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Abatacept/Ruxolitinib and Screening for Concomitant Respiratory Muscle Failure to Mitigate Fatality of Immune-Checkpoint Inhibitor Myocarditis

Short title: Management of immune checkpoint inhibitor myotoxicity

Joe-Elie SALEM¹; Marie BRETAGNE¹; Baptiste ABBAR^{1,2}; Sarah LEONARD-LOUIS³; Stéphane EDERHY⁴; Alban REDHEUIL⁵; Samia BOUSSOUAR⁵; Lee S NGUYEN^{1,6}; Adrien PROCUREUR¹; Frederic STEIN¹; Charlotte FENIOUX¹; Perrine DEVOS¹; Paul GOUGIS¹; Martin DRES⁷; Alexandre DEMOULE⁷; Dimitri PSIMARAS⁸; Timothee LENGLET⁸; Thierry MAISONOBE⁸; Marc PINETON DE CHAMBRUN⁹; Guillaume HEKIMIAN⁹; Christian STRAUS¹⁰; Jesus GONZALEZ-BERMEJO⁷; David KLATZMANN¹¹; Aude RIGOLET¹²; Perrine GUILLAUME-JUGNOT¹²; Nicolas CHAMPTIAUX¹²; Olivier BENVENISTE¹²; Nicolas WEISS¹³; Samir SAHEB¹⁴; Philippe ROUVIER¹⁵; Isabelle PLU³; Estelle GANDJBAKHCH¹⁶; Mathieu KERNEIS¹⁶; Nadjib HAMMOUDI¹⁶; Noel ZAHR¹; Claudia LLONTOP⁷; Capucine MORELOT-PANZINI⁷; Lorenz LEHMANN¹⁷; Juan QIN¹⁸; Javid J MOSLEHI¹⁸; Michelle ROSENZWAJG¹¹; Thomas SIMILOWSKI⁷; Yves ALLENBACH¹²

¹ Sorbonne Université, INSERM, UNICO-GRECO Cardio-oncology Program, Department of Pharmacology, GRC27, CIC-1901, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

² Sorbonne Université, INSERM, Department of Oncology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

³ Sorbonne Université, INSERM, Department of Neuropathology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

⁴ Sorbonne Université, INSERM, UNICO-GRECO Cardio-oncology Program, Department of Cardiology, GRC27, AP-HP, Hôpital Saint-Antoine, F-75012 Paris, France

⁵ Sorbonne Université, INSERM, ICAN, Department of Radiology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

⁶ Research and Innovation of CMC Ambroise Paré (RICAP), Centre Medico-Chirurgical Ambroise Paré, Neuilly-sur-Seine, France

⁷ Sorbonne Université, INSERM, Department R3S (Respiration, Réanimation, Réhabilitation, Sommeil), AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

⁸ Sorbonne Université, INSERM, Department of Neurology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

⁹ Sorbonne Université, INSERM, ICAN, Department of Medical Intensive Care Unit, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹⁰ Sorbonne Université, INSERM, UMRS1158, Service d'Explorations Fonctionnelles de la Respiration, de l'Exercice et de la Dyspnée (Département R3S), AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹¹ Sorbonne University, INSERM UMRS959, Assistance Publique Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, CIC-1901, Paris, France

¹² Sorbonne Université, INSERM, Department of Internal Medicine, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹³ Sorbonne Université, INSERM, Department of Neurology intensive care unit, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹⁴ Sorbonne Université, INSERM, Department of Hemobiology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹⁵ Sorbonne Université, INSERM, Department of Anatomopathology, AP-HP, Hôpital Pitié-Salpêtrière, F-75013 Paris, France

¹⁶ Sorbonne Université, ACTION Study Group, INSERM UMR_S 1166, Institute of Cardiometabolism And Nutrition (ICAN), and Hôpital Pitié-Salpêtrière (AP-HP), Boulevard de l'hôpital, F-75013, Paris, France.

¹⁷ Department of Cardiology, Angiology, and Pneumology, Heidelberg University Hospital, Heidelberg, Germany; German Centre for Cardiovascular Research (DZHK), Heidelberg/Mannheim partner site, Germany

¹⁸ Section of Cardio-Oncology & Immunology, Division of Cardiology and the Cardiovascular Research Institute, University of California San Francisco, 555 Mission Bay Blvd South, Box 3118, San Francisco, CA 94143-3118

Correspondence: joe-elie.salem@aphp.fr, +33(0)142178535, CIC-1901, Departement of Clinical Pharmacology, Pitié-Salpêtrière University Hospital, Sorbonne Universite; 47 Boulevard de l'Hopital, 75013 PARIS ;

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Abstract.

Immune-checkpoint-inhibitor (ICI)-associated myotoxicity involves the heart (myocarditis) and skeletal muscles (myositis), which frequently occur concurrently and is highly fatal. We report the results of a strategy that included identification of individuals with severe ICI-myocarditis by also screening for and managing concomitant respiratory muscle involvement with mechanical ventilation, as well as treatment with CTLA4-fusion protein abatacept and the Janus-kinase inhibitor ruxolitinib. Forty cases with definite ICI-myocarditis were included with pathological confirmation of concomitant myositis in the majority of patients. In the first 10 patients, using recommended guidelines, myotoxicity-related fatality occurred in 60%, consistent with historical controls. In the subsequent 30 cases, we instituted systematic screening for respiratory muscle involvement coupled with active ventilation and treatment using ruxolitinib and abatacept. Abatacept dose was adjusted using CD86-receptor occupancy on circulating monocytes. Myotoxicity-related fatality rate was 3.4%(1/30) in these 30 patients vs.60% in 1st quartile($p<0.0001$). These clinical results are hypothesis-generating and need further evaluation.

Statement of significance.

Early management of respiratory muscle failure using mechanical ventilation and high-dose abatacept with CD86 receptor occupancy monitoring combined with ruxolitinib may be promising to mitigate high fatality rates in severe Immune-Checkpoint Inhibitor myocarditis.

Introduction.

Immune-checkpoint inhibitors (ICI) have revolutionized cancer treatment and have been approved in multiple cancer types, and include antibodies that target CTLA4 (cytotoxic-T-lymphocyte-antigen-4), PD1 (Programmed-cell-Death-protein 1), the PD1 ligand PDL1, and LAG3 (lymphocyte-activating-3).(1) ICI restore T-cell-mediated host immunity against tumors,(1) but they may also induce immune related adverse events (irAE)(2) including ICI-myocarditis which occurs infrequently (~1%) but carries a 30-50% mortality rate.(3-7) Clinical suspicion for ICI-myocarditis often arises after associated symptoms and increases in cardiac biomarkers (troponins) or electrocardiographic alterations.(3,8-10) Endomyocardial biopsy is the gold-standard for establishing the diagnosis,(10,11) while cardiac magnetic resonance imaging (cMRI) and echocardiography may be misleadingly normal especially early in the clinical course.(10,12,13) Other forms of myotoxicity often accompany myocarditis and these include peripheral myositis, which can affect respiratory muscles or mimic myasthenia-gravis-like syndrome.(6,10,14) The pathophysiology of these myotoxicities is characterized by myocyte necrosis induced by lymphohistiocytic infiltrates secondary to activation of clonal autoreactive T-cells against muscle antigens, notably α -myosin expressed in the heart and peripheral muscles.(7,15). Preliminary data suggest that myositis, particularly when affecting respiratory muscles, accompanying ICI-myocarditis can negatively impact survival, and requires an aggressive patient care strategy.(5,6,10,14) The main causes of death attributed to ICI-myotoxicities are life-threatening ventricular arrhythmias, cardiogenic shock or hypercapnic respiratory muscle failure.(10)

Current treatment of ICI-myocarditis includes empiric corticosteroids, which are not universally effective; second line therapies, including immunosuppressive therapies targeting T-cells have been described.(16-18) In pre-clinical models, CTLA4 signaling appears to play a critical role in the development of myocarditis with CTLA4 immunoglobulin(Ig)-fusion-protein (abatacept) attenuating myocardial inflammation.(19) Preliminary case reports have suggested that the CTLA4 immunoglobulin-fusion-protein abatacept may be a promising option.(19-21) However, the optimal dosing scheme and

the effect of combining CTLA4-Ig with other immunosuppressants is unknown. Abatacept (a CTLA4-Ig), acts as a decoy receptor for CD80/CD86 expressed on antigen-presenting-cells (e.g., macrophages), interfering with T-cell co-stimulation through CD28. Therefore, abatacept leads to global T-cell anergy by reversing pathways activated by ICI.(1,19-21) When abatacept is used for approved indications such as rheumatoid arthritis, occupancy of the CD86 receptor on circulating monocytes (CD86 RO) is a pharmacodynamic biomarker of clinical activity, with CD86 RO \geq 80% as a target for maximal efficacy.(22,23) Another consideration is time of onset of abatacept which is slow-onset. Consistently, in a mouse model of ICI-myocarditis, myocardial immune infiltration is attenuated after 10 weeks of abatacept treatment; 2-week duration of treatment with abatacept had minimal effect on immune infiltration.(19) Preliminary reports suggest that abatacept clinical efficacy can be enhanced by addition of the Janus kinase (JAK) inhibitor ruxolitinib, which impairs T-cell activation via blockade of pro-inflammatory cytokines and is used to treat graft vs. host disease, a condition in which donor T-cells attack host tissue.(21,24) Ruxolitinib (a JAK1, JAK2 inhibitor) is thought to exert a rapid (within hours of start) synergistic effect with abatacept (slower time to onset of effect) by also decreasing CD86 expression on macrophages while also acting downstream in the immunological synapse, inactivating T-cells.(1,25-27)

With these considerations in mind, we have developed a strategy where we identify individuals with severe ICI-myocarditis by also screening for and managing concomitant respiratory muscle involvement with mechanical ventilation and treating these individuals with high dose CTLA4-fusion protein abatacept with CD86 RO monitoring and the Janus-kinase inhibitor ruxolitinib. We applied this strategy to 30 consecutive patients with ICI-myocarditis. By comparison, we also described a cohort of 10 patients seen previously where patients underwent usual recommended care with initiation of corticosteroids and addition of a secondary immunosuppressive therapy.

Results.

Study Cohort.

Of the 69 patients consecutively admitted for suspicion of ICI-myocarditis, 40 were confirmed as definite cases and were included in this report (**Figure 1** for flow-chart, **Table 1** for demographics & **Supplementary Table 1** for diagnostic certainty criteria). These patients were analyzed as the first 10 (quartile 1, Q1, admitted 05/2018-03/2020) treated as per current expert consensus guidelines starting with high dose corticosteroids boluses followed by second lines agents in case of cortico-resistance (16) versus the subsequent 30 (Q2-4, 03/2020-08/2021) treated with a mechanism-based approach. The mechanism-based approach included prompt use of high dose abatacept with real-time CD86 RO monitoring, ruxolitinib, lower doses of corticosteroids, and systematic screening for and early management of concomitant respiratory muscle failure. The median age of the overall cohort was 72 [IQR=62,79]years, 58% were male; the most represented cancers were lung (9/40, 23%), skin (9/40, 23%) and genitourinary cancers (10/40, 25%). A majority of patients (30/40, 75%) developed severe (grade \geq 3) myotoxicity (**Table 1** and **Figure 2** for details concerning severity features of these patients & **Supplementary Tables 2-5** for detailed severity adjudication criteria). During the course of the disease, all patients had increased troponin-T (**Figure 2**); most (34/40, 85%) developed abnormal ECG features and 9/40(23%) developed severe systolic cardiac dysfunction (grade \geq 3). A total of 13% (5/40) required inotropic agents or extra-corporeal hemodynamic support. At least one cMRI examination (median/patient: 2[1-3]) supported the diagnosis in 26/35 (74%) (**Table-1** for results and **Supplementary Table 1** for cMRI diagnostic criteria; 5 patients died prior to cMRI or refused it). Cardiac and muscular biopsies supported the ICI-myotoxicity diagnosis in 24/32 (75%; 8 patients died prior to cardiac biopsy or refused it) and 36/40 (90%), respectively (**Table 1** for results and **Supplementary Table 1** for pathology criteria). Cardiac and muscular histopathology findings consistently showed T-cell and macrophage infiltration with associated myocyte necrosis (**Supplementary Figure 1A-E**). A total of 12/40 (30%) developed ventricular tachycardias (6/12, 50% sustained) and 9/40 (23%) developed severe conduction

disorders (7 complete atrioventricular block, and 2 sinus node dysfunction requiring pacemaker implantation). Respiratory muscle involvement was present in 27/38 cases (71%; 2 patients in Q1 died prior to evaluation) with specific evidence for diaphragmatic involvement in 22/38 cases (**Supplementary Table 3** for the detailed framework used to evaluate the global respiratory muscle and more specifically the diaphragm function). Respiratory muscle involvement was severe and required mechanical ventilation in over a third of patients (15/38) (**Table 1**). Electromyogram showed a myopathic pattern in half (19/37; 3 died before evaluation) of patients with no associated neuromuscular junction disorder (0/28 abnormal repetitive nerve stimulation test) despite clinical features of myasthenia-gravis (**Table 1**). Acetylcholine receptor antibodies were positive ($>0.5\text{nmol/L}$) in 8/40(20%), of which 5/5(100%) were previously known abnormal before initiation of ICI. Anti-muscle specific kinase antibodies were negative in all patients. In the 4 patients autopsied during the index hospitalization for ICI-myotoxicity, myocarditis and diaphragmatic myositis was severe, associated with lesions in peripheral muscles (**Supplementary Figure 1A-E**).

Cardio-myotoxicities outcomes with evolving management protocol.

The overall clinical demographic and diagnostic characteristics of patients in Q1 vs. Q2-4 were similar with no difference in peak cardiac troponin-T reached and in the different myotoxicity severity features studied (arrhythmia, cardiac and respiratory muscle dysfunction) upon presentation or during follow-up (**Table 1**). In Q1, all patients were treated with corticosteroids ($\geq 500\text{mg/day}$ intravenous methylprednisolone pulse ≥ 2 days in 9/10(90%)) started within 2[0-4]days of presentation. 8/10 patients did not respond to corticosteroids (“cortico-resistant”) and subsequently received plasmapheresis (2-5 cycles, $n=8$), low-dose abatacept ($n=7$), or mycophenolate-mofetil ($n=4$; time to start from presentation and dose are detailed in **Supplementary Table 6** and **Supplementary Figure 2**). ICI-myotoxicity related mortality was 60% (6/10) in Q1, with deaths due to cardiogenic shock or respiratory muscle failure ($n=3$ each). Among the first 10 patients, the diagnosis of respiratory muscle involvement was confirmed on chart review in 6/8 (75%). From Q1, we also identified that plasmapheresis partially cleared ICI blood levels (**Supplementary Figure 3**), but identification of PD(L)1 expression on T-cells was markedly delayed

to few months after pheresis (**Figure 3**). We also inferred that plasmapheresis would clear abatacept (an example provided in **Supplementary Figure 4A**), so we delayed using the drug after plasmapheresis.(1) Moreover, in the first 10 patients, we found that the initial abatacept dosing strategy did not improve outcome (60% of ICI-related mortality in Q1). Indeed, even with higher abatacept doses used subsequently in Q2-4, CD86 RO peak levels were often below the objective of $CD86RO \geq 80\%$ and we identified that CD86 RO varied dose-dependently after abatacept injections (**Figure 4A & B**).

With the above information from Q1 patients, we modified treatment and monitoring strategy for patients in Q2-4. We stopped performing plasmapheresis in severe cases instead opting for initiation of higher dose abatacept if grade ≥ 3 (severe) myotoxicity criteria were met. The abatacept starting dose was increased to $\sim 20\text{mg/kg}$ on days 0, 5, and 14 and the total dose administered in the first 2 weeks was 60 [53-65]mg/kg in Q2-4 vs 41 [24-60]mg/kg in Q1 ($p=0.01$ for Q1 vs. Q2-4, **Supplementary Table 6** for evolution of abatacept dose used per specific quartile). In severe cases in Q2-4 (22/30 patients), we added ruxolitinib to abatacept and corticosteroids based on pre-clinical and translational studies outlined below, as well as given rapid onset of action of ruxolitinib (in contrast to abatacept which has delayed onset of action). Ruxolitinib was used in 0/8 of severe cases in Q1 vs. 17/22, 77% in Q2-4 ($p=0.0002$). In Q2-4, abatacept was used in all 22 severe cases. In Q2-4, we used lower concomitant doses of corticosteroids seeking for less related adverse events vs. Q1 (intravenous methylprednisolone equivalent pulse $\geq 500\text{mg/day}$ in 13/30, 43% in Q2-4 vs. 9/10, 90% in Q1, $p=0.01$; 155 [122-223]mg of mean daily dose in the 1st month of treatment in Q1 vs. 96[21-156] mg/day in Q2-4, $p=0.02$; **Supplementary Table 6** for evolution of treatment modalities per specific quartile). The median time between presentation, appearance of grade ≥ 3 (severe) ICI-myotoxicity criteria, and use of immunosuppressants are shown in **Supplementary Figure 2** and **Supplementary Table 6**. Frequencies of side effects observed on immunosuppressants including infections are detailed in **Supplementary Table 6**. In Q2-4, all patients were screened for respiratory muscle involvement for a decision concerning ventilator indication and management. While the proportion of severe cases (grade ≥ 3) was comparable in Q1 (8/10, 80%) and Q2-4 (22/30, 73%, $p=0.67$), the combined pharmacotherapeutic and ventilatory

strategy was associated with a decrease in ICI-related myotoxicity death from 6/10 (60%) in Q1 to 1/30 (3%) in Q2-4 ($p<0.0001$). Restricting analysis to severe (grade \geq 3) cases, results were similar with 6/8 (80%) ICI-related myotoxicity death in Q1 vs. 1/22 (5%) in Q2-4 ($p<0.0001$). With the use of screening for respiratory muscle failure in Q2-Q4, 10/30 (30%) were found eligible for elective ventilation and this was started in all but one who declined intubation (and who was the only death in Q2-Q4), and one with hepatic cancer who died of decompensated cirrhosis before ventilation was started. In these 8 ventilated patients (details on the modality of ventilation in **Supplementary Figure 2**), recovery was complete in 6/8 (median weaning time=17[2-111]days) and 2/8 were recovering (weaned from diurnal ventilation) being on residual nocturnal ventilation at 6month follow-up.

There were deaths due to other causes (**Figure 1**) including covid-19 (in Q2-4) and sepsis (Q1). All-cause mortality at 3 months was 6/10 (60%) in Q1 and dropped to 7/30 (23%, $p=0.03$) in Q2-4; the corresponding figures at 6 months were 7/10 (70%) vs. 9/30 (30%, $p=0.03$). Restricting analysis of all-cause mortality to severe cases showed similar results at 3 months (6/8, 75% in Q1 vs. 5/22, 23%; $p=0.009$) and 6 months (7/8, 88% in Q1 vs. 7/22, 32%; $p=0.007$). Median overall survival 6 months after presentation for ICI-myocarditis was not reached in ICI-myotoxicity survivor's ($n=33$) vs. 0.7 months in those dead from ICI-myotoxicity ($n=7$, $p<0.001$, **Figure 5A & B**). Among patients surviving ICI-related myotoxicity, 82% had stable disease or a partial response and median PFS was 5.7 months (**Figure 5C & D**). Best change from baseline in tumor burden as a function of their cancer type and immunosuppressants received are shown in **Figure 5E & F**, respectively. Pacemakers were placed in 8 patients and in 5/8 (63%), the conduction disorder resolved after 8[2-22] days. Three patients were still pacemaker-dependent at 1year follow-up; two were alive and the other died of COVID-19 with complete destruction of the sinus node at autopsy (**Supplementary Figure 1F**).

The Q2-4 group included 4 patients with asymptomatic myocarditis with grade-1 ICI-myotoxicity criteria, diagnosed by abnormal Troponin-T but with no concomitant symptomatic irAE; there was a mild increase of peak troponin-T of 5[3-8] fold-change above upper reference limit vs. 116[60-223] in the other 36 ($p=0.0002$, **Figure 2**). In these 4 patients, immunosuppressant drugs were not used but ICI

were withheld. One such patient was re-challenged with ICI 2 months after the first episode but developed severe hepatitis(16) leading to permanent ICI discontinuation.

Rationale for JAK-STAT pathway inhibition to treat ICI-myotoxicity.

To elucidate underlying signaling mechanisms of ICI-myocarditis, we have utilized a genetic mice model (*ctla4^{+/-} pd1^{-/-}*) of ICI-myocarditis, which recapitulates the clinical and pathological features seen in patients; and which was initially used to demonstrate the biological plausibility and efficacy of abatacept.(19) Despite efficacy, abatacept onset was slow with minimal clearance of cardiac immune infiltrates after 2 weeks of treatment with abatacept in mice.(19) We therefore felt that abatacept would need to be combined with an immunosuppressive with a shorter onset of action. In parallel, we performed bulk RNA sequencing assessing transcriptomics and pathways affected in our mouse model. We compared these identified pathways in mice to human tissue after performing bulk RNA-sequencing from endomyocardial biopsies of patients with ICI-myocarditis compared to ICI treated patients with no myocarditis.(28) In ICI-myocarditis mice (*ctla4^{+/-}pd1^{-/-}*), bulk RNA sequencing showed very distinct affected pathways in ICI-myocarditis mice compared to unaffected control mice (*ctla4^{+/+} pd1^{-/-}*) (**Figure 6A**). JAK-STAT signaling was especially upregulated with *Jak2* being the most significantly upregulated gene among the *Jak* family, and *Stat1* and *Stat4* were the most significantly upregulated genes among the *Stat* family (**Figure 6B**). *Interferon-gamma/Jak2/Stat1* signaling, *Interleukin12/Jak2/Stat4* signaling, and *Interleukin6/Jak2/Stat3* signaling were the most significantly upregulated signaling pathways in ICI-myocarditis mice compared to unaffected control mice (**Figure 6C**). Bulk RNA-sequencing from patients also indicated that *Jak2* was the only significantly upregulated gene in the *JAK* family in patients with ICI-myocarditis (n=9), compared to ICI-treated patients without evidence of myocarditis (n=4) (**Figure 6D**). These findings from both mice and human data support the potential beneficial effect of ruxolitinib (a JAK1/JAK2 inhibitor) in ICI-myocarditis.”

Discussion.

In this study, we showed that prompt high-dose abatacept with real-time assessment of CD86 RO immuno-monitoring combined with ruxolitinib, corticosteroids, and active management of respiratory muscle involvement was associated with a decrease in ICI-myotoxicity related death among ICI-myocarditis patients. With this strategy, ICI-myotoxicity related mortality decreased to ~3%, from historical controls (20-60% in other published cohorts worldwide with ICI-myocarditis) as well as the first 10 cases in our patient cohort (60% in our Q1 cases) who did not undergo screening and treatment with this strategy.(2-4,8,29,30) While our study is not a clinical trial, our results may provide a promising platform by which to best treat the growing population that presents with fulminant ICI-myocarditis, with a mechanism-based and personalized assessment of drug receptor occupancy. The concept of abatacept use for ICI-myocarditis initially came from successful treatment in a genetic mouse model with subsequent clinical case reports.(19,20) Our study presents a comprehensive approach to treatment of these patients with attention to extra-cardiac myotoxicity associated with disease and attention to abatacept pharmacodynamics, associated with ruxolitinib use.

One key element for improved care of ICI-myocarditis has been our recognition that skeletal muscle involvement is near-universal (95% abnormal muscular biopsy). With a very high frequency of respiratory muscle involvement comparable to that found in other types of systemic myositis,(31) screening for (serial arterial blood gases) and management of respiratory muscle involvement appeared critical in avoiding life-threatening hypoventilation. As T-cells and macrophages are critical in the development of ICI-myocarditis and ICI-myositis, optimizing immunosuppressant therapy was also a critical advance in our approach.(1,19,20) Abatacept antagonizes the activation of ICI pathways through blockade of T-cells activation by antigen-presenting-cells (ie blocking the interaction between CD86 on monocytes and CD28 on T-cells). (1,24-27,32) Moreover, using translational approaches in mice and human, we were able to show that JAK2 signaling pathways were specifically activated in the heart of ICI-myocarditis mice and patients (namely *Interferon-gamma/Jak2/Stat1*; *Interleukin12/Jak2/Stat4*; and

Interleukin6/Jak2/Stat3; **Figure 6A-D**). These findings supported the specific use of ruxolitinib (a JAK1, JAK 2 inhibitor notably blocking interleukin-6 and Interferon-gamma cytokine pathways) among available JAK inhibitors previously proposed in the treatment of other types of irAE such as ICI-colitis.(33-36) More research is needed to further decipher the full mechanistic spectrum of the potential beneficial effects of ruxolitinib to treat ICI-myotoxicity, including its potential to decrease the antigenic presentation by muscle cells (making them harder targets) or to block some of the pro-apoptotic functions of Interferon-gamma.(37,38)

All 8 patients in whom we implemented early mechanical ventilation for respiratory muscle failure had near complete recovery, to the point of full weaning in 6 cases and weaning during the day in 2 cases. This contrasts with the experience presented in a very similar cohort of severe ICI-myotoxicity,(14) in which 8/12 (67%) patients who developed respiratory failure attributable to respiratory muscle myositis died despite use of very high-dose corticosteroids, plasmapheresis, intravenous immunoglobulin and rituximab.(14) Notably, the time to recovery of respiratory muscle failure in our cohort varied from a few days to several months, apparently depending on severity of initial presentation. Very slow recovery of respiratory muscle function after control of the causative insult has been well described in other neuro-myopathies affecting respiratory muscles.(39,40) In the case of ICI-myotoxicities, the concomitant use of high-dose corticosteroids favoring sarcopenia may also interfere deleteriously with muscular recovery.(41,42)

Several limitations of our analysis need to be recognized. As any non-randomized observational study, there is a risk of unaccounted bias between compared groups. In our study, we did not find any difference between quartiles in known ICI-myotoxicity severity criteria including peak troponin levels, proportion of patients treated by combination ICI therapy or presenting with severe arrhythmias, heart and respiratory muscle failure (**Table 1**) but other unknown severity criteria (to date) may have been missed and different between quartiles.(10) Our population was too small to perform ancillary analysis better adjusted on the dose of corticosteroids received, or any other subtlety related to the type of anticancer treatment or immunosuppressant regimen received. Indeed, in Q2-4, both high-dose

abatacept and addition of ruxolitinib were started precluding comparison of each treatment modality individually. Abatacept dose used within the 2 first weeks of treatment start in Q2-4 was higher vs. Q1 (~60 vs. 40mg/kg, respectively), but it has to be acknowledged that both these doses are much higher as compared to the intravenous loading dose used in abatacept's approved indication (10mg/kg every 2 weeks in rheumatoid arthritis).(1) Indeed, these doses are closer to belatacept loading dose equivalent given in graft rejection prophylaxis.(1)

A major challenge to consider while treating patient with severe irAE is how to mitigate symptoms and mortality risk while preserving anti-tumor beneficial effects. In our cohort, abatacept and ruxolitinib were used only over a short period (~1-2 months) of active treatment to achieve resolution of severity criteria, and this also limited the corticosteroid dose. To date, it is unclear to what extent the approach we used to treat ICI-myotoxicities altered ICI anticancer efficacy or would be efficient in other life-threatening irAE.(2) Of note, it is also important to highlight that most irAE toxicities are not life-threatening and can be even detected while infra-clinical by active screening strategies (e.g., by systematic troponin surveillance).(10) These low grades irAE would probably require no or minimal immunosuppressive strategy contrasting with life-threatening irAE.(16) The PFS estimate in our cohort (~6months) is unique since most previous ICI-myocarditis cohorts focused on describing the deadly cardiac outcomes and did not report cancer response. The variety of cancers in our cohort (mainly metastatic melanoma, lung, and kidney cancers) precluded us from performing reliable comparison to expected PFS in historical cohorts. However, median PFS in ICI clinical trials focusing on these latter cancers were consistently below 6 months (3-5 months).(1) Altogether, our results are promising and should guide further research assessing the question of the optimal drug mix, dosage and duration to be used to preserve ICI therapeutic effect while treating a severe irAE, the subject of further ongoing clinical study ([NCT05195645](#)). Comparison to other alternative pharmacological approaches also targeting T-cells (e.g anti-thymoglobulin, alemtuzumab, anti-calcineurin drugs) and proposed in current treatment guidelines for irAE also deserve further assessment.(16) Lastly, the experimental part of the study in mice was done on a small number of animals. The latter data should be therefore considered as

preliminary and call for more detailed studies, particularly those at the protein level that would confirm what you have been suggested by RNA. Meanwhile, the findings reported here justify a prompt referral to expert centers, able to evaluate the severity of patients notably by providing a systematic assessment and very close monitoring of respiratory muscle function in patients with ICI-myocarditis, to avoid missing life-saving indications for ventilatory assistance.

Methods.

Study Cohort. This prospective single-center cohort included 40 consecutive patients with definite ICI-myocarditis admitted to our cardio-oncology unit (Pitié-Salpêtrière, Paris, France) among 69 evaluated for suspected ICI-related myocarditis between 10/05/2018 and 08/18/2021 (Flow chart in **Figure 1**). ICI-related myotoxicity was confirmed by endomyocardial or muscle biopsies in all patients. Diagnostic certainty for ICI-myocarditis was determined based on modified Bonaca et. al criteria (**Supplementary Table 1**).⁽¹¹⁾ The general diagnostic work-up strategy upon ICI-myocarditis suspicion is detailed in **Supplementary Table 2**. A diagnosis of respiratory muscle involvement was established using the 2019 European Respiratory Society statement.⁽⁴³⁾ All these patients were included after written informed consent in the *MASC* (Myositis, DNA, Serum, Cells: Clinical Database and Biobank of Patients With Inflammatory Myopathies) prospective cohort (ethical approval CPP#2013 Ile de France VI; *NCT04637672*). Patients were treated after providing their written consent for compassionate use for abatacept and ruxolitinib. This study was conducted in accordance with Declaration of Helsinki ethical guidelines. Follow-up exceeded 6 months. Data will be shared with other researchers upon reasonable request.

Adjudications of events. Patients' charts were reviewed by two respiratory physicians to evaluate respiratory muscle dysfunction and its severity using a multi-parametric approach detailed in **Supplementary Table 3**.⁽⁴³⁾ Cardiac and skeletal muscle involvement (**Supplementary Table 4 & Supplementary Table 5**) and oncological status (progression free survival, PFS) were prospectively

assessed and graded (adapted from current guidelines) by two cardio-oncologists, a medical oncologist, and a radiologist.(16,44) Cardiac involvement was evaluated by cardiac MRI, endomyocardial biopsy, coronary angiography, echocardiography, electrocardiography and Troponin-T (ultrasensitive assay, Elecsys ROCHE Diagnostics®) monitoring. Skeletal muscle involvement was evaluated by peripheral muscle and diaphragmatic MRI, muscular biopsy, electromyogram, diaphragmatic echography and pulmonary functional test specifically seeking for respiratory muscle dysfunction (**Supplementary Table 3**). All causes of death were evaluated by three independent investigators. Autopsies were performed to determine exact cause of death, if family consented. ICI-related myotoxicities were considered severe (grade \geq 3) if any of the following was present: severe arrhythmias (defined as appearance of ventricular tachyarrhythmias or high-degree atrioventricular block or sinus node dysfunction), heart failure (defined as heart failure symptoms requiring intravenous diuretics or inotropes or hemodynamic support), respiratory muscle failure leading to hypoventilation, or deterioration of bio-clinical status despite corticosteroids (**Supplementary Table 4 & Supplementary Table 5**).(16,44)

Therapeutic strategy. The first 10 patients (starting in 2018 until March 2020) were all treated with high dose boluses of corticosteroids regardless of symptoms and severity grade.(16,44) In severe cases (grade \geq 3, defined above), plasmapheresis, mycophenolate-mofetil and abatacept (~10mg/kg per injection approximately every 2 weeks) were used, according to current guidelines.(16,44) After the first 10 patients (March 2020 to August 2021), our strategy was modified to include systematic screening in these ICI-myocarditis patients for and management of concomitant respiratory muscle failure (myositis) including serial and repeated arterial blood gases to identify alveolar hypoventilation – increase carbon dioxide partial pressure, PaCO₂– (**Supplementary Table 3**) and in severe cases: (1) prompt initiation of higher dose abatacept (~20mg/kg, 3 doses within the first 2 weeks of treatment start) with dose-adjustment based on real-time assessment of CD86 RO targeting peak value of CD86 RO \geq 80% within 72hours of abatacept and residual CD86 RO \geq 50% (**Supplementary Figures 2 & 4A** for examples) until resolution of myotoxicity severity to grade \leq 2 (**Figure 2 & Supplementary Table 5** for details concerning severity grading features); (2) addition of ruxolitinib used with abatacept; and (3) decrease in

corticosteroids dose used to avoid associated side-effects (**Supplementary Table 6** for details concerning reporting of side-effects). (16,21,44) Also, starting from March 2020 until August 2021, asymptomatic cases detected by screening (Grade-1, **Figure 2 & Supplementary Table 5**) were monitored after withholding ICI with no systematic immunosuppressant treatment.(16,45,46) Summary of our general therapeutic management starting March 2020 depending on grade of severity of ICI-myotoxicity is in **Supplementary Table 5**.

Immune-monitoring. Details concerning the methodology for profiling of immune-checkpoint proteins on peripheral blood mononuclear cells (CD86 RO on monocytes, PD(L)1 expression on T-cells) and monitoring of abatacept or ICI plasma levels are detailed in **Supplementary Figure 4B** and **Appendix-Methods**.(47)

Statistics. Quantitative and qualitative data are expressed as median [interquartile-range, IQR] and n(%). Their comparison was performed by Mann-Whitney or Kruskal-Wallis tests (when comparing quantitative variables between two groups or more, respectively) and χ^2 -tests for comparison of qualitative variables (including trends by quartiles when appropriate). Correlation between quantitative variables were assessed by Spearman's correlation. $P < 0.05$ was deemed significant.

JAK-STAT pathway translational studies.

RNA-isolation and sequencing using mice cardiac tissues

ICI-myocarditis mice (n=3, *ctla4*^{+/-} *pd-1*^{-/-}) were compared to unaffected control mice (n=4, *ctla4*^{+/+} *pd-1*^{-/-}).⁽¹⁹⁾ Flash-frozen mouse tissue (cardiac ventricle) was homogenized using a TissueLyser II (Qiagen) for 2 min at 30 Hz. RNA was collected from dissociated tissue using Qiagen RNA Kit (Catalog#, 74136). Libraries were prepared using Illumina TruSeq Stranded Total RNA Kit (Illumina, San Diego, CA, USA). Sequencing was performed as paired-end sequencing, with a read length of 150 bps on the Illumina NovaSeq 6000 platform.

Bioinformatical analysis using human cardiac tissues

We used previously published bulk RNA-sequencing data from endomyocardial biopsies of patients with ICI-myocarditis ($n=9$) compared with ICI-treated patients without myocarditis ($n=4$) included at Heidelberg (Germany).(28) These data were obtained from the EBI ArrayExpress Database, with accession number E-MTAB-8867. The raw data files were aligned to the mouse or human genome using the RNA STAR software (Version 2.7.3). Reads (reads per kilobase million) were extracted from the mapped files using the Rsubread Bioconductor package (Version 3.1). Differential gene expression was calculated using the DESeq2 algorithms (European Molecular Biology Laboratory, Heidelberg, Germany) with a false discovery rate <0.05 . For visualizations, we used the ggplot2 and heatmap3 R packages.

Data Availability. Data will be shared with other researchers upon reasonable request to the corresponding author.

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Table 1. Demographics, diagnostic work-up & severity criteria of patients included by time period of inclusion

| Dates of inclusion in the consecutive quartiles (months/years) | Overall | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | p-value |
|--|---------------|---------------|---------------|---------------|-------------|---------|
| | 05/18 - 08/21 | 05/18 - 03/20 | 03/20 - 12/20 | 12/20 - 04/21 | 05/21-08/21 | |
| n/N (%), or median [interquartile range as appropriate] | | | | | | |
| Demographics | | | | | | |
| Age, years | 72 [62-79] | 67 [61-77] | 68 [51-79] | 75 [68-84] | 72 [64-75] | 0.33 |
| Sex, male (%) | 23 (58%) | 6 (60%) | 6 (60%) | 6 (60%) | 5 (50%) | 0.67 |
| Body mass index, kg/m ² | 24 [22-27] | 24 [19-26] | 24 [21-29] | 25 [20-26] | 24 [22-29] | 0.63 |
| Time to onset after first ICI dose | 33 [21-42] | 32 [16-64] | 34 [27-41] | 28 [21-41] | 34 [19-64] | 0.97 |
| - days | 2 [1-3] | 2 [1-4] | 2 [1-2] | 2 [1-2] | 2 [1-3] | 0.92 |
| - number of doses | | | | | | |
| Past medical history (%) | | | | | | |
| - heart failure | 2 (5%) | 0 (0%) | 2 (20%) | 0 (0%) | 0 (0%) | 0.52 |
| - hypertension | 20 (50%) | 4 (40%) | 5 (50%) | 5 (50%) | 6 (60%) | 0.40 |
| - diabetes | 7 (18%) | 2 (20%) | 1 (10%) | 2 (20%) | 2 (20%) | 0.85 |
| - dyslipidemia | 8 (20%) | 3 (30%) | 2 (20%) | 1 (10%) | 2 (20%) | 0.48 |
| - auto-immune disease | 7 (18%) | 2 (20%) | 2 (20%) | 0 (0%) | 3 (30%) | 0.85 |
| Cancer status (%) | | | | | | |
| - metastatic | 33 (83%) | 8 (80%) | 7 (70%) | 9 (90%) | 9 (90%) | 0.91 |
| Type of ICI, anti-PD(L)1 (%) | | | | | | 0.10 |
| - monotherapy | 30 (75%) | 9 (90%) | 8 (80%) | 7 (70%) | 6 (60%) | |
| - combination with anti-CTLA4 | 10 (25%) | 1 (10%) | 2 (20%) | 3 (30%) | 4 (40%) | |
| Diagnostic work-up | | | | | | |
| Cardiac symptoms | | | | | | |
| - chest pain | 11 (28%) | 6 (60%) | 3 (30%) | 2 (20%) | 0 (0%) | 0.003 |
| - syncope/faintness | 7 (18%) | 3 (30%) | 1 (10%) | 2 (20%) | 1 (10%) | 0.35 |
| - dyspnea | 24 (60%) | 9 (90%) | 5 (50%) | 5 (50%) | 5 (50%) | 0.08 |
| - palpitations | 6 (15%) | 2 (20%) | 3 (30%) | 1 (10%) | 0 (0%) | 0.11 |
| Muscular symptoms | | | | | | |
| - muscular pain or weakness | 28 (70%) | 8 (80%) | 8 (80%) | 8 (80%) | 4 (40%) | 0.06 |
| - ptosis | 16 (40%) | 4 (40%) | 5 (50%) | 3 (30%) | 4 (40%) | 0.77 |
| - diplopia | 16 (40%) | 5 (50%) | 5 (50%) | 2 (20%) | 4 (40%) | 0.39 |
| - dysphagia | 13 (33%) | 5 (50%) | 3 (30%) | 2 (20%) | 3 (30%) | 0.29 |
| - dysphonia | 14 (35%) | 4 (40%) | 4 (40%) | 2 (20%) | 4 (40%) | 0.77 |
| Electrocardiogram abnormal | 34 (85%) | 9 (90%) | 10 (100%) | 8 (80%) | 7 (70%) | 0.11 |
| Creatinine kinase abnormal | 36 (90%) | 9 (90%) | 8 (80%) | 9 (90%) | 10 (100%) | 0.35 |
| cMRI tissular analysis* | | | | | | 0.33 |
| - definite | 16/35 (46%) | 5/8 (63%) | 6/9 (67%) | 2/10 (20%) | 3/8 (38%) | |
| - suggestive | 10/35 (29%) | 2/8 (25%) | 2/9 (22%) | 3/10 (30%) | 3/8 (38%) | |
| - normal | 9/35 (26%) | 1/8 (13%) | 1/9 (11%) | 5/10 (50%) | 2/8 (25%) | |
| Endomyocardial pathology* | | | | | | 0.03 |
| - definite | 3/32 (9%) | 2/9 (22%) | 0/8 (0%) | 0/9 (0%) | 1/6 (17%) | |
| - suggestive | 21/32 (66%) | 3/9 (33%) | 4/8 (50%) | 9/9 (100%) | 5/6 (83%) | |
| - normal | 8/32 (25%) | 4/9 (44%) | 4/8 (50%) | 0/9 (0%) | 0/6 (0%) | |
| Peripheral muscle pathology* | | | | | | 0.74 |
| - definite | 32 (80%) | 8 (80%) | 8 (80%) | 8 (80%) | 8 (80%) | |
| - suggestive | 4 (10%) | 1 (10%) | 2 (20%) | 0 (0%) | 1 (10%) | |
| - non-specific lesions | 2 (5%) | 0 (0%) | 0 (0%) | 1 (10%) | 1 (10%) | |
| - normal | 2 (5%) | 1 (10%) | 0 (0%) | 1 (10%) | 0 (0%) | |

| Severity Grade (at presentation)** | | | | | | |
|--|--------------|--------------|-------------|--------------|--------------|------|
| Cardiac dysfunction | | | | | | 0.65 |
| - Grade ≤2 | 39/40 (98%) | 10/10(100%) | 9/10 (90%) | 10/10 (100%) | 10/10 (100%) | |
| - Grade ≥3 (severe) | 1/40 (2%) | 0/10 (0%) | 1/10 (10%) | 0/10 (0%) | 0/10 (0%) | |
| Cardiac Arrhythmia | | | | | | 0.16 |
| - Grade ≤2 | 36/40 (90%) | 8/10 (80%) | 9/10 (90%) | 9/10 (90%) | 10/10 (100%) | |
| - Grade ≥3 (severe) | 4/40 (10%) | 2/10 (20%) | 1/10 (10%) | 1/10 (10%) | 0/10 (0%) | |
| Respiratory muscle dysfunction | | | | | | 1 |
| - Grade ≤2 | 36/40 (90%) | 9/10 (90%) | 9/10 (90%) | 9/10 (90%) | 9/10 (90%) | |
| - Grade ≥3 (severe) | 4/40 (10%) | 1/10 (10%) | 1/10 (10%) | 1/10 (10%) | 1/10 (10%) | |
| Severity maximal grade** | | | | | | |
| Cardiac dysfunction | | | | | | 0.06 |
| - Grade ≤2 | 31/40 (78%) | 5/10 (50%) | 8/10 (80%) | 10/0 (100%) | 8/10 (80%) | |
| - Grade ≥3 (severe) | 9/40 (22%) | 5/10 (50%) | 2/10 (20%) | 0/10 (0%) | 2/10 (20%) | |
| Cardiac Arrhythmias | | | | | | 0.26 |
| - Grade ≤2 | 23/40 (58%) | 4/10 (40%) | 5/10 (50%) | 7/10 (70%) | 7/10 (70%) | |
| - Grade ≥3 (severe) | 18/40 (45%) | 6/10 (60%) | 5/10 (50%) | 3/10 (30%) | 4/10 (40%) | |
| Respiratory muscle dysfunction | | | | | | 0.84 |
| - Grade ≤2 | 23/38 (61%) | 3/8 (38%) | 6/10 (60%) | 9/10 (90%) | 5/10 (50%) | |
| - Grade ≥3 (severe) | 15/38 (39%) | 5/8 (63%) | 4/10 (40%) | 1/10 (10%) | 5/10 (50%) | |
| Myotoxicity (overall) | | | | | | 0.74 |
| - Grade ≤2 | 10/40 (25%) | 2/10 (20%) | 2/10 (20%) | 4/10 (40%) | 2/10 (20%) | |
| - Grade ≥3 (severe) | 30/40 (75%) | 8/10 (80%) | 8/10 (80%) | 6/10 (60%) | 8/10 (80%) | |
| Other severity criteria | | | | | | |
| Respiratory muscle involvement [‡] | | | | | | 0.23 |
| - definite | 20/38 (53%) | 4/8 (50%) | 5/10 (50%) | 8/10 (80%) | 3/10 (30%) | |
| - probable | 7/38 (18%) | 2/8 (25%) | 1/10 (10%) | 0/10 (0%) | 4/10 (40%) | |
| - absent | 11/38 (29%) | 2/8 (25%) | 4/10 (40%) | 2/10 (20%) | 3/10 (30%) | |
| Troponin-T peak value (ratio vs. 99 th upper reference limit) | 111 [31-175] | 245 [50-991] | 96 [34-157] | 64 [18-153] | 115 [43-150] | 0.18 |

Abbreviations: cMRI: cardiac magnetic resonance imaging

* See **Supplementary-Table-1** for the detailed features used for this assessment

** See **Supplementary-Table-4 & 5** for the detailed features used for this assessment

‡ See **Supplementary-Table-3** for the detailed features used for this assessment

Statistics: p-values are those calculated for the comparison between quartiles, using Kruskal-Wallis and χ^2 -tests for trend when applied to quantitative and qualitative variables, respectively.

Figure legends.

Figure 1. Flow-Chart and evolution of the mortality in our prospective cohort of patients admitted for suspicion of ICI-myocarditis in Pitié-Salpêtrière Hospital, Paris, France

Figure 2. Troponin-T circulating levels as a function of ICI-myotoxicity severity. Evolution of circulating Troponin-T levels (cTnT, ng/L) as a surrogate for myotoxicity severity grade in the 40 ICI-myocarditis patients included in our study. All patients had increased Troponin-T levels on admission. Peak values of Troponin-T levels per patient are represented by * and track the severity grading of these patients. Five patients had grade 1 asymptomatic myocarditis, 4 of whom were not treated by any immunosuppressant (but ICI withhold) and evolved favorably.

Figure 3. Effects of plasmapheresis on ICI circulating levels and immune checkpoint expression on peripheral blood mononuclear cells. While ICI concentrations were decreased by plasmapheresis, there was a surge few days after each plasmapheresis with ICI blood levels staying well above the limit of detection for months ($<1\mu\text{g/mL}$ for nivolumab; $<2\mu\text{g/mL}$ for durvalumab); i.e. well above their expected IC50 (half maximal inhibitory concentration) for their PD(L)1 targets ($\sim 0.3\mu\text{g/mL}$ for nivolumab and $\sim 0.02\mu\text{g/mL}$ for durvalumab).(1) This phenomenon may explain why PD1 and PDL1 receptors on T-cells (CD3^+) and B-cells (CD19^+) are blocked by ICI for months after plasmapheresis. *BID: bis in die; MMF: mycophenolate mofetil*

Figure 4. Abatacept binds its target CD86 on circulating monocytes dose-dependently. (A) Evolution of CD86 receptor occupancy (CD86 RO) on circulating monocytes after abatacept injections as a function of the various dose injected in the severe patients treated by abatacept in Q2-4 (n=22). Data after the 7th injection are not shown (n=2 patients). (B) Correlation (spearman) between delta CD86 RO (maximal value within 72h after each abatacept injection compared to the baseline just before each injection) and doses of abatacept injected.

Figure 5. Overall survival and cancer related outcomes in our cohort. Overall survival since ICI start (A) or ICI-myocarditis presentation (B). Cancer progression free survival since ICI start (C) or ICI-myocarditis

presentation (D) in patients surviving ICI-myotoxicity. Best change from baseline in tumor burden in patients surviving myotoxicity as a function of their cancer type (E) and immunosuppressants already received (F). For the latter analysis, 4 patients receiving ICI in adjuvant setting were excluded and one patient died before its evaluation. Among the 28 remaining patients, eight (8/28, 29%) had partial response, fifteen (15/28, 54%) had stable disease and five (5/28, 18%) had progressive disease using RECIST 1.1 criteria.(48)

Figure 6. JAK-STAT pathway, particularly JAK2 (i.e ruxolitinib target) is upregulated in ICI-myocarditis mice and patients. (A) Heatmap of differentially regulated genes in ICI-myocarditis (*Ctla4*^{+/-} *Pd1*^{-/-} mice, n=3) in comparison with unaffected control (*Ctla4*^{+/+} *Pd1*^{-/-} mice, n=4). (B) Gene expression of JAK and STAT family in ICI-myocarditis (*Ctla4*^{+/-} *Pd1*^{-/-} mice, n=3) in comparison with unaffected control (*Ctla4*^{+/+}; *Pd1*^{-/-} mice, n=4). (C) Expression of the genes in each JAK-STAT signaling pathway in ICI-myocarditis (*Ctla4*^{+/-} *Pd1*^{-/-} mice, n=3) in comparison with unaffected control (*Ctla4*^{+/+}; *Pd1*^{-/-} mice, n=4). (D) Gene expression of JAK and STAT family in ICI-myocarditis patients (ICIM, n=9), in comparison with ICI-treated patients without myocarditis (ICI-control, n=4).

Figure 1

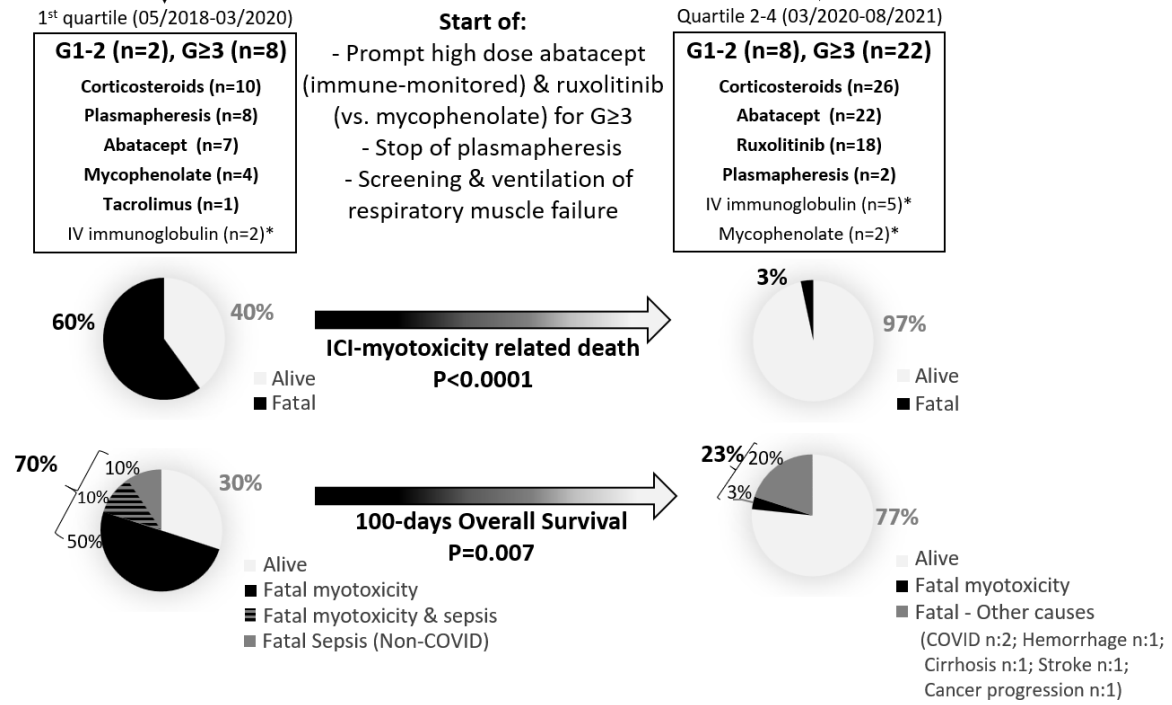
Suspected ICI-Myocarditis prospectively included (n=69)

n=27 excluded for ICI-myocarditis
n= 2 refused diagnosis work-up and care

Definite ICI-myocarditis (n=40)

Severity grading (G) criteria detailed in Supp-Table-5 (severe if G≥3)

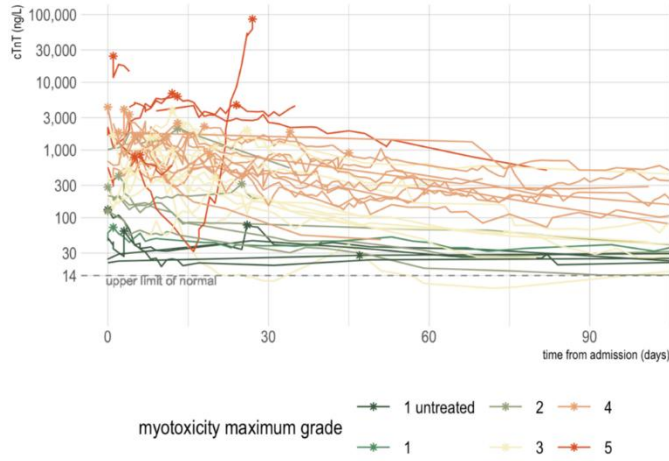
Per chronological admission period



* Started before admission in our unit & transferred for poor evolution on these drugs (5/7 for intravenous (IV) immunoglobulin & 2/2 for mycophenolate). They were stopped upon admission in our unit

Figure 2

Troponin-T evolution over time after admission



*See Supplementary Table 5 for extended details concerning severity grading criteria.

Myotoxicity severity criteria*

| | |
|-----------------------------------|---|
| Grade 1 (mild) | Asymptomatic with abnormal cardiac biomarker testing (troponin) potentially associated with mild ECG or LVEF abnormalities (no severity criteria) |
| Grade 2 (moderate) | Mild symptoms with abnormal cardiac biomarker testing (troponin) with moderate LVEF or ECG abnormalities |
| Grade 3 (Severe) | Abnormal cardiac biomarker testing (troponin) with appearance of severe cardiac pro-arrhythmias, or acute heart failure (but not cardiogenic shock) or concurrent respiratory muscle failure leading to hypoventilation (but no urgent need for mechanical ventilation) or cortico-resistance |
| Grade 4 (life-threatening) | Abnormal cardiac biomarker testing (troponin) with sustained/symptomatic cardiac arrhythmias or cardiogenic shock; or concurrent overt respiratory muscle failure requiring urgent mechanical ventilation. |
| Grade 5 (fatal) | Fatal |

Figure 3

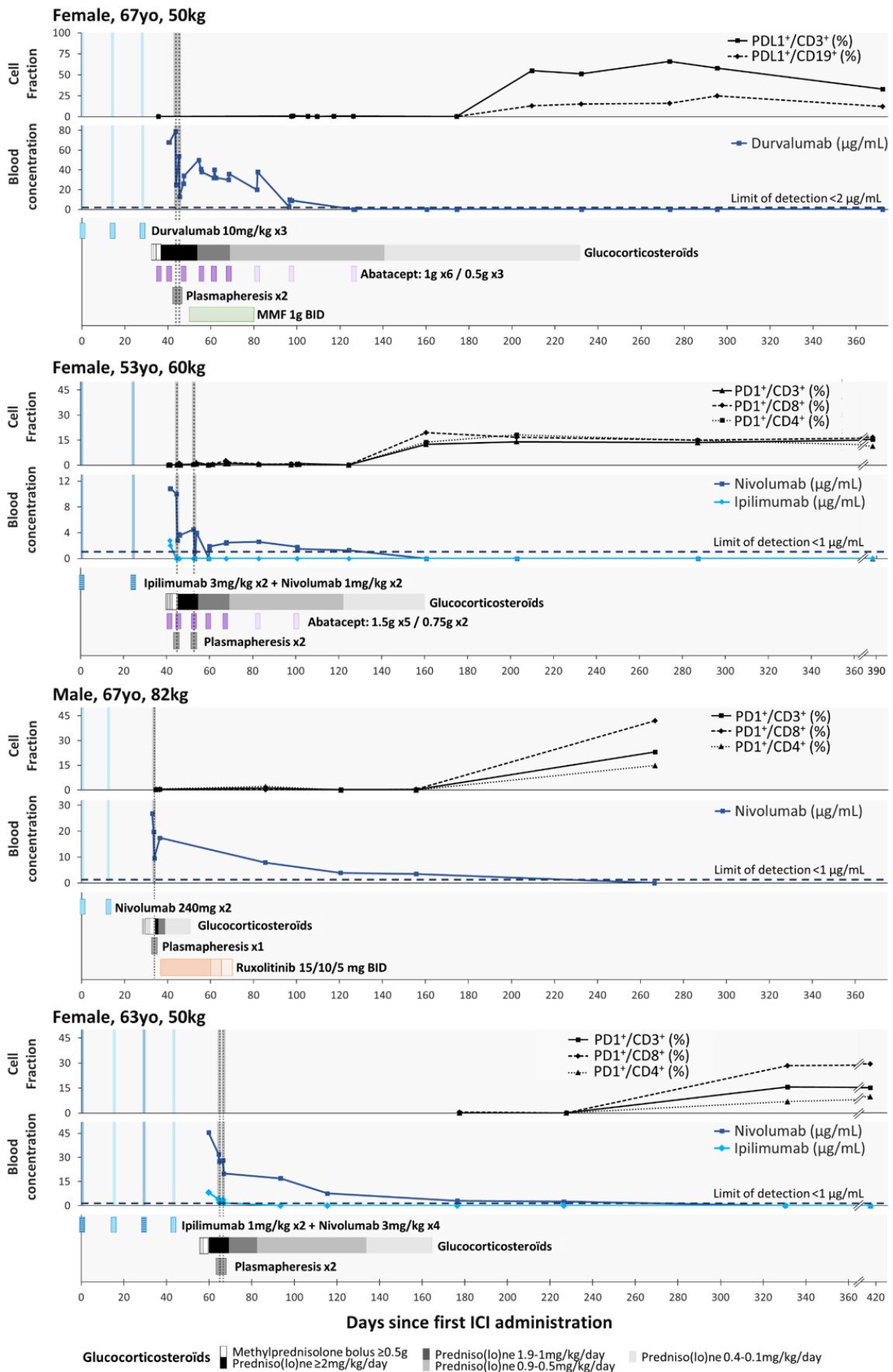
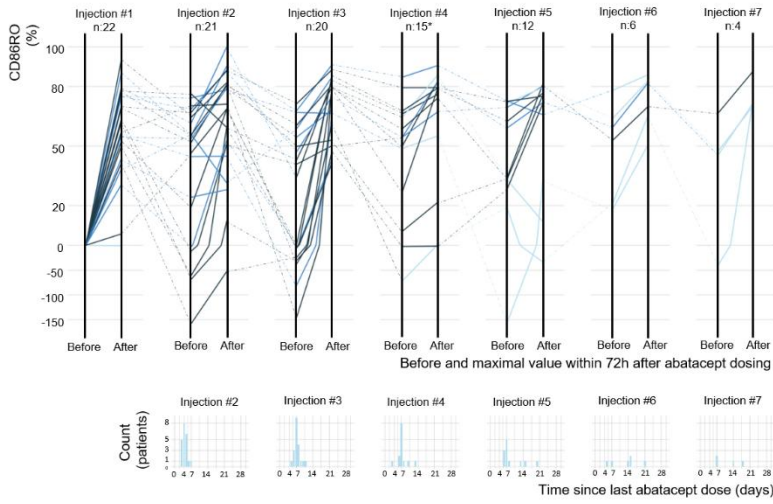


Figure 4

A

| Abatacept range (mg/kg/dose) | 9 - 14.9 | 15 - 19.9 | 20 - 28 |
|-------------------------------------|-------------|--------------|--------------|
| Delta CD86RO before-after abatacept | n:16 | n:32 | n:56 |
| Median [interquartile range] (%) | +20 [1; 42] | +27 [11; 53] | +43 [16; 76] |



* One CD86RO value was not evaluated due to technical issues

B

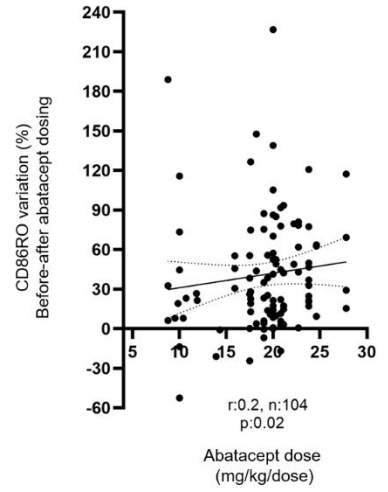
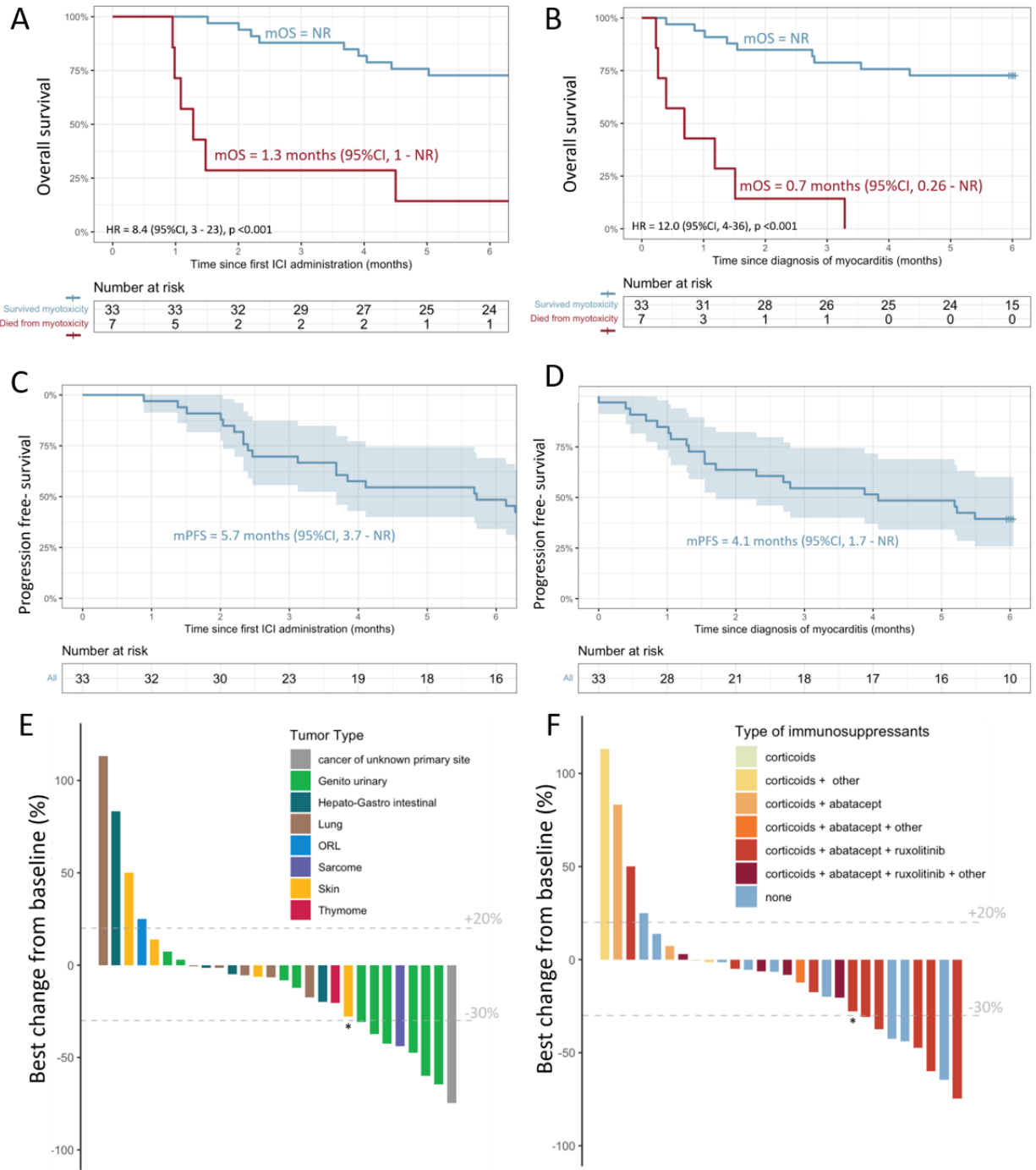


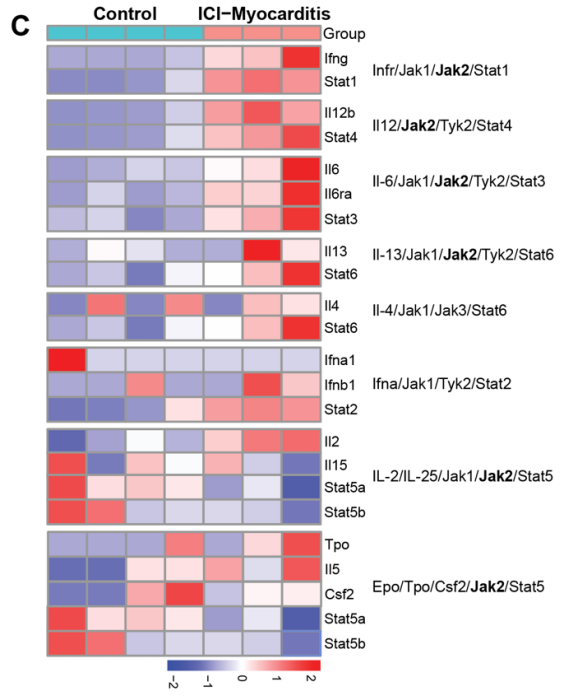
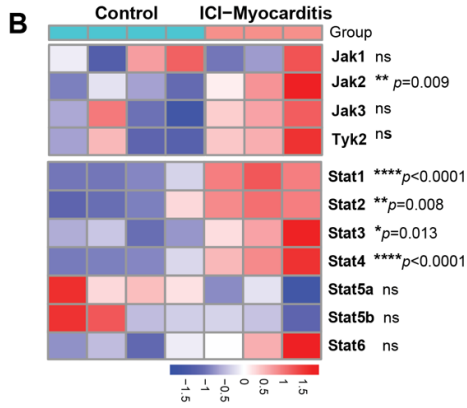
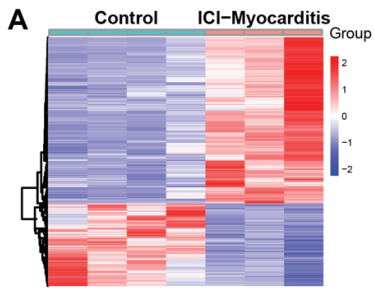
Figure 5



* Progressive disease due to appearance of new tumoral lesions

Figure 6

RNA-seq of mouse ICI-myocarditis model



RNA-seq of ICI-myocarditis patients

