxicity. Symptoms, especially edema and dyspnea, can also be attributed to CLS, and extra attention should be paid to a possible infectious cause as patients are immunocompromised. The challenge sits in using available diagnostics and detecting risk factors while definite risk factors and screening strategies have not been established. Moreover, if the patient's condition does not permit treatment of toxicity, or when the treatment strategy will not change by carrying out additional diagnostic tests, we refrain from burdening the patient, as diagnostics may also be invasive or potentially harmful. In every case, diagnostics must be justified and proportional to the possible treatment. Likewise, if a patient develops signs of cardiotoxicity early on in the treatment, such as during or after the lymphodepleting chemotherapy or TIL infusion, extra caution is necessary and the risk—benefit ratio of continuing with the treatment should be carefully considered.

### *Pre-treatment*

The evaluation of patients who are potential candidates for TIL treatment should at minimum follow the latest European Society of Cardiology (ESC) Guidelines on cardio-oncology.[42] This starts with risk assessment and includes cardiac serum biomarkers [cardiac troponin (TropT/I), BNP, NT-proBNP], a 12-lead ECG, and a TTE (LVEF and global longitudinal strain). A functional test, such as an (exercise) stress test, may be considered when in doubt of physical performance and symptoms. When abnormalities are observed in these assessments, or if the patient has a history of cardiac disease or has a high risk of developing cardiotoxicity (see ESC Guidelines on cardio-oncology), the patient should be referred to a cardiologist for further examination and risk assessment.[42] Furthermore, all patients should be encouraged to maintain physical activity, and if applicable, quit smoking and restrict their alcohol consumption.[43] If the time between screening and TIL treatment is long, it is recommended to repeat cardiac serum biomarkers to identify subclinical cardiac injury during TIL treatment.[42]

### *Lymphodepleting chemotherapy*

As the TIL infusion is preceded by a lymphodepleting chemotherapy regimen, the first cardiotoxicities to be expected are related to the chemotherapeutic agents and the concomitant fluid challenge to the cardiovascular system. Special attention has to be paid to cyclophosphamide-induced acute heart failure. Symptoms and signs suggesting this should prompt direct evaluation, consisting of physical examination, 12-lead ECG, cardiac biomarkers, and cardiac imaging.[42] Although CMR is the diagnostically superior tool, a transthoracic echocardiogram is usually preferred due to its availability, and the possibility to carry it out at the bedside. Depending on the severity of symptoms, continuous hemodynamic monitoring at the ICU or referral to a specialized cardio-oncology center with a cardiac care unit (CCU) may be required. In general, management should follow the corresponding ESC Clinical Practice Guidelines.[42] In patients with obesity, it can be considered to use the (adjusted) ideal body weight or capping the body surface area at 2.0 $m^2$ in order to potentially reduce the risk of chemotherapy-induced toxicities in this patient population.[44-46]

### *TIL infusion*

During the TIL infusion, patients can experience shortness of breath as a result of TIL accumulation in the lung capillaries. This is a temporary sensation and can be managed effectively by oxygen supplementation. When patients experience symptoms potentially related to cardiotoxicity, it is important to keep in mind that patients are immunocompromised and are/will be thrombocytopenic at this point.

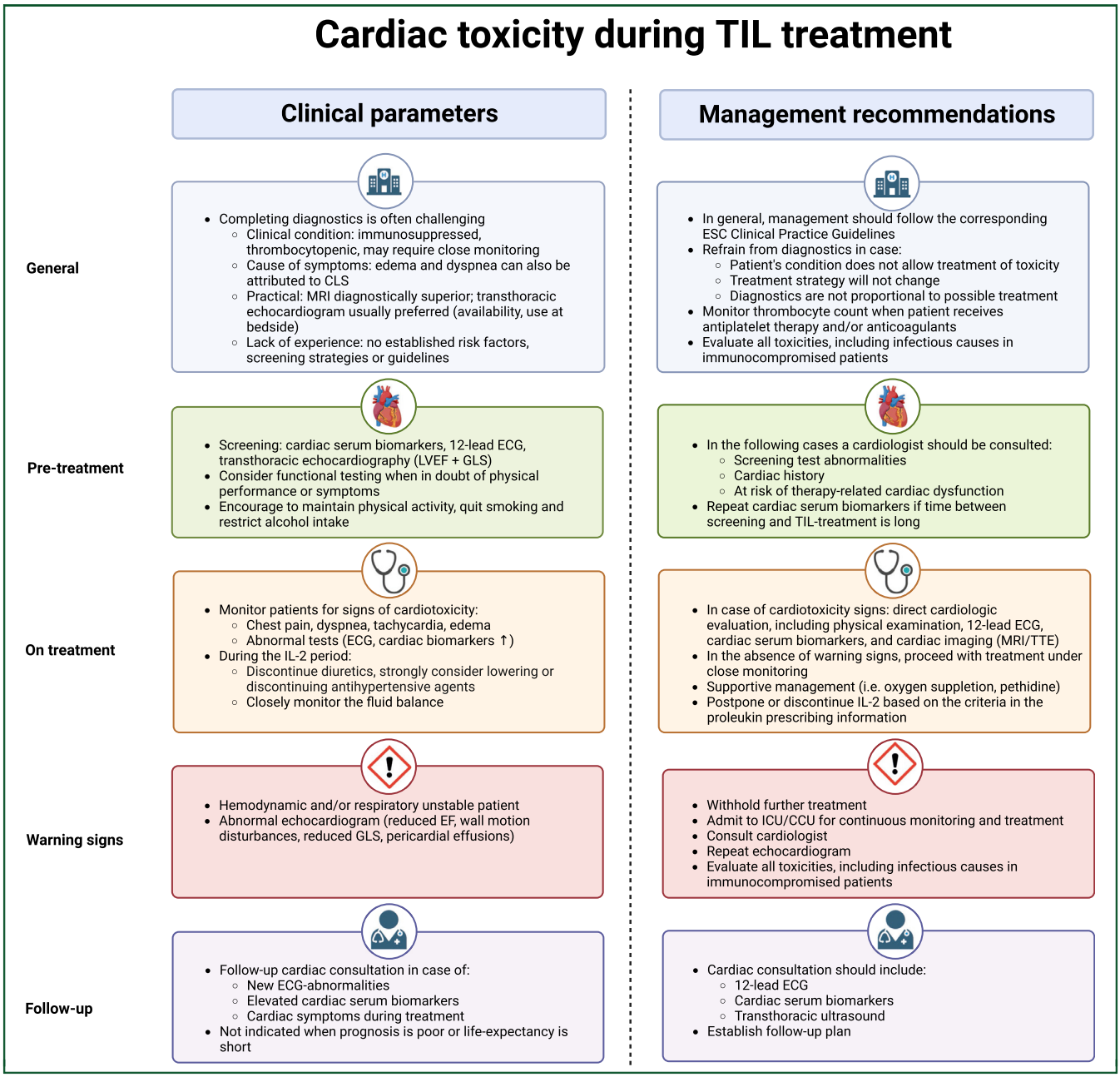


**Figure 4. Guidelines.** A summary of the guidelines, both focused on the clinical parameters and the corresponding management recommendation, from the initial screening of the patient until the follow-up after treatment.
CCU, coronary care unit; CLS, capillary leak syndrome; ECG, electrocardiogram; EF, ejection fraction; ESC, European Society of Cardiology; GLS, global longitudinal strain; ICU, intensive care unit; IL-2, interleukin-2; LVEF, left-ventricular ejection fraction; MRI, magnetic resonance imaging; TIL, tumor-infiltrating lymphocyte; TTE, transthoracic echocardiography.

### *High-dose IL-2*

Patients receiving HD IL-2 after the TIL infusion are at risk of developing CLS, characterized by extravascular fluid accumulation. This, in combination with the systemic inflammatory response after TIL infusion further triggered by IL-2, may evoke myocardial ischemia, infarction, arrhythmias, and peri- or myocarditis. When patients develop signs of pulmonary congestion, tachycardia, hypotension, or experience symptoms of chest pain or dyspnea, possible cardiotoxicity should be evaluated. At a minimum, a cardiology consultation including a TTE is recommended. Symptoms typically peak ∼4-6 h after the HD IL-2 administration and are reversible upon IL-2 discontinuation in most cases.[40] A factor complicating the diagnostics is the increase of cardiac enzymes observed in almost all patients on the days of IL-2 administrations (Figure 1), making this diagnostic tool unsuitable in the evaluation of possible cardiotoxicity. Experienced personnel, trained in the management of symptoms and who are familiar with the criteria when doses should be postponed, restarted or permanently discontinued, as described in the Proleukin (aldesleukin, IL-2) prescribing information, is essential.[41] With appropriate treatment and

monitoring, a stable patient with non-life-threatening toxicity can proceed with treatment. In patients with severe cardiotoxicity, or potentially life-threatening toxicity such as MI or myocarditis, treatment should be interrupted and patients should be admitted to an ICU or CCU for continuous hemodynamic monitoring. In patients who develop acute coronary syndrome or arrhythmias and who are still thrombocytopenic, the benefits and risks of anticoagulation should be carefully considered. Of note, in contrast to symptoms of systemic inflammation mediated by IL-6 as seen in CAR-T cell treatments, tocilizumab, an anti-IL-6 receptor antibody, is not typically administered to treat IL-2-induced systemic inflammation.

### *Follow-up*

For patients who develop new abnormalities on ECG, elevated cardiac serum biomarkers, or other cardiac symptoms during the TIL treatment period, a cardiac consultation after discharge is recommended to establish a follow-up plan.[42] Here, 12-lead ECG, repeated TTE, and serum biomarkers should be considered to guide diagnosis and treatment. However, if the prognosis is poor or their life expectancy is short, this follow-up consultation is not indicated.[42] Importantly, while the cardiotoxicities observed vary greatly in presentation and severity, they did not seem to significantly affect survival in our patients or in the patients described in the study by Fradley and colleagues.[29]

### *Conclusions*

In the absence of evidence-based guidelines for the treatment of TIL therapy-associated cardiotoxicity, we provided an overview of literature, case descriptions, and recommendations for diagnosis and management. These recommendations are intended to help physicians in their daily practice as the number of patients qualifying for TIL treatment is rapidly increasing. Thus far, there are limited data available on the prevalence of cardiotoxicity in patients treated with TIL, as most studies have not reported these toxicities. To better understand TIL-induced cardiotoxicities and develop evidence-based guidelines, systematic registration and publication of these data is required.

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