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(71) Applicant: CHARITÉ - UNIVERSITÄTSMEDIZIN
BERLIN [DE/DE]; Charitéplatz 1, 10117 Berlin (DE).

(72) Inventors: HEIDECKER, Bettina; Düsseldorf Str. 14,
10719 Berlin (DE). BEULE, Dieter; Cantianstr. 11, 10437
Berlin (DE). WEINER, January Mikolaj; Hönoweg
6, 15366 Hoppegarten (DE). LANDMESSER, Ulf; Falken-
ried 6, 14195 Berlin (DE).

(74) Agent: HERTIN UND PARTNER RECHTS- UND
PATENTANWÄLTE PARTG MBB; Kurfürstendamm
54/55, 10707 Berlin (DE).

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(54) Title: CYTOKINES AS THERAPEUTIC TARGET IN THE TREATMENT OF CARDIOMYOPATHY

(57) Abstract: The invention relates to an inhibitor of cytokine signaling for use in the treatment of cardiomyopathy, wherein the inhibitor of cytokine signaling is selected from a cytokine receptor inhibitor, a tyrosine kinase inhibitor or an antibody targeting a cytokine and wherein the cardiomyopathy is an inflammatory cardiomyopathy (myocarditis).

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CYTOKINES AS THERAPEUTIC TARGET IN THE TREATMENT OF CARDIOMYOPATHY

DESCRIPTION

5 The invention lies in the field of biochemistry and medicine, especially in the field of pharmaceutical treatments of heart diseases. The invention relates in particular to an inhibitor of cytokine signaling for use in the treatment of cardiomyopathy, wherein the inhibitor of cytokine signaling is selected from a cytokine receptor inhibitor, a tyrosine kinase inhibitor or an antibody targeting a cytokine.

10 BACKGROUND OF THE INVENTION

Despite currently available state-of-the art therapies, a substantial proportion of patients with inflammatory cardiomyopathy progresses to advanced heart failure. There is an urgent need for novel therapies to improve outcomes. We hypothesized that elevated cytokine levels in inflammatory cardiomyopathy may lead to cardiac injury and that specific cytokines are
15 associated with severely decreased left ventricular function consequently, thereby suggesting their potential as therapeutic targets.

Inflammatory cardiomyopathy, including myocarditis, is one of the leading causes of sudden cardiac death (SCD) in young adults, with autopsy studies demonstrating that myocarditis is present in up to 30% of SCD cases¹. Despite being a significant public health concern with
20 potentially devastating outcomes, the pathophysiology of inflammatory cardiomyopathy is not well understood and the condition is often underdiagnosed, leading to suboptimal treatment outcomes for many patients²⁻⁴.

The development of drugs for inflammatory cardiomyopathy has been stagnant for more than two decades⁵, with steroids remaining a mainstay of therapy for severe cases⁵⁻⁸. However, steroids
25 are a non-specific treatment that can have various side effects⁶. Despite available treatments, approximately 15% of patients with inflammatory cardiomyopathy develop chronic heart failure and suffer from cardiovascular complications⁹. A recent study following patients with chronic inflammatory cardiomyopathy over 20 years found that 41% experienced major adverse cardiovascular events (MACE) including 28% cardiovascular death, 16% heart transplantation,
30 and 42% implantable cardioverter defibrillator (ICD) implantation, with standard heart failure therapy alone¹⁰. Immunosuppressive therapy with azathioprine and prednisone improved outcomes, including a significantly lower risk of cardiovascular death and heart transplantation in the group that received immunosuppressive therapy as compared to placebo, as well as an improvement in left ventricular ejection fraction (LVEF) and a lower likelihood of needing an ICD.
35 However, approximately 12% of patients did not respond to immunosuppression and worsened¹⁰.

No gold-standard therapies exist currently for patients with chronic inflammatory cardiomyopathy who do not respond to current undirected immunosuppressive therapy using azathioprine and prednisolone or even show deterioration. At present, there are no suitable therapeutic options for

a patient group of relevant size - 12% of patients with inflammatory cardiomyopathy (which affects about 4 million newly diagnosed patients worldwide/year), i.e. about half a million new patients in the untreated patient group every year.

In recent years, there has been a growing interest in the role of inflammation in heart failure of various etiologies. It is increasingly recognized that a systemic inflammatory state, particularly in inflammatory cardiomyopathy, may contribute to a decline in left ventricular ejection fraction¹¹. The impact of systemic inflammation on cardiac injury, and the role of cytokines in particular, has been increasingly reported in the literature¹²⁻¹⁶.

In light of the prior art there remains a significant need in the art to provide additional means for the treatment of cardiomyopathy, especially for inflammatory cardiomyopathy (myocarditis).

SUMMARY OF THE INVENTION

In light of the prior art the technical problem underlying the present invention is to provide improved means for treating cardiomyopathy, especially for inflammatory cardiomyopathy (myocarditis).

This problem is solved by the features of the independent claims. Preferred embodiments of the present invention are provided by the dependent claims.

The invention therefore relates to an inhibitor of cytokine signaling for use in the treatment of cardiomyopathy, wherein the inhibitor of cytokine signaling is selected from a cytokine receptor inhibitor, a tyrosine kinase inhibitor or an antibody targeting a cytokine.

In embodiments the inhibitor of cytokine signaling is a cytokine receptor inhibitor. In embodiments the inhibitor of cytokine signaling is a protein kinase inhibitor, preferably a tyrosine kinase inhibitor. In embodiments the inhibitor of cytokine signaling is a janus kinase (JAK) inhibitor.

In embodiments the inhibitor of cytokine signaling is an antibody targeting a cytokine. In embodiments the inhibitor of cytokine signaling is an antibody targeting a cytokine receptor.

Using the state of the art in inflammatory heart disease, the inventors hypothesized that myocardial damage in inflammatory heart disease is mediated by cytokines. The inventors examined the association between cytokines and inflammatory heart disease severity in two cohorts totaling more than 400 patients with inflammatory heart disease and found clear and robust associations. Because cytokine modulation has been successfully used in many other diseases, the presently analyzed cohorts also included 159 patients who were treated with different cytokine blocking drugs due to comorbidity. This subgroup showed a significantly better outcome relative to the overall cohort and thus impressively confirms that these cytokine targets are suitable for a successful treatment. Further experiments using cell lines further functionally characterized the relevant cytokines. In addition, the inventors explored specific blockade and modulation strategies comprising inhibition of single cytokines or combinations.

The present invention provides in embodiments a new therapeutic approach for inflammatory cardiomyopathy or general heart failure comprising targeted inhibition of cytokines (individually or in combination, if of advantage). This can be achieved in embodiments by (individual) blocking of

single cytokines or by combination blocking of multiple cytokines. In embodiments the preferred ten cytokines are COLEC12, PLAUR, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, and CCL3. In embodiments the inhibition could also involve the intracellular kinases of these cytokines further downstream. Furthermore, in embodiments inhibition of the other cytokines disclosed in the invention disclosure, individually or in combination, is also a possible treatment approach. One known method/procedure for inhibiting cytokines comprises their inhibition by neutralizing antibodies. In other embodiments the removal of these cytokines from the patient's bloodstream may be accomplished by filtration or immune absorption techniques alternatively or in addition to the inhibition of cytokines. Modulating cytokines, their receptors, or intracellular kinases further downstream of cytokine signaling is pharmacologically well established in general and has been used successfully in autoimmune diseases and other conditions of systemic inflammation such as inflammatory sepsis, COVID-19, and cytokine storm after chimeric antigen receptor (CAR) T-cell treatment.

In embodiments the cardiomyopathy is an inflammatory cardiomyopathy (myocarditis). In embodiments the cardiomyopathy is an inflammatory cardiomyopathy (myocarditis) associated with a viral infection. In embodiments the cardiomyopathy is an inflammatory cardiomyopathy (myocarditis) associated with an organ or system-specific autoimmune or inflammatory disease.

In embodiments the cytokine is selected from interleukins, TNF, COLEC12, PLAUR, CHRDL1, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, CCL3, CLSTN2, LY6D, PRSS8, KRT19, VEGFD and FSTL3.

In embodiments the cytokine is selected from interleukins, TNF, IL-6, COLEC12, PLAUR, CHRDL1, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, CCL3, CLSTN2, LY6D, PRSS8, KRT19, VEGFD and FSTL3.

In embodiments the cytokine is selected from interleukins, such as e.g., interleukins from the IL-1 and/or IL6-family, or tumor necrosis factor (TNF).

In embodiments the cytokine is selected from interleukins of the IL6-family comprising IL-6, IL-11, IL-27 ciliary neurotrophic factor (CNTF), cardiotrophin 1 (CT-1), cardiotrophin-like cytokine (CLC), leukemia inhibitory factor (LIF) and oncostatin M (OSM).

In embodiments the cytokine is selected from interleukins of the IL1-family comprising IL-1 (IL-1 α , IL-1 β , IL-1RA), IL-18, IL-33, IL-36 (IL-36 α , IL-36 β , IL-36 γ , IL-36RA), IL-37 and IL-38.

In embodiments the cytokine is selected from VEGFD, CLSTN2, FSTL3, KRT19 and CRIM1.

In embodiments the cytokine is selected from COLEC12, CHRDL1, LAIR1, CRIM1, CLSTN2, LY6D, PRSS8, KRT19 and FSTL3.

In embodiments the cytokine is selected from interleukins, TNF-alpha, COLEC12, PLAUR, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, CCL3, VEGFD, CLSTN2, FSTL3, KRT19, CXCL17, AGRN, CD4, FABP1, LAIR1, LGALS9, CRIM1, TNFSF13, FSTL3, CCL7, TGFA, CLSTN2, HGF, ANGPTL4, SPON1, IL17D, LRRN1, LY6D, PON3, HLA-E, SPINK4, LGALS4, IL6, NPPC, CHRDL1, TREM2, CXCL10, EPO, CXCL8, MATN2, CXCL14, GAL, TFF2, PREB,

SULT2A1, ENPP7, IL15, DNER, CXCL9, IL4R, REG4, BTN3A2, FST, EGLN1, TNF, IL1RN, NTF3, TNFRSF13B, CXADR, CD276, TNFRSF11A, SMOC2, CCL25, NFASC, CCL28, SIGLEC10, PRSS8, LAMA4, PRELP, ENPP5, CCL21, TNFRSF4, HLA-DRA, CCL23, FASLG, TPP1, CCL11, CCL13, KRT19, LIFR, ITM2A, CKAP4.

- 5 In embodiments the cytokine is selected from interleukins, TNF-alpha, COLEC12, PLAUR, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, CCL3, VEGFD, CLSTN2, FSTL3, KRT19, CXCL17, AGRN, CD4, FABP1, LAIR1, LGALS9, CRIM1, TNFRSF13, FSTL3, CCL7, TGFA, CLSTN2, HGF, ANGPTL4, SPON1, IL17D, LRRN1, LY6D, PON3, HLA-E, SPINK4, LGALS4, NPPC, CHRDL1, TREM2, CXCL10, EPO, CXCL8, MATN2, CXCL14, GAL, TFF2, PREB,
- 10 SULT2A1, ENPP7, IL15, DNER, CXCL9, IL4R, REG4, BTN3A2, FST, EGLN1, TNF, , NTF3, TNFRSF13B, CXADR, CD276, TNFRSF11A, SMOC2, CCL25, NFASC, CCL28, SIGLEC10, PRSS8, LAMA4, PRELP, ENPP5, CCL21, TNFRSF4, HLA-DRA, CCL23, FASLG, TPP1, CCL11, CCL13, KRT19, LIFR, ITM2A, CKAP4.

- In embodiments the cytokine is selected from AGRN, ANGPTL4, BTN3A2, CCL11, CCL13,
- 15 CCL21, CCL23, CCL25, CCL28, CCL3, CCL7, D276, CD4, CHRDL1, CKAP4, CLSTN2, COLEC1, 2CRIM1, CXADR, CXCL10, CXCL14, CXCL17, CXCL8, XCL9, DNER, EGLN1, ENPP5, ENPP7, EPO, FABP1, FASLG, FST, FSTL3, GAL, HGF, HLA-DRA, HLA-E, IL15, IL17D, IL1RN, IL4R, IL6, ITM2A, KRT19, LAIR1, LAMA4, LGALS4, LGALS9, LIFR, LILRB4, LRRN1, LY6D, MATN2, NFASC, NPPC, NTF3, PLAUR, PON3, PREB, PRELP, ,RSS8, REG4,
- 20 SIGLEC10, SMOC2, SPINK4, SPON1, SULT2A1, TFF2, TGFA, TNF, TNFRSF11A, TNFRSF13B, TNFRSF4, TNFSF13, TPP1, TREM2, VEGFD and WNT9A.

- In embodiments the cytokine is selected from AGRN, ANGPTL4, BTN3A2, CCL11, CCL13, CCL21, CCL23, CCL25, CCL28, CCL3, CCL7, D276, CD4, CHRDL1, CKAP4, CLSTN2, COLEC1, 2CRIM1, CXADR, CXCL10, CXCL14, CXCL17, CXCL8, XCL9, DNER, EGLN1,
- 25 ENPP5, ENPP7, EPO, FABP1, FASLG, FST, FSTL3, GAL, HGF, HLA-DRA, HLA-E, IL15, IL17D, IL1RN, IL4R, ITM2A, KRT19, LAIR1, LAMA4, LGALS4, LGALS9, LIFR, LILRB4, LRRN1, LY6D, MATN2, NFASC, NPPC, NTF3, PLAUR, PON3, PREB, PRELP, ,RSS8, REG4, SIGLEC10, SMOC2, SPINK4, SPON1, SULT2A1, TFF2, TGFA, TNF, TNFRSF11A, TNFRSF13B, TNFRSF4, TNFSF13, TPP1, TREM2, VEGFD and WNT9A.

- 30 Herein the inventors hypothesized that cytokines in the context of inflammatory cardiomyopathy would lead to myocardial injury and decreased LVEF consequently. Investigating cytokines that are associated with a severe clinical course of inflammatory cardiomyopathy may help to identify novel drug targets that could be blocked with monoclonal antibodies^{17, 18} or potentially removed through filtration or immunoadsorption techniques^{19, 20}. Increasing evidence in the literature
- 35 suggests that this is a reasonable approach, as blocking cytokines, their receptors, or intracellular kinases further downstream of cytokine signaling were successfully applied in autoimmune diseases and other states of systemic inflammation such as inflammatory cardiomyopathy^{4, 18, 21}, sepsis²², COVID-19²³, and in cytokine storm after chimeric antigen receptor (CAR)-T cell treatment²⁴. The derivation cohort used in the present examples consisted exclusively of patients
- 40 with biopsy-proven inflammatory cardiomyopathy to ascertain the main results of our study in a cohort with a definitive diagnosis of inflammatory cardiomyopathy. The validation cohort also

included patients who were diagnosed based on cardiac magnetic resonance imaging (CMR). Patients were divided into a group with severe inflammatory cardiomyopathy (LVEF $\leq 35\%$) and patients with mild to moderate inflammatory cardiomyopathy (LVEF $> 35\%$). The choice for a cutoff of 35% for LVEF was guided based on the European Society of Cardiology (ESC) guidelines²⁵, in which this cutoff is applied as an indicator for clinical severity and risk of severe arrhythmias. In a third step, we evaluated if our findings were broadly applicable to idiopathic dilated cardiomyopathy (IDCM). After that, a post-hoc analysis was performed on all samples included in the examples. Finally, to evaluate for potential causality of the discovered cytokines, the inventors evaluated if already existing therapeutic inhibitors of cytokines of interest have a potential cardioprotective effect. For that purpose, the inventors successfully confirmed their hypothesis using data extracted from electronic medical records of patients who received cytokine inhibitors for various indications at our hospital.

In embodiments the inhibitor of cytokine signaling is selected from Axitinib, Etanercept, Cabozantinib (L-malat), Certolizumab pegol, Etanercept, Golimumab, Infliximab, Nivolumab, Golimumab, Upadacitinib (-0,5-Wasser), Upadacitinib (-0,5-Wasser), Certolizumab (pegol), Baricitinib, Tofacitinib (citrate), Upadacitinib (-0,5-Wasser), Golimumab, Ustekinumab, Infliximab, Ustekinumab, Ustekinumab, Infliximab, Tocilizumab, Mepolizumab, Dupilumab, Guselkumab, Risankizumab, Ixekizumab, Secukinumab, Ustekinumab, Canakinumab, Cetuximab, Belimumab, Anakinra, Benralizumab, Bimekizumab, Filgotinib (maleat), Ruxolitinib (phosphate), Sarilumab and Tildrakizumab.

In embodiments the inhibitor of cytokine signaling is a cytokine receptor inhibitor or a protein kinase inhibitor, preferably a tyrosine kinase inhibitor. In embodiments the inhibitor of cytokine signaling is selected from Axitinib, Etanercept, Cabozantinib, Cabozantinib, Etanercept, Upadacitinib, Upadacitinib-0,5-Wasser, Upadacitinib, Upadacitinib-0,5-Wasser, Baricitinib, Tofacitinib, Tofacitinib citrate, Upadacitinib, Upadacitinib-0,5-Wasser, Anakinra, Filgotinib, Filgotinib maleat, Ruxolitinib and Ruxolitinib phosphate.

In embodiments the inhibitor of cytokine signaling is an antibody targeting a cytokine or an antibody targeting a cytokine receptor. In embodiments the inhibitor of cytokine signaling is selected from Certolizumab pegol, Golimumab, Infliximab, Nivolumab, Golimumab, Certolizumab (pegol), Golimumab, Ustekinumab, Infliximab, Ustekinumab, Ustekinumab, Infliximab, Tocilizumab, Mepolizumab, Dupilumab, Guselkumab, Risankizumab, Ixekizumab, Secukinumab, Ustekinumab, Canakinumab, Cetuximab, Belimumab, Benralizumab, Bimekizumab, Sarilumab and Tildrakizumab.

In embodiments the inhibitor of cytokine signaling is selected from the group comprising adalimumab, certolizumab pegol, etanercept, golimumab, golimumab, upadacitinib(-0,5-water), upadacitinib(-0,5-water), certolizumab pegol and infliximab and the cytokine is TNF α (tumor necrosis factor alpha).

In embodiments the inhibitor of cytokine signaling is selected from the group comprising golimumab, infliximab, ustekinumab and infliximab and the cytokine is selected from TNF α , IL-12 and IL-23.

In embodiments the inhibitor of cytokine signaling is Golimumab and the cytokines is TNF α .

In embodiments the inhibitor of cytokine signaling is Ustekinumab and the cytokines are IL-12 and IL-23.

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In embodiments the inhibitor of cytokine signaling is Infliximab and the cytokine is TNF α .

In embodiments the inhibitor of cytokine signaling is Ustekinumab and the cytokines are IL-12 and IL-23.

10

In embodiments the inhibitor of cytokine signaling is Upadacitinib and the cytokines is TNF α .

In embodiments the inhibitor of cytokine signaling is Certolizumab and the cytokines is TNF α .

15

In embodiments the inhibitors of cytokine signaling are Golimumab and Ustekinumab and the cytokines are TNF α and IL-12 and IL-23.

In embodiments the inhibitors of cytokine signaling are Infliximab and Ustekinumab and the cytokines are TNF α and IL-12 and IL-23.

In embodiments the inhibitors of cytokine signaling are Upadacitinib and Certolizumab and the cytokines are TNF α and IL-12 and IL-23

20

In embodiments the inhibitor of cytokine signaling is selected from the group comprising Benralizumab, Golimumab, Ustekinumab, Infliximab, Ixekizumab, Mepolizumab, Guselkumab, Risankizumab, Secukinumab, Canakinumab, Anakinra, Bimekizumab, Tildrakizumab and the cytokine is an interleukin selected from IL-12, TNF α , IL-5, IL-23, IL-17A, IL-1 β , IL-5, IL-17 (IL-17A, IL-17F, IL-17AF), and IL-23.

25

In embodiments the inhibitor of cytokine signaling is selected from the group comprising Golimumab and Upadacitinib(-0,5-water), Upadacitinib(-0,5-water), Certolizumab pegol, Baricitinib, Tofacitinib (citrate), Upadacitinib(-0,5-water) and the cytokine is a Janus kinase (JAK) and/or TNF α .

30

In embodiments the inhibitor of cytokine signaling is selected from the following table 1, which enlists the respective inhibitor of cytokine signaling and its target cytokine:

Table 1:

Inhibitor of cytokine signaling	Cytokine
Axitinib	VEGFR
Etanercept and Cabozantinib (L-malat)	tyrosine kinase
Adalimumab	TNF-alpha
Certolizumab pegol	TNF-alpha
Etanercept	TNF-alpha
Golimumab	TNF-alpha
Infliximab	TNF-alpha
Nivolumab	PD-1R
Golimumab and Upadacitinib(-0,5-water)	Janus kinase, TNF α
Upadacitinib(-0,5-water) and Certolizumab pegol	Janus kinase, TNF α
Baricitinib	Janus kinase

Tofacitinib (citrate)	Janus kinase
Upadacitinib(-0,5-water)	Janus kinase
Golimumab and Ustekinumab	IL-12, IL-23, TNFa
Infliximab and Ustekinumab	IL-12, IL-23, TNFa
Ustekinumab and Infliximab	IL-12, IL-23, TNFa
Tocilizumab	IL-6R
Mepolizumab	IL-5
Dupilumab	IL-4R and IL-13R
Guselkumab	IL-23
Risankizumab	IL-23
Ixekizumab	IL-17A
Secukinumab	IL-17A
Ustekinumab	IL-12 and IL-23
Canakinumab	IL-1beta
Cetuximab	EGFR
Belimumab	BLyS
Anakinra	IL-1
Benralizumab	IL-5
Bimekizumab	IL-17A_IL-17F_IL-17AF
Filgotinib (maleat)	Janus kinase
Ruxolitinib (phosphate)	Janus kinase (JAK1/2)
Sarilumab	IL-6
Tildrakizumab	IL-23

In embodiments the patient exhibits one or more of chest pain, shortness of breath, an irregular and/or elevated heartbeat, decreased ability to exercise, heart muscle weakness and/or dysfunction, heart failure or cardiac arrest.

- 5 In embodiments the patient exhibits one or more of chest pain, shortness of breath, an irregular and/or elevated heartbeat, decreased ability to exercise, heart muscle weakness, systolic dysfunction, ventricular arrhythmias, ventricular tachycardia, (high-degree) atrioventricular block, (acute or severe) myocardial dysfunction, (persistent or relapsing) release of biomarkers of myocardial necrosis, (severe) heart failure, cardiogenic shock and/or cardiac arrest.
- 10 In embodiments the cardiomyopathy is associated with a current or previous inflammation of the myocardium.

In embodiments the inflammatory cardiomyopathy (myocarditis) is accompanied by a pericarditis.

- 15 In embodiments the patient is experiencing severe cardiomyopathy with cardiac dysfunction and a left ventricular ejection fraction (LVEF) of ≤ 35 %. In embodiments the LVEF is or has been determined by echocardiography preferably by applying the Simpson's Biplane method.

In embodiments the patient is experiencing a mild to moderate cardiomyopathy with a left ventricular ejection fraction (LVEF) of > 35 %. In embodiments the LVEF is or has been determined by echocardiography preferably by applying the Simpson's Biplane method.

In embodiments ACE inhibitors, beta blockers, diuretics, corticosteroids, angiotensin receptor and/or neprilysin inhibitors, SGLT2 inhibitors and/or intravenous immunoglobulin (IVIG) are administered in combination and/or simultaneously to a subject.

5 In another aspect the present invention relates to a pharmaceutical composition for use in the treatment of cardiomyopathy comprising the inhibitor according to the present invention.

In another aspect the present invention relates to a combination medication for use in the treatment of cardiomyopathy, comprising an inhibitor according to the present invention and a compound selected from the group comprising ACE inhibitors, beta blockers, diuretics, angiotensin receptor and/or neprilysin inhibitors, sodium-glucose cotransporter 2 (SGLT2)
10 inhibitors, corticosteroids and/or intravenous immunoglobulin (IVIG).

In embodiments the inhibition of cytokine signaling according to the invention is considered to be an advantageous approach, e.g., as drugs or antibodies targeting cytokine signaling, e.g., by modulating single or multiple cytokines, their receptors or intracellular kinases further downstream of cytokine signaling, are already available and have been successfully applied and approved for
15 treating autoimmune diseases and other conditions of systemic inflammation such as inflammatory sepsis, COVID-19 and in cytokine storm after chimeric antigen receptor (CAR) T cell treatment.

In general, both "drug repurposing" of established cytokine blockers and specifically directed new development are conceivable in the context of embodiments of the present invention.

20 One target group for a new cytokine-targeting therapeutic approach according to the present invention are patients with inflammatory cardiomyopathy and chronic inflammatory cardiomyopathy who do not respond to current nondirected immunosuppressive therapy using azathioprine and prednisolone or even show deterioration. For such patients, there are no suitable therapeutic options, particularly for a patient group of relevant size-12% of patients with
25 inflammatory cardiomyopathy (which affects about 4 million newly diagnosed patients worldwide/year), or about half a million new patients in the untreated patient group each year. In addition, targeted therapies according to the invention - even in patients who respond to the currently used nondirected therapy - cause fewer side effects and/or better efficacy, which will provide a significant benefit for said patients.

30 It is further noteworthy that the presently provided data implies that cytokine-targeting therapy is also successful in patients with general heart failure. Underlying this conclusion is the observation that the present data (see examples) is also applicable to this group of patients. Furthermore, patients with generalized heart failure comprise those who actually have inflammatory cardiomyopathy, in which the diagnosis was previously overlooked. Therefore, the true benefit
35 and scope of cytokine-directed therapy are expected to span beyond myocarditis. Increasing physician awareness of inflammatory cardiomyopathy, combined with the continued improvement and precision of newly developed diagnostic methods, is expected to lead to a substantial increase in the incidence and prevalence of patients with inflammatory cardiomyopathy. This indicates an increasingly widening scope of application. The possible use of cytokines to improve
40 diagnosis or differential diagnosis has been investigated by the inventors as well.

In some embodiments in addition or alternative to the use of an inhibitor of cytokine signaling in the treatment of cardiomyopathy cytokines may be removed from the blood stream of a patient through immunoabsorption or immunofiltration, e.g., as plasmapheresis. Therefore, the invention relates in another aspect to immunoabsorption for use in the treatment of cardiomyopathy, wherein cytokines are at least partially removed by the immunoabsorption from the blood stream of a patient. Moreover, the invention further relates in another aspect to immunofiltration for use in the treatment of cardiomyopathy, wherein cytokines are at least partially removed by the immunofiltration from the blood stream of a patient.

DETAILED DESCRIPTION OF THE INVENTION

All cited documents of the patent and non-patent literature are hereby incorporated by reference in their entirety.

Herein the terms “subject” and “patient” may be used interchangeably. A subject or patient can be selected from the group comprising vertebrae, animals, livestock, mammals, humans, preferably mammal or human.

Myocarditis is an inflammatory disease of the heart that can be triggered by infections, activation of the immune system or by drugs/as side effect of drugs. Myocarditis describes the inflammation of, and the injury to heart tissue, partially due to infiltration by lymphocytes and monocytes. Myocarditis can be differentiated according to etiology, phase and severity of the disease, predominant symptoms, and pathologic findings. Clinically, acute myocarditis (AM) implies a short period between the onset of symptoms and diagnosis (commonly less than one month). On the contrary, chronic inflammatory cardiomyopathy indicates myocarditis with established dilated cardiomyopathy or a hypokinetic, nondilated phenotype that progresses to fibrosis without demonstrable inflammation at advanced stages. Classification of myocarditis may be performed as reviewed by Ammirati et al., 2020 (Circ Heart Fail. 2020;13:e007405. DOI: 10.1161/CIRCHEARTFAILURE.120.007405). Endomyocardial biopsy is considered the standard for the diagnosis of myocarditis.

Cardiomyopathy is a group of diseases that affect the heart muscle. In the early stages, there may be few or no symptoms. As the disease worsens, shortness of breath, fatigue and swelling of the legs may occur, reflecting the onset of heart failure. An irregular heartbeat and fainting may occur. Affected individuals are at increased risk for sudden cardiac death. Types of cardiomyopathy include hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic right ventricular dysplasia, and Takotsubo cardiomyopathy.

In hypertrophic cardiomyopathy, the heart muscle enlarges and thickens. In dilated cardiomyopathy, the heart chambers enlarge and weaken. In restrictive cardiomyopathy, the ventricle stiffens. In addition, immunohistochemistry-specific antibodies for leukocytes (CD45), macrophages (CD68), T cells (CD3) and their major subtypes, helper (CD4) and cytotoxic (CD8) cells, and B cells (CD19/CD20) can increase the sensitivity of endomyocardial biopsy.

Herein patients may be classified as suffering from severe inflammatory cardiomyopathy when experiencing a left ventricular ejection fraction (LVEF) of $\leq 35\%$ and as suffering from mild to moderate inflammatory cardiomyopathy when experiencing a left ventricular ejection fraction (LVEF) of $> 35\%$. The choice for a cutoff of 35% for LVEF was guided based on the European Society of Cardiology (ESC) guidelines ²⁵, in which this cutoff is applied as an indicator for clinical severity and risk of severe arrhythmias.

Left ventricular ejection fraction (LVEF) is the key indicator of left ventricular systolic function. The left ventricular ejection fraction is the fraction of ventricular volume ejected during a systole (resembling the 'stroke volume') relative to the volume of blood in the ventricle at the end of the diastole (resembling the 'end-diastolic volume'). The stroke volume is commonly determined as the difference between the end-diastolic volume (EDV) and end-systolic volume (ESV).

The Simpson's Biplane method or Simpson's biplane rule (SBR) is the standard approach for the quantification of the left ventricle (LV) volume from echocardiography, which depends on a summation-of-disks approach, which makes assumptions about LV orientation and cross-sectional shape.

Cytokines are peptides or glycoproteins secreted locally in the heart or systemically in a wide range of cardiac diseases, from heart failure and ischemia-reperfusion to myocarditis, graft rejection, and dysfunction caused by sepsis. Leukocytes migrating into the heart are a major source of cytokines in most of these situations, but they are also secreted by virtually all endogenous cell populations in the heart. By binding their cognate receptors, cytokines have important effects on the extracellular matrix and cardiomyocyte. The proinflammatory cytokines TNF, IL-1, and IL-6 all play important roles in the heart. Cytokines can directly modulate contractility through NOS3 activity, effects on excitation-contraction coupling, phospholipase A2 activity (arachidonic acid production), sphingomyelinase signaling, and adrenoreceptor sensitivity. Inflammatory signaling in cardiomyocytes may be triggered by canonical pathways in which extracellular cytokines stimulate cell surface receptors. Cytokines may also be released from neighboring cardiomyocytes after inflammatory activation, thereby transmitting the inflammatory signal to multiple cells. Cytokines can also be released by immune cells recruited to the myocardium. Myocardial stresses induce production of interleukin (IL)-6, leading to activation of janus kinase (e.g., JAK/STAT) signaling. Mechanical stresses in the vessel wall (as well as in the myocardium) are considered to contribute through production of ROS, MCP-1, and TGF1, by inducing macrophage infiltration.

Chemokines are a subgroup of peptide cytokines which may function as chemo-attractants for leukocytes. Chemokines were shown to play a role also in the heart.

The toll-like receptors (TLRs) TLR2, TLR3, and TLR4 are expressed in cardiomyocytes and elevated TLR 4-expression has been observed in patients with heart failure. Intriguingly, elevated TLR signaling has been shown to contribute to worsening of cardiac dysfunction and to induce recruitment of inflammatory cells.

Tumor necrosis factor (TNF) signaling and NF-B activation in the heart may also play a role in myocarditis. Stimuli from infectious agents and ischemic or other tissue injury have been shown

to induce release of TNF from various immune cells in the myocardium, including macrophages, mast cells, but also fibroblasts and endothelial cells. TNF receptor activation by TNF-alpha is presently considered to have both beneficial and adverse effects. TNF gene transcription is mediated, in part, by activation of NF-kappa B. In previous studies cardiomyocyte expression of TNF was implicated to lead to cardiomyopathy. On the contrary, deletion of the NFB subunit p50 was shown to abrogate left ventricular dysfunction and mortality after myocardial infarction. Chronic inactivation of NFB after myocardial infarction has been shown to abrogate inflammation, heart failure, and apoptosis. Glucocorticoids as dexamethasone and prednisone commonly suppress inflammation by blocking the translation of mediators of inflammation. TNF may in embodiment be neutralized by soluble TNF antagonists, such as etanercept or by neutralizing antibodies, such as infliximab, thereby inhibiting the binding of TNF to receptors.

Herein, an "antibody" generally refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. Where the term "antibody" is used, the term "antibody fragment" may also be considered to be referred to. Immunoglobulin genes include the alpha, lambda, kappa, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as alpha, gamma, delta, mu, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD, and IgE, respectively. The basic immunoglobulin (antibody) structural unit comprises a tetramer or dimer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (L) (about 25 kD) and one "heavy" (H) chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids, primarily responsible for antigen recognition. The terms "variable light chain" and "variable heavy chain" refer to these variable regions of the light and heavy chains respectively. Optionally, the antibody or the immunological portion of the antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins.

Antibodies or antibody fragments according to the invention therefore include, but are not limited to human, humanized, polyclonal, monoclonal, bispecific or chimeric antibodies, single variable fragments (ssFv), single chain fragments (scFv), single domain antibodies (such as VHH fragments from nanobodies), Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, epitope-binding fragments and anti-idiotypic antibodies or combinations thereof, preferably comprising the corresponding CDRs, or VL and VH regions. Further mini-antibodies and multivalent antibodies such as diabodies, triabodies, tetravalent antibodies and peptabodies may be used in the context of the invention. The immunoglobulin molecules of the invention can be of any class (i.e. IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecules.

Pharmaceutical compositions for administration to a subject can include at least one further pharmaceutically acceptable additive such as carriers, diluents, buffers, preservatives, thickeners, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more additional active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like. The pharmaceutically acceptable carriers useful for these formulations are conventional. Remington's Pharmaceutical Sciences, by

E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition (1995), describes compositions and formulations suitable for pharmaceutical delivery of the compounds herein disclosed.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually contain injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, aqueous dextrose, balanced salt solutions, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of lactose, starch, mannitol, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate.

According to the present invention, the term "combined administration", or "co-administration" comprises in some embodiments the administration of separate formulations of the compounds described herein, whereby treatment may occur within minutes of each other, in the same hour, on the same day, in the same week or in the same month as one another. Alternating administration of two agents is considered as one embodiment of combined administration. Staggered administration is encompassed by the term combined administration, whereby one agent may be administered, followed by the later administration of a second agent, optionally followed by administration of the first agent, again, and so forth. Simultaneous administration of multiple agents is considered as one embodiment of combined administration. Simultaneous administration encompasses in some embodiments, for example the taking of multiple compositions comprising the multiple agents at the same time, e.g., orally by ingesting separate tablets simultaneously. A combination medicament, such as a single formulation comprising multiple agents disclosed herein, and optionally additional immuno-suppressing (e.g., steroids) or immuno-modulating medicaments, may also be used in order to co-administer the various components in a single administration or dosage. A combined therapy or combined administration of one agent may precede or follow treatment with the other agent to be combined, by intervals ranging from minutes to weeks. In embodiments where the second agent and the first agent are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the first and second agents would still be able to exert an advantageously combined synergistic effect on a treatment site. In such instances, it is contemplated that one would contact the subject with both modalities within about 12-96 hours of each other and, in embodiments preferably, within about 6-48 hours of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12) lapse between the respective administrations.

It is understood that substituents and substitution patterns of the compounds described herein can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art and further by the methods set forth in this disclosure. One aspect of the present disclosure includes "pharmaceutical compositions" prepared for administration to a subject and which include a "therapeutically

effective amount" of one or more of the compounds disclosed herein. The therapeutically effective amount of a disclosed compound (e.g., antibody, agent, substance) will depend on the route of administration, the species of subject and the physical characteristics of the subject being treated. Specific factors that can be taken into account include disease severity and stage, weight, diet and concurrent medications. The relationship of these factors to determining a therapeutically effective amount of the disclosed compounds is understood by those of skill in the art.

The pharmaceutical compositions can be administered by intramuscular, intravenous, intra-arterial, intra-articular, intraperitoneal, intrathecal, intracerebroventricular, or parenteral routes. Optionally, compositions can be administered to subjects by a variety of administration modes, including by oral or intravenous delivery. In accordance with the treatment methods according to the invention, the compound can be delivered to a subject in a manner consistent with conventional methodologies associated with management of the disorder for which treatment or prevention is sought. In accordance with the disclosure herein, a prophylactically or therapeutically effective amount of the compound and/or other biologically active agent is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent, inhibit, and/or ameliorate a selected disease or condition or one or more symptom(s) thereof.

"Administration of and "administering a" compound should be understood to mean providing a compound, a prodrug of a compound, a combination of at least two compounds or a pharmaceutical composition as described herein. The compound(s) or composition(s) can be administered by another person to the subject (e.g., intravenously) or it can be self-administered by the subject (e.g., tablets). Dosage can be varied by the attending clinician to maintain a desired concentration at a target site. Higher or lower concentrations can be selected based on the mode of delivery, for example, intravenous, or oral delivery. Dosage can also be adjusted based on the release rate of the administered formulation, for example, of an sustained release oral versus injected formulations, and so forth.

The present invention also relates to a method of treatment of subjects suffering from the medical conditions disclosed herein. The method of treatment comprises preferably the administration of a therapeutically effective amount of a compound disclosed herein to a subject in need thereof.

A "therapeutically effective amount" refers to a quantity of a specified compound sufficient to achieve a desired effect in a subject being treated with said compound. For example, this may be the amount of a compound disclosed herein. The therapeutically effective amount or diagnostically effective amount of a compound will be dependent on the subject being treated, the severity of the affliction, and the manner of administration of the therapeutic composition. Dosage regimens can be adjusted to provide an optimum prophylactic or therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental side effects of the compound and/or other biologically active agent is outweighed in clinical terms by therapeutically beneficial effects.

The instant disclosure also includes kits, packages and multi-container units containing the herein described pharmaceutical compositions, active ingredients, and/or means for administering the

same for use in the prevention and treatment of diseases and other conditions in mammalian subjects.

FIGURES

The invention is further described by the following figures. These are not intended to limit the scope of the invention, but represent preferred embodiments of aspects of the invention provided for greater illustration of the invention described herein.

Figure 1: Relative cytokine concentrations in patients with inflammatory cardiomyopathy in both derivation and validation cohort (n = 488). The patients were grouped according to cohort and left ventricular ejection fraction (LVEF). The selected cytokines are the top 15 cytokines identified in the derivation cohort. Blue dots correspond to male patients, red dots correspond to female patients. We tested whether there was a significant interaction between LVEF and sex. We did not find any proteins which showed both, a significant main effect of LVEF and a significant interaction between LVEF and sex, neither in the derivation cohort nor in the validation cohort. Association between LVEF and cytokine levels was significant for all cytokines shown at FDR < 0.001.

Figure 2: Comparison of NT-proBNP values in ng/L between patients who were prescribed IL-6 receptor inhibitors or janus kinase inhibitors with matched controls without use of this particular drugs. IL-6 receptor inhibitor or janus kinase inhibitor intake was associated with lower NT-proBNP values.

Figure 3: Comparison of high sensitive Troponin-T (HS-TNT) values in ng/L between patients who were prescribed janus kinase inhibitors with matched controls without use of this particular drug. Janus kinase inhibitor intake was associated with lower HS-TNT values.

Figure 4: Visual abstract of one embodiment of the invention. The scheme depicts the procedure of diagnosis of cardiomyopathy, the selection of a suitable inhibitor of cytokine signaling and the use of an inhibitor of cytokine signaling in the treatment of cardiomyopathy according to the invention.

Figure 5: NPX values of all protein assays in the first batch (X axis, derivation and generalizability cohorts) and second batch (Y axis, validation cohort) data sets for the 16 bridging samples. Each dot represents one protein assay in one sample. Left panel, raw data. Right panel, data normalized using the olink_normalization function from the OlinkAnalyze R package. In total, there were 16 samples, 368 assays and 5888 total measurements. Blue line shows a fitted LOESS model.

Figure 6: Comparison of 10 randomly chosen protein assays across the different data sets and different normalizations for the 16 bridging samples. Top: raw data. Bottom: normalized data. In total, 16 unique samples and 640 measurements are shown.

Figure 7: Principal component analysis of the 529 measurements before normalization (left panel) and the same measurements after normalization (right panel). Each dot represents one sample. The first two principal components are shown in the top row, and the third and fourth

principal components are shown in the bottom row. The color of the dots indicates the batch of the sample.

Figure 8: Plot of the principal component analysis of the 529 measurements before normalization (red) and the same measurements after normalization (blue). The x-axis shows the principal components and the y-axis.

Figure 9: Principal component analysis of cardiomyocytes.

Figure 10: Principal component analysis of endothelial cells.

Figure 11: Gene expression of selected genes in cardiomyocytes.

Figure 12: Gene expression of selected genes in endothelial cells. Top row: selected genes showing a large difference between 48 h and 1 h in all treatments. Middle row: selected genes showing a difference between COLEC and control at 48 h. Bottom row: selected genes corresponding to significant enrichment in the interferon transcriptional module for comparison CRIM vs control at 48 h.

Figure 13: Evidence plots for gene set enrichments. Left, leukocyte differentiation (LI.M160) transcriptional module for comparison 48 h vs 1 h in IL6 treated HAEC. This enrichment is common for all comparisons 48 h vs 1 h, also in the controls. Right, interferon transcriptional module (DC.M1.2) for comparison CRIM 48 h vs control in HAEC. Genes with higher expression at 48h are shown in a lighter color, genes with lower expression in a darker color.

EXAMPLES

The invention is further described by the following examples. These are not intended to limit the scope of the invention, but represent preferred embodiments of aspects of the invention provided for greater illustration of the invention described herein.

5 The inventors hypothesized that some cytokines in the context of inflammatory cardiomyopathy may lead to myocardial injury and decreased LVEF consequently. Investigating cytokines that are associated with a severe clinical course of inflammatory cardiomyopathy may help to identify novel drug targets that could be blocked with monoclonal antibodies^{17, 18} or potentially removed through filtration or immunoadsorption techniques^{19, 20}. Increasing evidence in the literature
10 suggests that this is a reasonable approach, as blocking cytokines, their receptors, or intracellular kinases further downstream of cytokine signaling were successfully applied in autoimmune diseases and other states of systemic inflammation such as inflammatory cardiomyopathy^{4, 18, 21}, sepsis²², COVID-19²³, and in cytokine storm after chimeric antigen receptor (CAR)-T cell treatment²⁴. The presently used derivation cohort consisted exclusively of patients with biopsy-
15 proven inflammatory cardiomyopathy to ascertain the main results of our study in a cohort with a definitive diagnosis of inflammatory cardiomyopathy. The validation cohort also included patients who were diagnosed based on cardiac magnetic resonance imaging (CMR). Patients were divided into a group with severe inflammatory cardiomyopathy (LVEF $\leq$ 35%) and patients with mild to moderate inflammatory cardiomyopathy (LVEF $>$ 35%). The choice for a cutoff of 35% for
20 LVEF was guided based on the European Society of Cardiology (ESC) guidelines²⁵, in which this cutoff is applied as an indicator for clinical severity and risk of severe arrhythmias. In a third step, the inventors evaluated if our findings were broadly applicable to idiopathic dilated cardiomyopathy (IDCM). After that, a post-hoc analysis was performed on all samples included in this study. Finally, to evaluate for potential causality of the discovered cytokines, the inventors
25 evaluated if already existing therapeutic inhibitors of our cytokines of interest have a potential cardioprotective effect. For that purpose, we tested our hypothesis using real world data extracted from electronic medical records of patients who received cytokine inhibitors for various indications at our hospital.

METHODS

30 Study participants and recruitment

For our study, we investigated samples from 104 patients who were collected in the biobank of the Deutsches Herzzentrum der Charité (DHZC), Campus Benjamin Franklin, Berlin between 2014 and 2021. Endomyocardial biopsies (EMB) were obtained during routine diagnostic workup as indicated by the ESC Consensus Statement²⁶ and American Heart Association Guidelines²⁷.
35 As recommended, EMBs were obtained in clinical scenarios, in which the results of histology were expected to change management. Coronary artery disease was excluded in all patients via coronary angiography. Blood samples were systematically collected in EDTA plasma tubes at the time of EMB and stored at -80 °C.

Based on the results of histology and comprehensive immunohistochemistry of EMBs, we divided
40 the patients into 2 groups: 1) Patients with inflammatory cardiomyopathy (n=63) and 2) patients

with IDCM (n=41) based on the definition of the ESC²⁶. Patients taking immunosuppressive or anti-inflammatory therapy at the time of EMB were excluded to avoid potential confounders. Based on echocardiographic data, patients were further divided into a group with severe inflammatory cardiomyopathy (LVEF $\leq$ 35%) and patients with mild-moderate inflammatory cardiomyopathy (LVEF > 35%). The same was applied to patients with IDCM.

Replication of results in an independent cohort

To evaluate if our results can be replicated in an independent cohort, we investigated blood samples from patients with inflammatory cardiomyopathy collected in a German national registry for cardiomyopathies. This registry contained samples collected within the Translational registry for cardiomyopathies (TORCH) network of the German Center for Cardiovascular Research e.V. (eingetragener Verein) /Deutsches Zentrum für Herz-Kreislaufforschung (DZHK).

Patients were recruited at cardiovascular centers across Germany within the DZHK network since 2014. Samples were collected in EDTA tubes and stored within one hour after collection at -80 °C. All samples retrieved from the TORCH registry were obtained from patients with a diagnosis of inflammatory cardiomyopathy. Similar to the derivation cohort, patients who had received immunosuppression prior to sample collection were excluded from the analyses. Furthermore, patients of whom relevant clinical data such as LVEF were missing were excluded. Samples that qualified for our study were derived from 11 German centers.

Cytokine analysis

For cytokine analysis, EDTA blood from patients was analyzed with the Proximity Extension Assay (PEA) by Olink. In summary, the method is based on the binding of proteins, i.e. cytokines, by antibodies that carry unique oligonucleotide sequences. When two complementary oligonucleotide sequences come close, they hybridize and can be extended by a DNA polymerase to form a complementary strand. The DNA amplicon can then be quantified using real-time PCR or next generation sequencing (NGS). A major advantage of PEA technology is that it has no impact on data quality while enabling biomarker analysis with high multiplex and fast throughput²⁸.

Olink's Explore 384 – "Inflammation" subpanel, a part of the Explore 3072 panel, was used for our analyses. The preparation of the sample libraries, randomization, quality control and data processing took place in the process standardized by Olink (<https://www.olink.com>). Data are presented as normalized protein expression (NPX), providing relative quantification between samples. Log-transformed values were used to obtain a normal distribution.

Statistical analysis

Statistical analysis was conducted using R. The two batches of Olink data were integrated using the `olink_normalization` function from the `OlinkAnalyze` package (v. 3.1) and 16 bridging samples were present in both data sets. Cytokine level influence on LVEF was analysed with a linear regression model (R function `lm`), using the normalized protein levels (NPX) as the predictor variable and LVEF as the response variable, with cohort (derivation / validation) and patient sex as covariates. P-values were corrected for multiple testing using a false discovery rate controlling

procedure (Benjamini-Hochberg method)²⁹. For random forest analysis, we used the R package randomForest, version 4.7. R markdown.

Hypothesis testing: Evaluation for potential causality of cytokines in electronic medical record (EMR) real-world data

5 To further test our hypothesis, we sought to investigate a potential causative effect of cytokines on cardiac injury by analyzing laboratory markers of cardiac injury in patients treated with cytokine inhibitors. These data were collected from three rheumatology departments at the Charité Universitätsmedizin, Berlin. Data were extracted from the electronic medical records (EMR) for the period from January 2018 to May 2023. The analysis concentrated on patients who
10 were treated with inhibitors specifically targeting the cytokines identified in our study, or the pathways these cytokines influence downstream. This encompassed patients who had been administered IL-6 or Janus Kinase inhibitors, and for whom high-sensitivity troponin T (hs-TnT) and NT-proBNP measurements were obtainable.

Only laboratory values obtained after the initiation of cytokine inhibitors were included in the
15 analysis. From these laboratory values, the median was calculated for each patient. For patients who did not receive any cytokine inhibitors and therefore belonged to the control group of this analysis, the median was calculated from all available measurements. For each cytokine being investigated, a control group was established at a 1:4 ratio using the nearest matching procedure, identifying comparable individuals who had not undergone treatment with cytokine inhibitors.

20 Matching criteria included cardiac, rheumatologic, autoimmune comorbidities, and renal insufficiency. Patients with a glomerular filtration rate (GFR) below 30 ml/min/m² were excluded from the analysis, as hs-TnT and NT-proBNP are affected by renal function. Outliers were identified using the boxplot method³⁰ and were excluded from both the respective control group and cytokine inhibitor group. Subsequently, the medians of the respective laboratory values were
25 compared using a Kruskal-Wallis test. Additionally, a linear model was computed.

Ethics committee approval

This study was reviewed and approved by the ethics committee of DHZC Universitätsmedizin Berlin (EA4/056/20, EA1/187/22) and the Ethikkommission Medizinische Fakultät Heidelberg (S-344/2014). All patients provided their written informed consent.

30 Results

Study population

We enrolled a total of 529 patients (table 2). The derivation cohort was composed of 63 patients who were recruited from the DHZC, while the validation cohort consisted of 425 patients who were enrolled within the TORCH registry of the DZHK. The derivation (n=63) and validation (n =
35 425) cohort contained only patients with inflammatory cardiomyopathies. The third group, referred to as the "Generalizability" cohort, encompassed patients diagnosed with IDCM (n=41). There were no significant differences among the groups concerning key baseline characteristics such as sex, age, BMI, LVEF, and renal function. Women comprised between 28.2% and 31.7% of the

participants across all cohorts. The median age of the participants spanned from 49 to 54 years. Moreover, the median Body Mass Index (BMI) was in the range of 26-27 kg/m², categorizing the participants, on average, as overweight.

The three groups were categorized based on the extent of reduction in LVEF, with patients classified as having either severe LVEF reduction ($\leq 35\%$) or mild to moderate LVEF reduction ($>35\%$). Between 36.5 and 41.5% of patients exhibited severely diminished left ventricular function with an LVEF $\leq 35\%$. The median LVEF was in the range of 42-45%. Furthermore, the median creatinine levels ranged from 0.93 to 0.94 mg/dl, indicative of normal renal function.

Table 2: Demographic data: Derivation cohort; independent validation cohort; generalizability cohort: Cohort of patients with idiopathic dilated cardiomyopathy, in whom general applicability of the findings was assessed.

	Derivation cohort (n=63)	Validation cohort (n=425)	Generalizability cohort (n=41)
Cardiomyopathy	Inflammatory Cardiomyopathy	Inflammatory Cardiomyopathy	Idiopathic Dilated Cardiomyopathy
Registry	DHZC	DZHK	DHZC
Sex female (n, %)	19 (30.2%)	120 (28.2%)	13 (31.7%)
Age in years (median, IQR)	54 (36 – 62)	49 (37 – 58)	54 (48 – 65)
BMI in kg/m ² (median, IQR)	27 (25 – 29)	26 (24 – 30)	27 (24 – 32)
LVEF $\leq 35\%$ (n, %)	23 (36.5%)	169 (39.8%)	17 (41.5%)
LVEF (median, IQR)	45 (31 – 58)	42 (30 – 55)	42 (30 – 58)
Creatinine in mg/dl (median, IQR)	0.93 (0.83 – 1.1)	0.93 (0.82 – 1.1)	0.94 (0.85 – 1.1)

Cytokine Analysis in the Derivation Cohort

First, we tested the hypothesis that cytokine levels are associated with severe inflammatory cardiomyopathy, defined by an LVEF $\leq 35\%$. A linear regression model was employed to analyze the relationship between cytokine levels and LVEF in patients with inflammatory cardiomyopathy.

Following correction for multiple testing, a total of 5 cytokines were found to be significantly associated with LVEF in the derivation cohort of patients with inflammatory cardiomyopathy (DHZC, n= 63) (FDR < 0.05; Table 3). The relationship between relative cytokine concentration and LVEF was found to be inverse. This indicates that higher relative cytokine concentrations are associated with lower LVEF in patients with inflammatory cardiomyopathy.

Table 3: Derivation cohort (n=63): Top 10 cytokines associated with left ventricular ejection fraction (LVEF). Higher relative cytokine concentrations are associated with lower LVEF values. This inverse relationship with LVEF was significant for 5 cytokines (FDR < 0.05). Estimate, the coefficient estimate from the linear regression model; r^2 , coefficient of determination (r^2) value for the model; P-value, raw p value for the model; FDR, false discovery rate calculated using Benjamini-Hochberg correction.

Cytokine	Estimate	r^2	P-value	FDR
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VEGFD	-15,418	0,421638	5,47E-08	2,01E-05
CLSTN2	-12,3104	0,315359	1,01E-05	0,003696
FSTL3	-14,615	0,313893	1,08E-05	0,00394
KRT19	-8,64163	0,31284	1,13E-05	0,004121
CRIM1	-20,57	0,287229	3,54E-05	0,012901
COLEC12	-14,5942	0,244778	0,000219	0,079625
CHRD1	-13,612	0,228297	0,000436	0,15771
LAIR1	-10,3162	0,222985	0,000542	0,195762
PRSS8	-12,4807	0,22271	0,000548	0,197437
LY6D	-11,7192	0,217577	0,000677	0,243021

Normalization and Cytokine Analysis in Validation cohort

In order to validate the results obtained from the derivation cohort, we utilized patients diagnosed with inflammatory cardiomyopathy from the TORCH registry of the DZHK as a validation cohort.

- 5 The samples were collected in two batches. The first batch comprised of the derivation and generalizability cohorts, and the second batch included the validation cohort as well as 16 bridging samples. The normalization procedure followed the recommendations of the manufacturer.

- 10 The data were normalized using the OlinkAnalyze package, version 3.1.0, using the first batch data set as the reference sample and 16 bridging samples. Results of the normalization process are presented in Figure 5 and 6. Principal component analysis was performed to ensure that no subgroups differentiated the derivation and validation datasets. No significant differences were observed for the first and second principal components between the datasets. As such, the datasets were considered comparable (Figure 7 and Figure 8). Similar to the derivation cohort, a
- 15 linear regression model was employed to analyze the relationship between cytokine levels and LVEF in the independent validation cohort of patients with inflammatory cardiomyopathy collected in the national registry (TORCH, n= 425). Following correction for multiple testing, a total of 77 cytokines were found to be significantly associated with LVEF (FDR < 0.05; Table4). The relationship between relative cytokine concentration and LVEF was again found to be inverse.
- 20 The replication in the validation cohort confirms that higher relative cytokine concentrations of the top 10 cytokines identified in the derivation cohort (DHZC) are associated with lower LVEF in patients with inflammatory cardiomyopathy from the independent cohort (TORCH; Table 5).

- Table 4:** Validation cohort (n=452): Similar to the derivation cohort, there is an inverse relationship between cytokine concentrations and left ventricular ejection fraction (LVEF) with
- 25 high cytokine levels being associated with lower LVEF. There were 77 cytokines significantly associated with LVEF (FDR < 0.05). Estimate, the coefficient estimate from the linear regression model; r^2 , coefficient of determination (r^2) value for the model; P-value, raw p value for the model; FDR, false discovery rate calculated using Benjamini-Hochberg correction.

Cytokine	Estimate	r ²	P-value	FDR
COLEC12	-12,1741	0,164675	4,46E-16	1,64E-13
PLAUR	-11,1866	0,163439	6,12E-16	2,24E-13

Cytokine	Estimate	r2	P-value	FDR
CCL3	-7,14421	0,152285	1,04E-14	3,81E-12
LILRB4	-9,01034	0,151545	1,25E-14	4,58E-12
CXCL17	-5,54511	0,149364	2,18E-14	7,92E-12
AGRN	-10,9161	0,148426	2,75E-14	1E-11
WNT9A	-9,78385	0,141907	1,41E-13	5,11E-11
CD4	-11,4165	0,137435	4,31E-13	1,56E-10
FABP1	-4,20844	0,136129	5,96E-13	2,15E-10
LAIR1	-7,74702	0,133987	1,01E-12	3,64E-10
LGALS9	-8,96591	0,123841	1,24E-11	4,44E-09
CRIM1	-12,4333	0,122375	1,78E-11	6,35E-09
TNFSF13	-10,0253	0,121528	2,19E-11	7,79E-09
FSTL3	-7,4228	0,113642	1,5E-10	5,33E-08
CCL7	-4,43945	0,109818	3,8E-10	1,35E-07
TGFA	-7,94094	0,109175	4,44E-10	1,57E-07
CLSTN2	-6,20831	0,107495	6,67E-10	2,35E-07
HGF	-4,49271	0,104511	1,37E-09	4,82E-07
ANGPTL4	-6,68144	0,104232	1,47E-09	5,14E-07
SPON1	-7,40211	0,103125	1,92E-09	6,69E-07
IL17D	-9,61421	0,101249	3,01E-09	1,05E-06
LRRN1	7,205829	0,101132	3,1E-09	1,08E-06
LY6D	-7,43169	0,098996	5,18E-09	1,79E-06
PON3	11,39412	0,095766	1,13E-08	3,88E-06
HLA-E	-11,4467	0,095722	1,14E-08	3,91E-06
SPINK4	-4,40881	0,095501	1,2E-08	4,11E-06
LGALS4	-5,27358	0,09436	1,58E-08	5,39E-06
IL6	-2,99671	0,093813	1,8E-08	6,13E-06
NPPC	-4,98094	0,093211	2,08E-08	7,06E-06
CHRD1	-7,91593	0,092942	2,21E-08	7,51E-06
TREM2	-4,77254	0,091431	3,18E-08	1,07E-05
CXCL10	-4,17866	0,088631	6,21E-08	2,09E-05
EPO	-3,74666	0,087718	7,72E-08	2,59E-05
CXCL8	-4,01822	0,087691	7,77E-08	2,6E-05
MATN2	-9,39644	0,086945	9,28E-08	3,1E-05
CXCL14	-4,49222	0,08555	1,29E-07	4,31E-05
GAL	4,65288	0,082948	2,4E-07	7,98E-05
TFF2	-4,06551	0,081606	3,31E-07	0,000109
PREB	-10,3113	0,081157	3,68E-07	0,000121
SULT2A1	-4,04533	0,078762	6,5E-07	0,000214
ENPP7	-3,32201	0,077855	8,06E-07	0,000264
IL15	-6,78804	0,077687	8,39E-07	0,000274
DNER	9,589636	0,077289	9,22E-07	0,0003
CXCL9	-3,22193	0,074958	1,6E-06	0,000521
IL4R	-6,07854	0,074123	1,95E-06	0,000633
REG4	-4,97668	0,073114	2,48E-06	0,000801

Cytokine	Estimate	r2	P-value	FDR
BTN3A2	-6,09672	0,071531	3,61E-06	0,001162
FST	-5,18705	0,071502	3,63E-06	0,001166
EGLN1	-3,95482	0,07108	4,01E-06	0,001284
TNF	-5,94492	0,070676	4,42E-06	0,001409
IL1RN	-3,4451	0,070089	5,08E-06	0,001614
NTF3	-5,07255	0,069915	5,29E-06	0,001677
TNFRSF13B	-6,8624	0,069553	5,76E-06	0,001821
CXADR	-4,82297	0,068877	6,76E-06	0,00213
CD276	-5,47858	0,068871	6,77E-06	0,00213
TNFRSF11A	-4,2661	0,06851	7,38E-06	0,002308
SMOC2	-5,99596	0,068308	7,73E-06	0,002413
CCL25	-4,11859	0,067723	8,88E-06	0,002763
NFASC	-7,98799	0,067721	8,89E-06	0,002763
CCL28	-3,05271	0,067265	9,9E-06	0,003059
SIGLEC10	-5,64472	0,066844	1,09E-05	0,003368
PRSS8	-5,97756	0,066519	1,18E-05	0,003626
LAMA4	-6,9675	0,066442	1,2E-05	0,00368
PRELP	-8,7097	0,065305	1,57E-05	0,004801
ENPP5	5,817113	0,063325	2,51E-05	0,007643
CCL21	-4,3005	0,063229	2,57E-05	0,007793
TNFRSF4	-4,64774	0,061676	3,71E-05	0,011215
HLA-DRA	-5,76163	0,061424	3,94E-05	0,011865
CCL23	-5,004	0,061069	4,29E-05	0,012863
FASLG	4,476295	0,060662	4,72E-05	0,014115
TPP1	-5,9269	0,059997	5,53E-05	0,016466
CCL11	-4,03204	0,059247	6,6E-05	0,019599
CCL13	-2,60901	0,057756	9,39E-05	0,0278
KRT19	-3,53618	0,057209	0,000107	0,031541
LIFR	-6,6545	0,05658	0,000124	0,036485
ITM2A	-4,51045	0,056553	0,000125	0,036589
CKAP4	-5,15816	0,056407	0,000129	0,037749

Table 5: Replication in the validation cohort confirms that higher relative cytokine concentrations of the top 10 cytokines from the derivation cohort are associated with lower LVEF in patients with inflammatory cardiomyopathy. Estimate, the coefficient estimate from the linear regression model; r^2 , coefficient of determination (r^2) value for the model; P-value, raw p value for the model; FDR, false discovery rate calculated using Benjamini-Hochberg correction.

Cytokine	Estimate	r2	p value	FDR
COLEC12	-12.17	0.1647	4.5e-16	1.6e-13
LAIR1	-7.747	0.134	1.0e-12	3.6e-10
CRIM1	-12.43	0.1224	1.8e-11	6.3e-09
FSTL3	-7.423	0.1136	1.5e-10	5.3e-08
CLSTN2	-6.208	0.1075	6.7e-10	2.3e-07
LY6D	-7.432	0.099	5.2e-09	1.8e-06

Cytokine	Estimate	r2	p value	FDR
CHRD1	-7.916	0.09294	2.2e-08	7.5e-06
PRSS8	-5.978	0.06652	1.2e-05	0.0036
KRT19	-3.536	0.05721	0.00011	0.0315
VEGFD	-3.324	0.04488	0.00202	0.5383

Generalizability of findings

Following the identification of cytokines that showed an inverse relationship with LVEF in patients with inflammatory cardiomyopathy, generalizability of these findings was evaluated in patients with IDCM (DHZC, n=41). A Welch two-sided t-test was used to compare the groups with severe vs mild to moderately reduced LVEF in IDCM. Similar to the derivation and validation cohort, there was a trend for top cytokines of both cohorts being associated with more severely reduced LVEF in IDCM as well (Figure 1). After correction for multiple testing, no cytokines were significant. However, there was a significant correlation between the effect sizes of the derivation and generalizability cohort (Pearson's correlation coefficient = 0.29).

Post hoc analysis for all patients with inflammatory cardiomyopathy

After normalization, the relative cytokine values of all 488 patients with inflammatory cardiomyopathy were collectively analyzed and compared to each other with regards to their ability to distinguish between severe and mildly reduced LVEF categories. Covariables sex and dataset of origin were used in the analysis. A total of 82 proteins were found to be significant after correction for multiple testing. The top values for the top cytokines from the derivation cohort are presented in Table 6.

Table 6: Top 10 cytokines associated with the left ventricular ejection fraction (LVEF) in the derivation and validation cohorts when analyzed together (n = 488). Higher relative cytokine concentrations are associated with lower LVEF values.

Cytokine	Estimate	r2	p value	FDR
COLEC12	-12.58	0.1762	< 2e-16	< 2e-16
PLAUR	-11.25	0.163	< 2e-16	4.2e-15
AGRN	-11.2	0.1549	< 2e-16	4.6e-14
WNT9A	-10.03	0.1492	6.5e-16	2.4e-13
LAIR1	-8.063	0.144	2.9e-15	1.0e-12
LILRB4	-8.768	0.1439	3.0e-15	1.1e-12
FABP1	-4.191	0.1416	5.8e-15	2.1e-12
CRIM1	-13.45	0.1403	8.4e-15	3.0e-12
CCL3	-6.865	0.1389	1.3e-14	4.5e-12
FSTL3	-8.187	0.1324	8.1e-14	2.9e-11

EMR Data Evaluation

We screened 18,566 patient records from three rheumatology departments of Charité University Berlin to test our hypothesis in EMR data. Among these, hs-TnT and/or NT-proBNP measurements were available for 8,767 patients. Within this patient pool, 348 individuals had been administered the cytokine inhibitors of interest. These included IL-6 inhibitors and janus kinase inhibitors. For the other cytokines identified in our study, there are either no specific

inhibitors presently in clinical use, or the number of patients treated was insufficient to permit robust statistical analysis. Out of this subset, 190 patients had hs-TnT and/or NT-proBNP measurements recorded post-administration of the cytokine inhibitors. After excluding statistical outliers, the final analysis consisted of a total of 159 patients.

5 *NT-proBNP:*

Treatment with IL-6 receptor inhibitors (n=69) resulted in a notable reduction in median NT-proBNP levels by 136 ng/L ($p < 0.001$). Similarly, in the group receiving janus kinase inhibition (n=90) the median NT-proBNP level was significantly reduced by approximately 170.5 ng/L ($p < 0.001$). The results are presented in Figure 2.

10 *Hs-TnT:*

In the group receiving janus kinase inhibitors (n=52) a significant decrease in median hs-TnT levels by 9 ng/L ($p = 0.001$) was detectable. The results are presented in Figure 3.

DISCUSSION

15 This study aimed to fill a crucial clinical gap in the treatment of inflammatory cardiomyopathy, by exploring new potential therapeutic targets for patients who do not respond to existing, advanced therapies. We hypothesized that some cytokines released in the context of inflammatory cardiomyopathy may lead to myocardial injury and decreased LVEF as a consequence. Therefore, we investigated the association of cytokines with LVEF. Our investigation proved our hypothesis that there was an inverse correlation of specific cytokines with LVEF. We first
20 identified our findings within a derivation cohort from our medical center (n=63). Then, we replicated our results in an independent validation cohort (n=425), which encompassed samples gathered from 11 different cardiovascular centers across Germany, as part of the national TORCH registry. Furthermore, testing of our results in patients with IDCM (n=41) suggested generalizability of our findings. A post-hoc analysis further confirmed the robustness of our data.
25 Real-world data from our EMR system, specifically from patients treated with inhibitors targeting the cytokines identified in our study or the pathways they influence downstream, suggested a potential cardioprotective effect of such specific cytokine inhibition. These data support the validity of our hypothesis and findings.

30 All three cohorts, including derivation, validation, and generalizability cohort, demonstrated a balanced distribution of key baseline parameters including sex, age, BMI, LVEF, and renal function. The sex distribution, with approximately one third of the participants being women is in agreement with prior literature^{4, 12, 15, 31}. Based on the median BMI, a large part of the patients was overweight. This is an observation that aligns with findings from prior work on inflammatory cardiomyopathy³¹. One may speculate that proinflammatory characteristics of adipose tissue
35 could contribute to a predisposition of developing inflammatory cardiomyopathy³¹. A considerable proportion of patients (36.5% in the derivation cohort, 39.8% in the validation cohort and 41.5% in the generalizability cohort) exhibited severely compromised left ventricular function, ensuring a balanced representation of the degree of illness. Kidney function was overall normal in all cohorts. According to ESC guidelines²⁵, patients with LVEF $\leq 35\%$ received more angiotensin-

receptor blockers (ARBs) and diuretics compared to those with a higher LVEF. If this disparity in medication regimen influenced our study outcome, it likely concealed the observed association between specific cytokines and low LVEF, given that ARBs are known to possess anti-inflammatory properties^{32, 33}. Our investigation confirmed the hypothesis that specific cytokines correlate with severe inflammatory cardiomyopathy as defined by severely reduced LVEF ($\leq 35\%$). This correlation was inverse, highly significant, and reproducible in a large independent set of samples that were collected at 11 different sites in Germany in a national registry. Furthermore, our data suggested that results may be generalizable to IDCM.

Several of the cytokines identified had been suggested or evaluated as potential therapeutic targets in inflammatory cardiomyopathy previously, supporting validity and plausibility of our results. Similar to prior studies in patients with heart failure overall³⁴⁻³⁸, higher serum levels of IL6 or CRIM1 were associated with more severe forms of inflammatory cardiomyopathy in our study. Importantly, it had been shown previously that in IL6 deficient mice, prevalence and severity of myocarditis were reduced in the absence of IL6³⁵. In myocarditis, IL6 is produced by monocytes infiltrating the myocardium and differentiating into inflammatory macrophages, contributing to tissue degradation and T cell activation⁴. IL6 inhibitors tocilizumab and sarilumab have been administered successfully to patients with severe COVID-19 to mitigate the overactive cytokine response, improving survival³⁹.

Janus kinase inhibitors block downstream signaling of inflammatory pathways of IL6 and other cytokines. Investigation of our current EMR data demonstrating lower NT-proBNP and hs-TnT levels in patients receiving IL6 or janus kinase inhibitors is in agreement with existing literature suggesting that this drug classes reduce cardiac inflammation and injury^{35, 40, 41}. The inventors study identified numerous novel cytokines that had either not been previously associated with severe inflammatory cardiomyopathy or had only been minimally explored within this context. These included COLEC 12 - a scavenger receptor that plays a role in host defense, LAIR1 – a protein expressed on human leukocytes and LILRB4 – a leukocyte immunoglobulin-like receptor (www.genenames.org). FSTL3 was recently identified as relevant prognostic marker for major adverse cardiovascular events in stable coronary artery disease. Higher levels of FSTL3 were associated with poor outcomes⁴². Also in the current study, higher blood levels of FSTL3 were associated with severe inflammatory cardiomyopathy. LGALS9 is a protein that modulates cell-cell and cell-matrix interactions. It has been shown to promote innate immune cell activation by potentiating or synergizing toll-like receptor signaling⁴³. Also, LGALS9 has been associated with the severity of myocarditis in mice infected with Coxsackievirus B3 and to have an immunomodulatory effect when administered in experimental myocarditis⁴⁴. Our findings have important implications for future treatment of patients with inflammatory cardiomyopathy, who clinically deteriorate despite currently available state-of-the art medical therapy. Cytokines are feasible therapy targets as prior research has shown. Blocking of cytokines, their receptors, or intracellular kinases downstream of cytokine signaling has been successfully applied^{4, 16, 19, 20, 21, 22}. Also, removal through immunoabsorption or -filtration may be applied. Drug-repurposing of already existing drugs that target the cytokines of interest such as Ixekizumab and Secukinumab to neutralize IL17A is assumed to be a novel treatment approach.

The inventors herein tested the hypothesis that there is excess of specific cytokines in severe inflammatory cardiomyopathy, which may lead to cardiac injury and decreased LVEF as a result. Our investigation confirmed that 82 cytokines were elevated in patients with severe inflammatory cardiomyopathy. Furthermore, elevation of cytokine levels correlated inversely with LVEF. Some of these cytokines had been previously reported as potential therapy targets in inflammatory cardiomyopathy and heart failure in general, supporting validity and biological plausibility of our data. In addition, the inventors discovered numerous novel cytokines of interest that may be used as therapeutic targets through binding and neutralizing agents, filtration, immunoadsorption or drug repurposing of already existing drugs in patients, who clinically deteriorate despite current state-of-the art therapies.

Example 2

The aim of the present example was to test the effect of cytokines, such as COLEC, CRIM and IL-6, on two different cell types in order to evaluate the possible causality of these markers for myocardial cell damage.

Methods

Experimental design

Cardiomyocytes (KM) and endothelial cells (HAEC) were treated with cytokines (IL6, CRIM or COLEC) except for the control group. The cells were harvested after 1 h, 24 h or 48 h. The experiment was performed in duplicates only, so the total number of samples was $2 \times 4 \times 3 \times 2 = 48$. The two replicates for cardiomyocytes were harvested at different times, B1 (September 2023) and B2 (March 2023), except for COLEC treated cells, which were all harvested in the second batch (B2). For endothelial cells, both replicates were harvested at approximately the same date (16.4.2024, 17.4.2024, 18.4.2024).

Statistical analysis

Reads were processed with the 'seasnap' pipeline (<https://github.com/bihealth/seasnap-pipeline>). In brief, reads were aligned to the human genome using the STAR aligner, and gene counts were generated using 'featureCounts'. Features were filtered by counts: minimum total 10 counts per feature and at least 5 counts in at least 3 samples. Differential expression analysis was performed using 'DESeq2'. To this end, the two cell types were analyzed separately. For each cell type, a model including both the treatment (one of cytokines or placebo) and time point (1 h, 24 h, 48 h) was fit, thus allowing in theory for an analysis of interactions. We have considered contrasts pertaining to difference between cytokine (IL6, CRIM or COLEC) and control at each time point, difference between cytokines at time point 24 h vs 1 h, 48 h vs. 1 h and 48 h vs. 24 h, as well as an interaction between treatment (cytokine) and time point. For each time point, gene set enrichment analysis was performed using the 'CERNO' gene set enrichment algorithm as implemented in the 'tmod' package, using the built-in transcription module database 'tmod' as well as other gene sets derived from 'MSigDB' by means of the 'msigdb' R package. These included 'GO/BP', 'REACTOME', 'Hallmark' and 'KEGG' gene sets.

Results

Principal component analysis (PCA)

PCA showed that the main effect seen overall in the cardiomyocyte data was the batch effect corresponding to the sample collection date. For endothelial cells, the main effect was the time point of the harvest. The treatment effect was visible in the endothelial cell data in that the first cell harvest coincided with the time point 1 sample.

Responses to cytokines in cardiomyocytes

With the exception for comparisons involving COLEC at 48 h, there were no significantly differentially expressed genes at FDR < 0.05.

The two COLEC 48 h KM samples differed from both, the 1 h and 24 h samples, and from the control samples. When compared to the control at the same time point, the COLEC 48 h samples showed several differentially expressed genes (DEGs) related to protein synthesis, most of them ribosomal proteins. This was confirmed by the gene set enrichment analysis, which showed a significant enrichment in terms such as GO/BP “ribosome assembly”.

Apart from the increase in expression of ribosomal proteins, the COLEC 48h also showed a decrease in expression of a number of other genes. These included the serum response factor (SRF; ENSG00000112658), ephrin receptor B3 (EPHB3; ENSG00000182580) and myocardin (MYOCD; ENSG00000141052). Correspondingly, the gene set enrichments of the contrast comparing COLEC treatment at 48 h and 1 h showed a significant enrichment in GO/BP gene sets “sarcomere organisation” and “cardiac muscle cell myoblast differentiation”.

While there were no significant DEGs for the comparison in other cytokines, gene set enrichment analysis showed that also in the 48 h IL6 and CRIM treated samples the genes related to translation, specifically ribosome proteins, were significantly enriched.

Responses to cytokines in endothelial cells

In endothelial cells (HAEC), the response to cytokines were more complex. Firstly, all cells irrespective of treatment showed significant DEGs and corresponding gene set enrichments between 1 h and later time points. These responses were very similar across all treatments including controls (Figure 12). A number of genes were affected by this phenomenon (Figure 13, left), and included such diverse genes as SMAD family member 6 (SMAD6; ENSG00000137834), fatty acid binding protein 4 (FABP4; ENSG00000170323) or DEPP autophagy regulator 1 (DEPP1; ENSG00000165507). These DEGs corresponded to a set of similar gene set enrichment terms across all experimental conditions.

The differences between the cytokine treatments and the control were more nuanced. At 48h, only the comparison between COLEC and control showed any DEGs (Figure 12), including SON DNA and RNA binding protein (SON; ENSG00000159140) and kinectin 1 (KTN1; ENSG00000126777). These differences did not correspond to any significant gene set enrichments and, furthermore, were not confirmed by the interaction contrast.

The opposite was true for the comparison between CRIM and control at 48h. While there were no significant DEGs, there was a weak, but statistically significant enrichment in the tmod interferon

transcriptional module (effect size, AUC = 0.77, FDR = 0.02). This was not confirmed by a corresponding enrichment in the interaction contrast. The genes related to this enrichment included interferon induced protein 44 like (IFI44L; ENSG00000137959) and MX dynamin like GTPase 1 (MX1; ENSG00000157601); see Figure 13, right.

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CLAIMS

1. An inhibitor of cytokine signaling for use in the treatment of cardiomyopathy, wherein the inhibitor of cytokine signaling is selected from a cytokine receptor inhibitor, a tyrosine kinase inhibitor or an antibody targeting a cytokine.
- 5 2. The inhibitor for use according to any one of the preceding claims, wherein the cardiomyopathy is an inflammatory cardiomyopathy (myocarditis).
3. The inhibitor for use according to any one of the preceding claims, wherein the cytokine is selected from interleukins, TNF, COLEC12, IL6, PLAUR, CHRDL1, AGRN, WNT9A, LAIR1, LILRB4, FABP1, CRIM1, CCL3, CLSTN2, LY6D, PRSS8, KRT19, VEGFD and FSTL3.
- 10 4. The inhibitor for use according to any one of the preceding claims, wherein the inhibitor of cytokine signaling is selected from Axitinib, Cabozantinib (L-malat), Etanercept, Filgotinib (maleat), Ruxolitinib (phosphate), Anakinra, Baricitinib, Tofacitinib (citrate), Upadacitinib (-0,5-Wasser), Golimumab, Infliximab, Nivolumab, Golimumab, Certolizumab (pegol), Golimumab, Ustekinumab, Infliximab, Ustekinumab, Ustekinumab, Infliximab, Tocilizumab, Mepolizumab, 15 Dupilumab, Guselkumab, Risankizumab, Ixekizumab, Secukinumab, Ustekinumab, Canakinumab, Cetuximab, Belimumab, Benralizumab, Bimekizumab, Sarilumab and Tildrakizumab.
5. The inhibitor for use according to any one of the preceding claims, wherein the patient exhibits one or more of chest pain, shortness of breath, an irregular and/or elevated 20 heartbeat, decreased ability to exercise, heart muscle weakness and/or dysfunction, heart failure or cardiac arrest.
6. The inhibitor for use according to any one of the preceding claims, wherein the cardiomyopathy is associated with a current or previous inflammation of the myocardium.
7. The inhibitor for use according to any one of the preceding claims, wherein the inflammatory 25 cardiomyopathy (myocarditis) is accompanied by a pericarditis.
8. The inhibitor for use according to any one of the preceding claims, wherein the patient is experiencing severe cardiomyopathy with cardiac dysfunction and a left ventricular ejection fraction (LVEF) of $\leq 35\%$.
9. The inhibitor for use according to any one of the preceding claims, wherein the patient is 30 experiencing a mild to moderate cardiomyopathy with a left ventricular ejection fraction (LVEF) of $> 35\%$.
10. The inhibitor for use according to any one of the preceding claims, wherein ACE inhibitors, beta blockers, angiotensin receptor and/or neprilysin inhibitors, SGLT2 inhibitors, diuretics, corticosteroids and/or intravenous immunoglobulin (IVIG) are administered in combination 35 and/or simultaneously to a subject.
11. A pharmaceutical composition for use in the treatment of cardiomyopathy comprising the inhibitor according to any one of claims 1-8.

12. A combination medication for use in the treatment of cardiomyopathy, comprising an inhibitor according to any one of claims 1-8 and a compound selected from the group comprising ACE inhibitors, beta blockers, angiotensin receptor and/or neprilysin inhibitors, SGLT2 inhibitors, diuretics, corticosteroids and/or intravenous immunoglobulin (IVIG).

Fig. 1

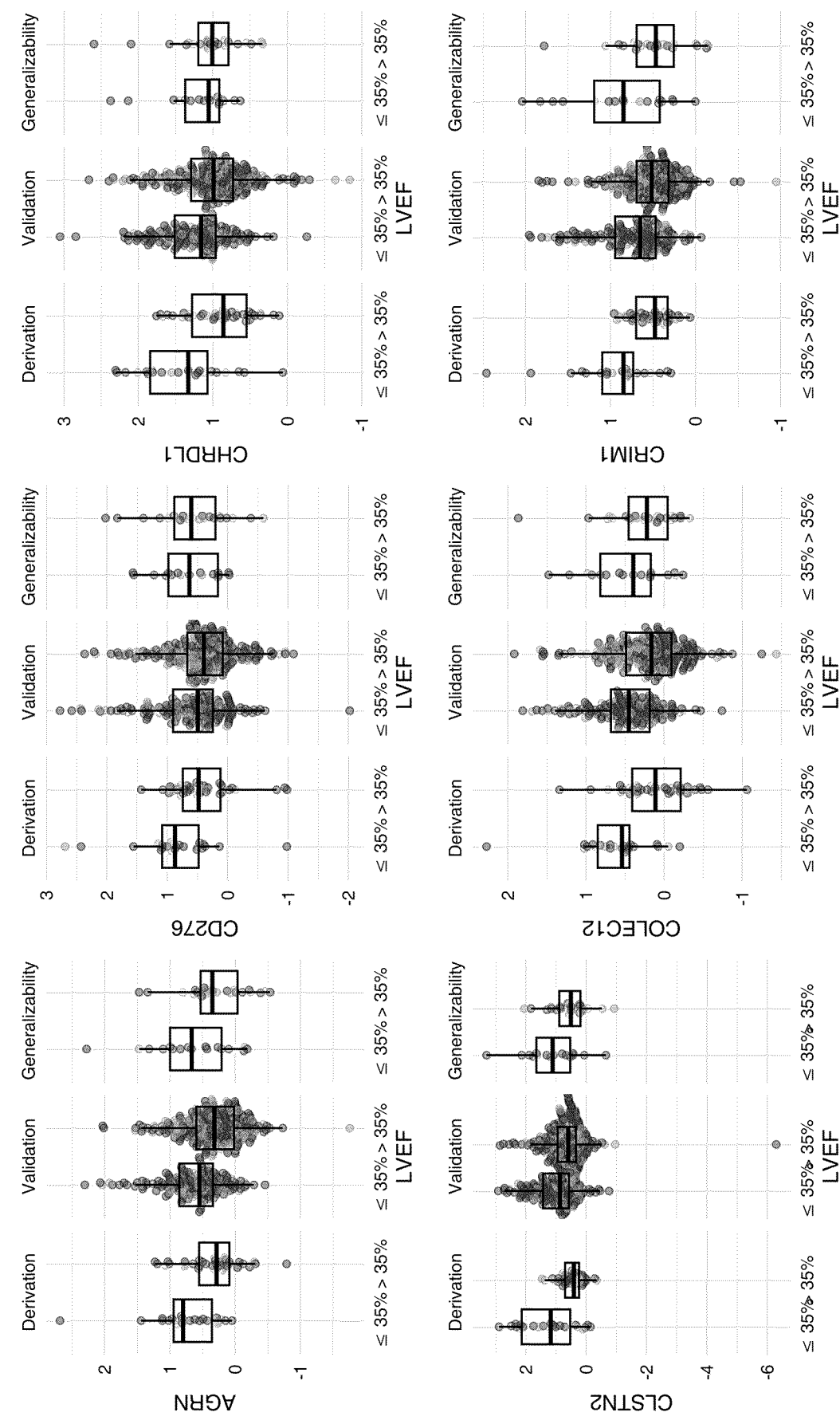


Fig. 1 continued

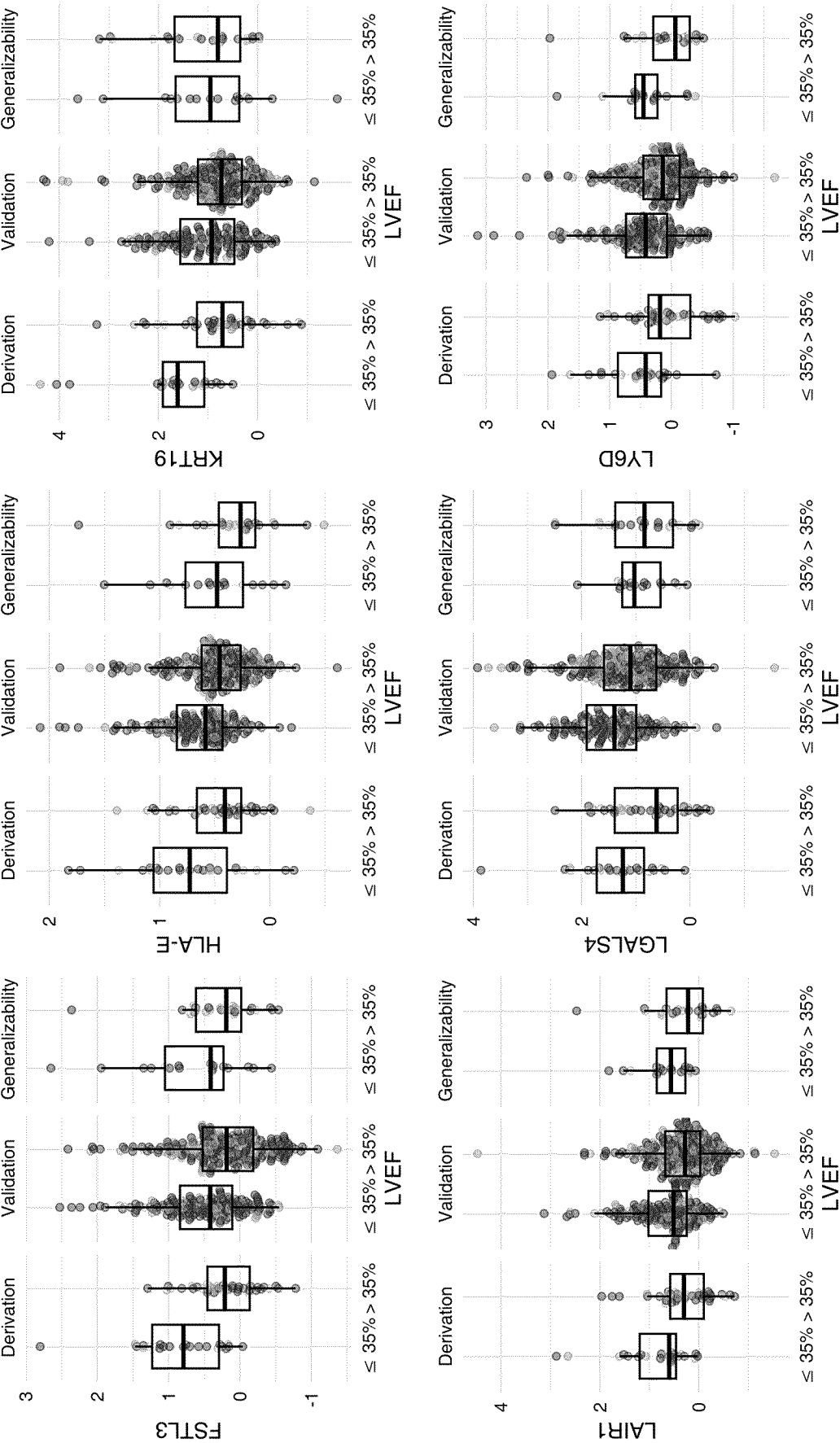


Figure 1 continued

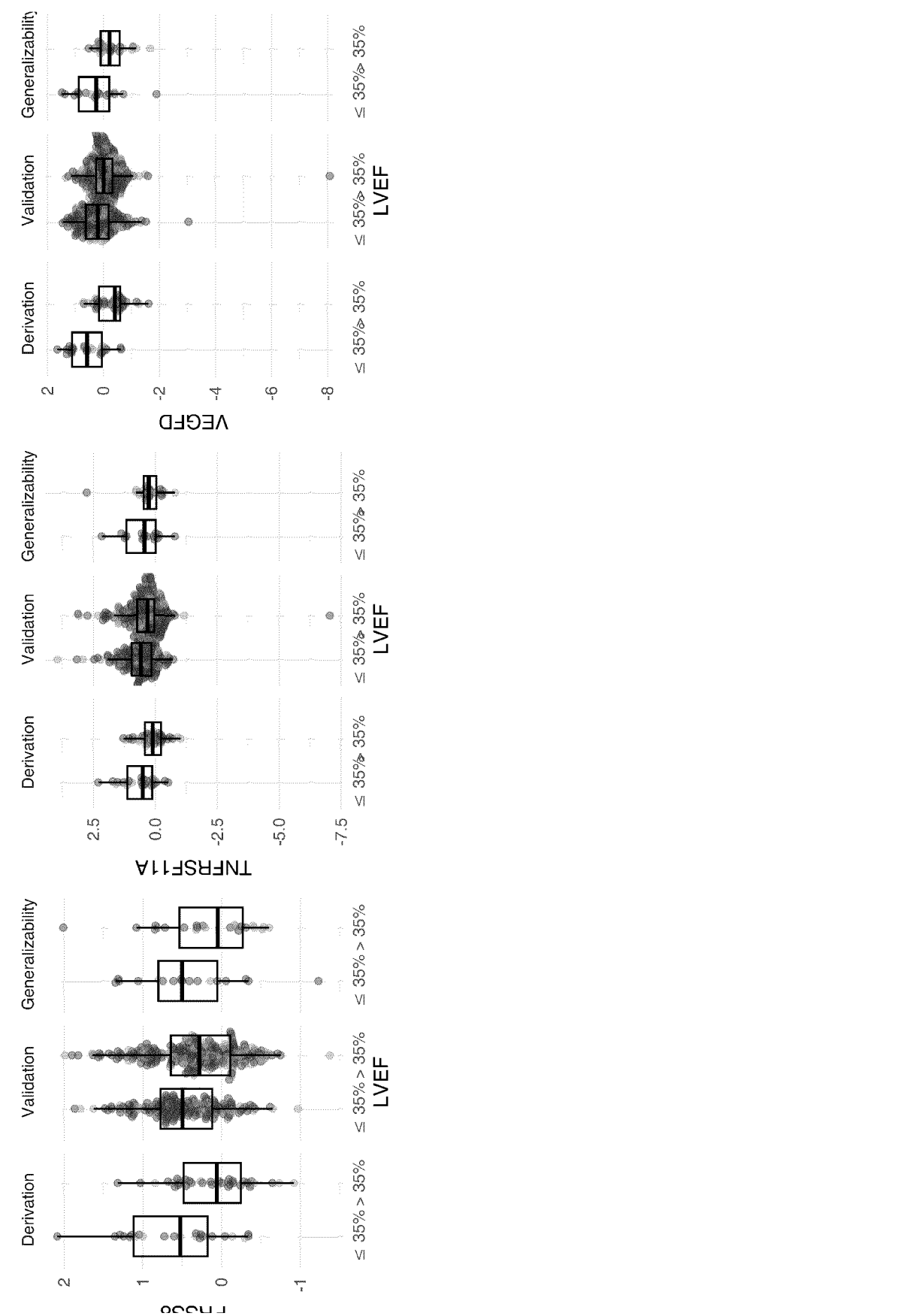


Fig. 2

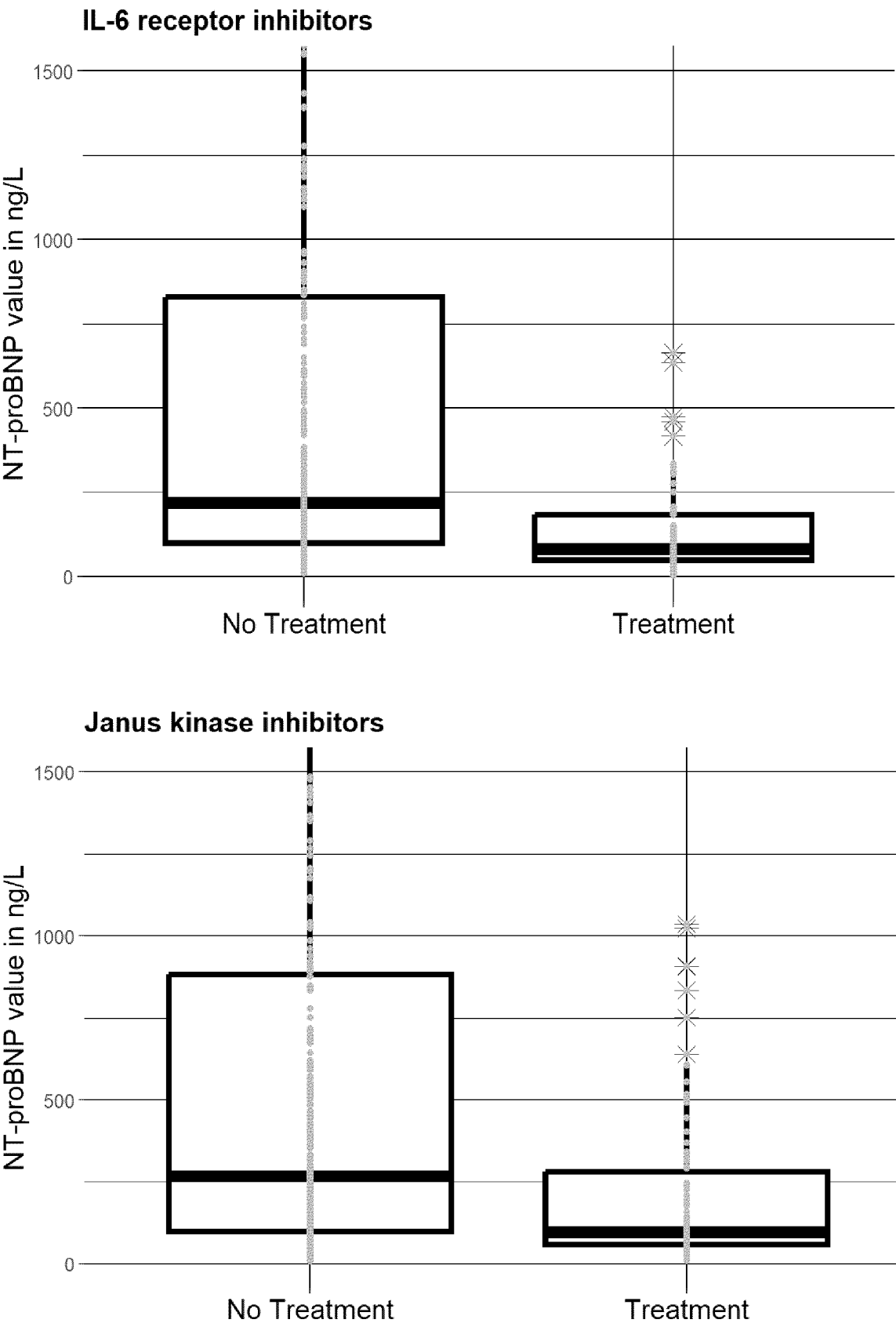


Fig. 3

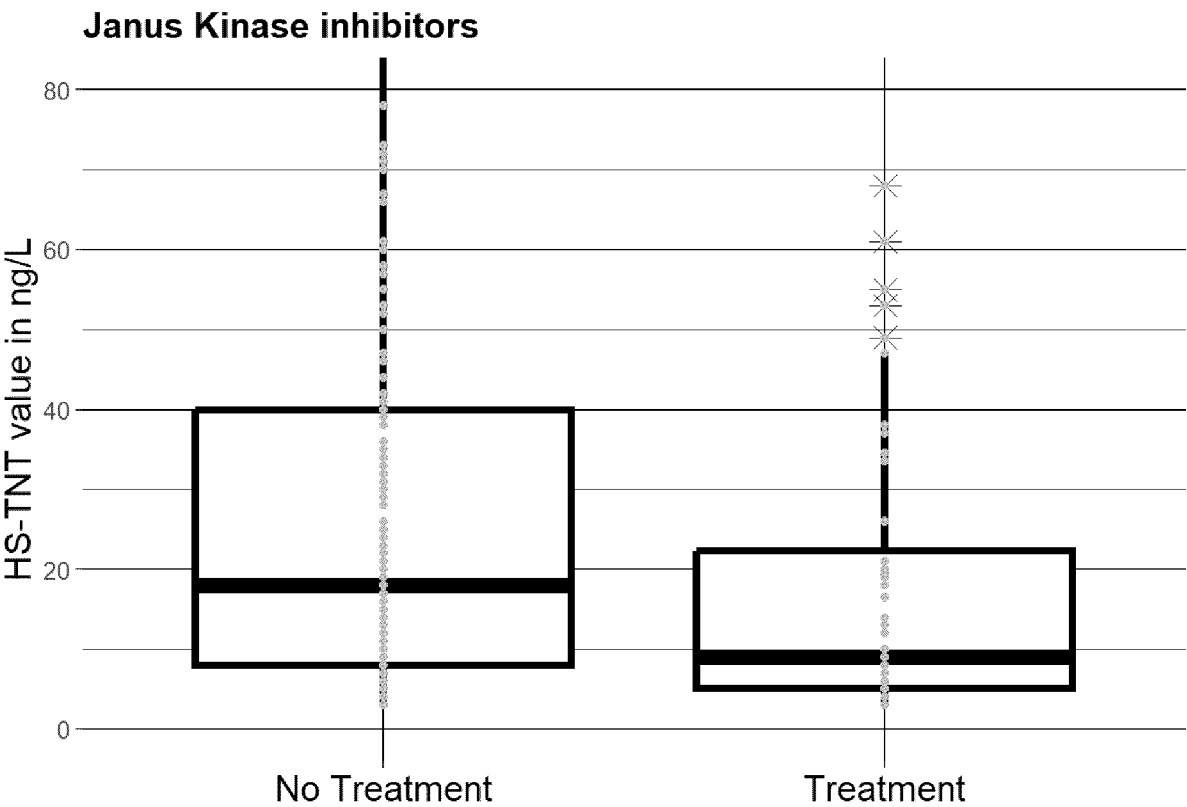


Fig. 4

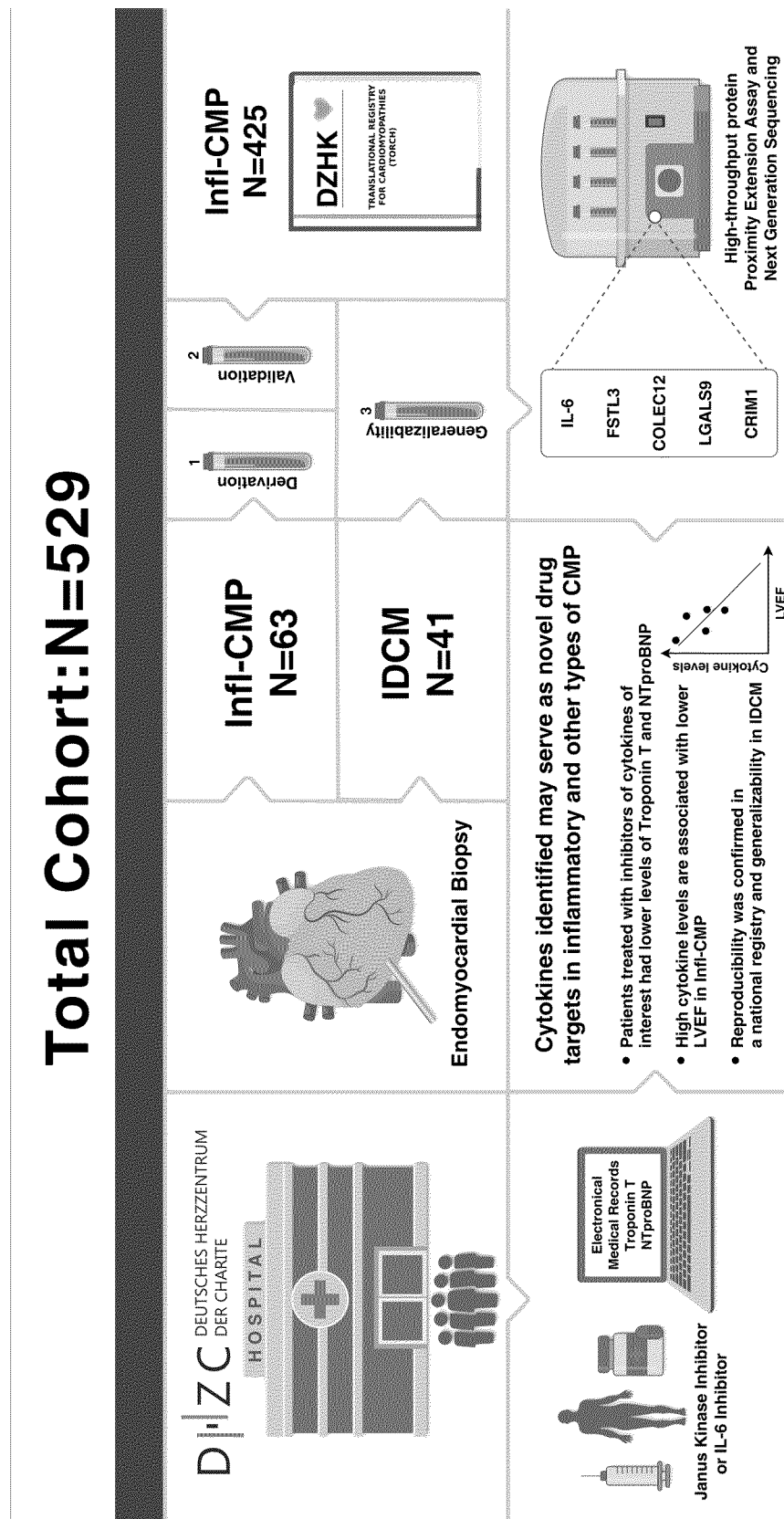


Fig. 5

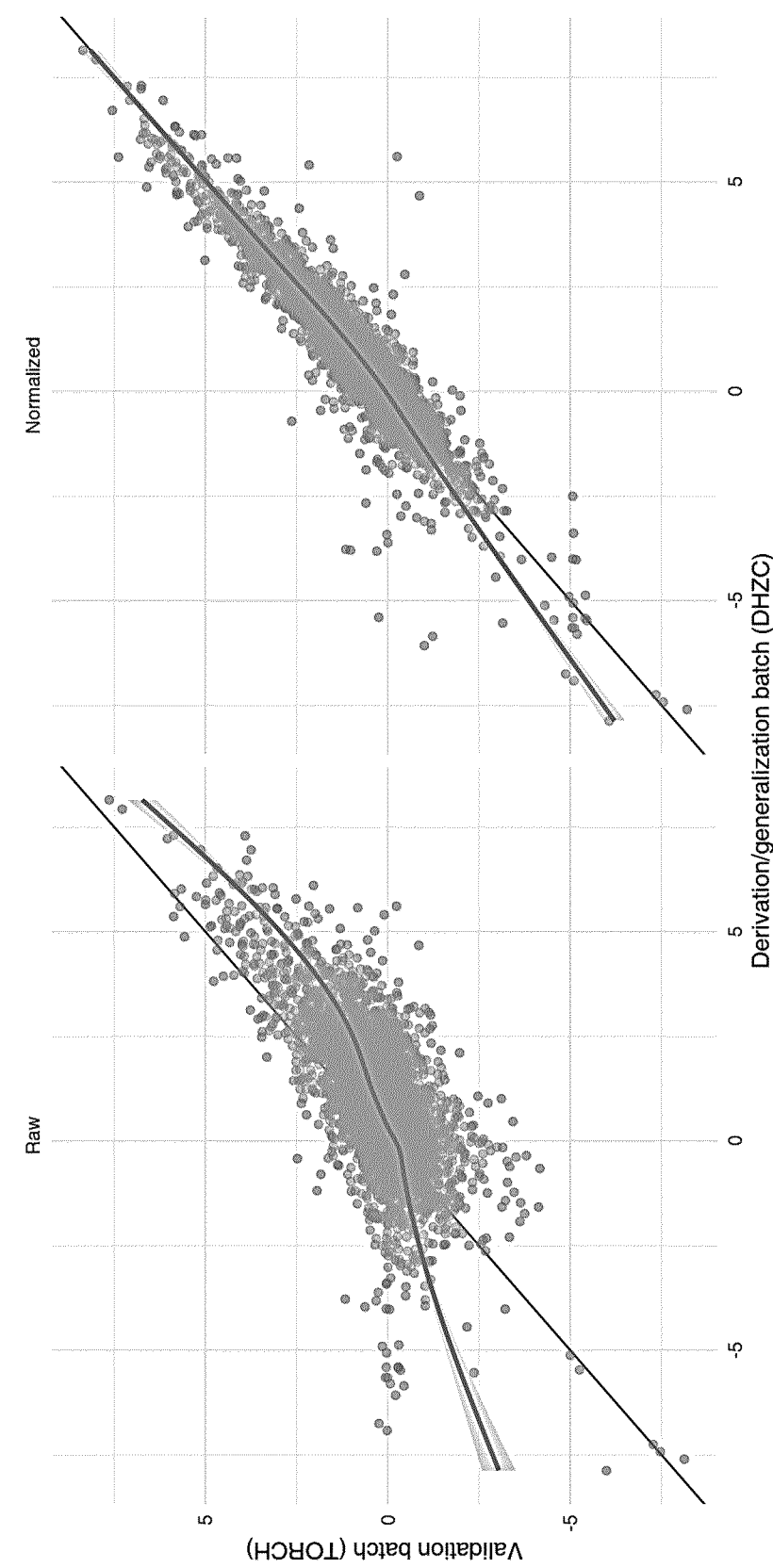


Fig. 6

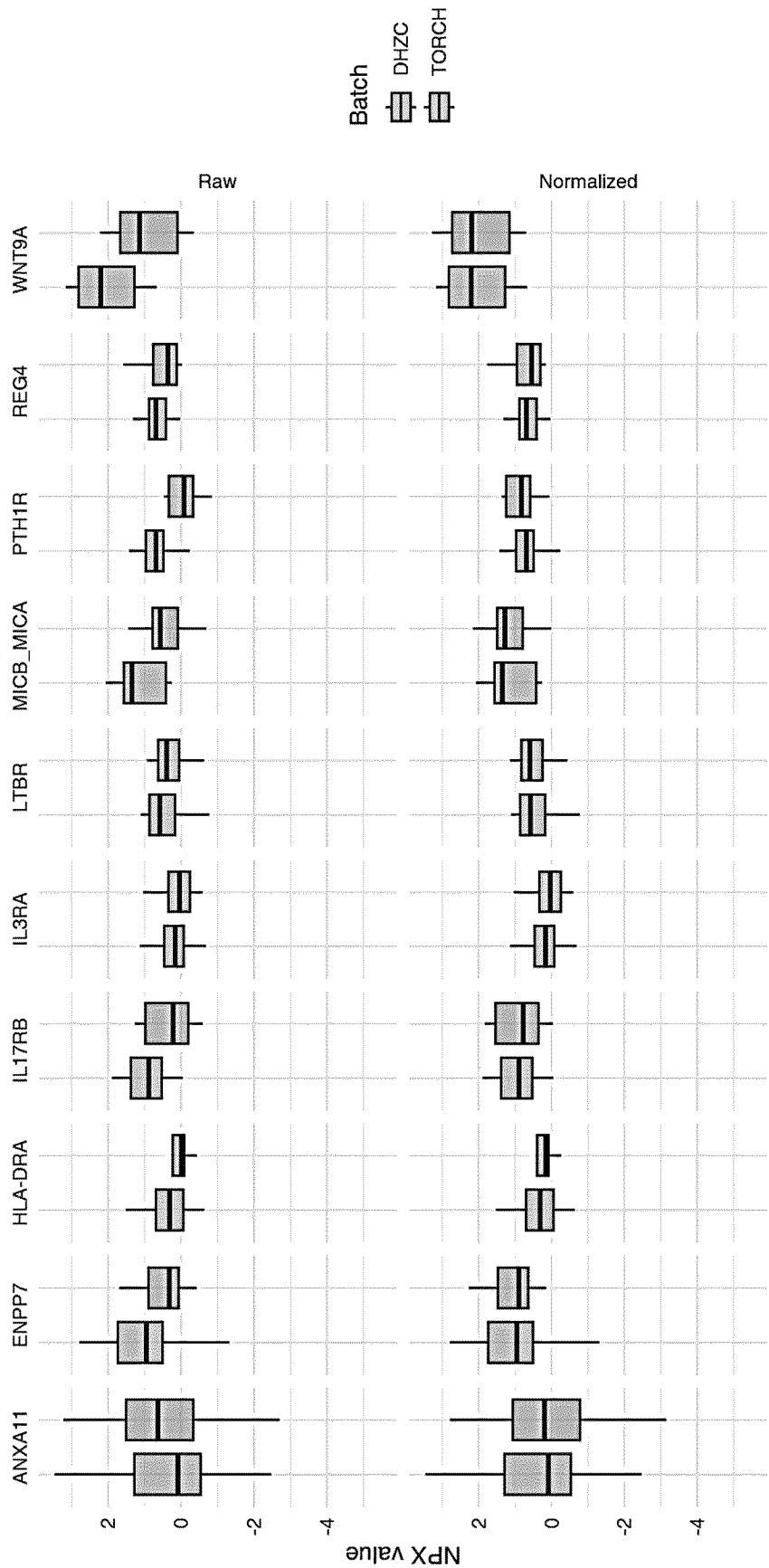


Fig. 7

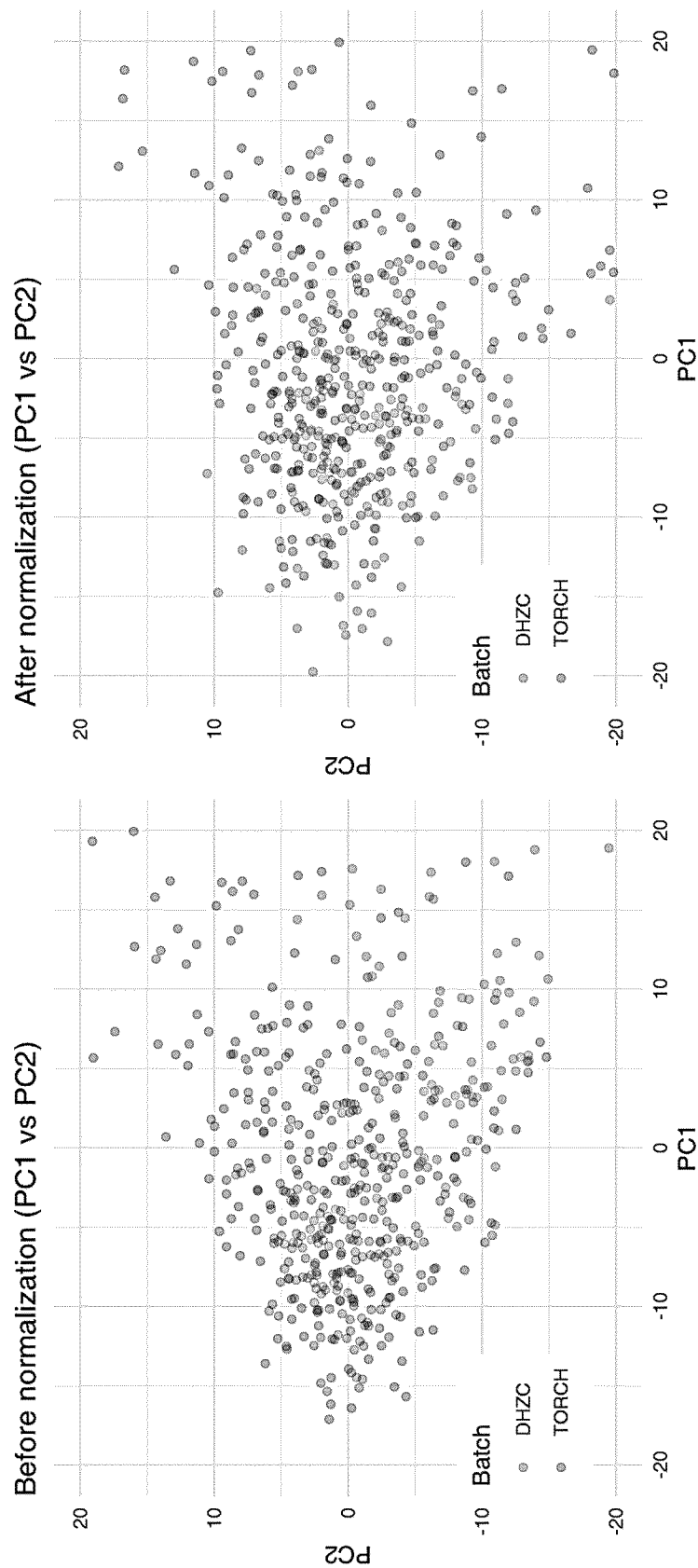


Fig. 7 continued

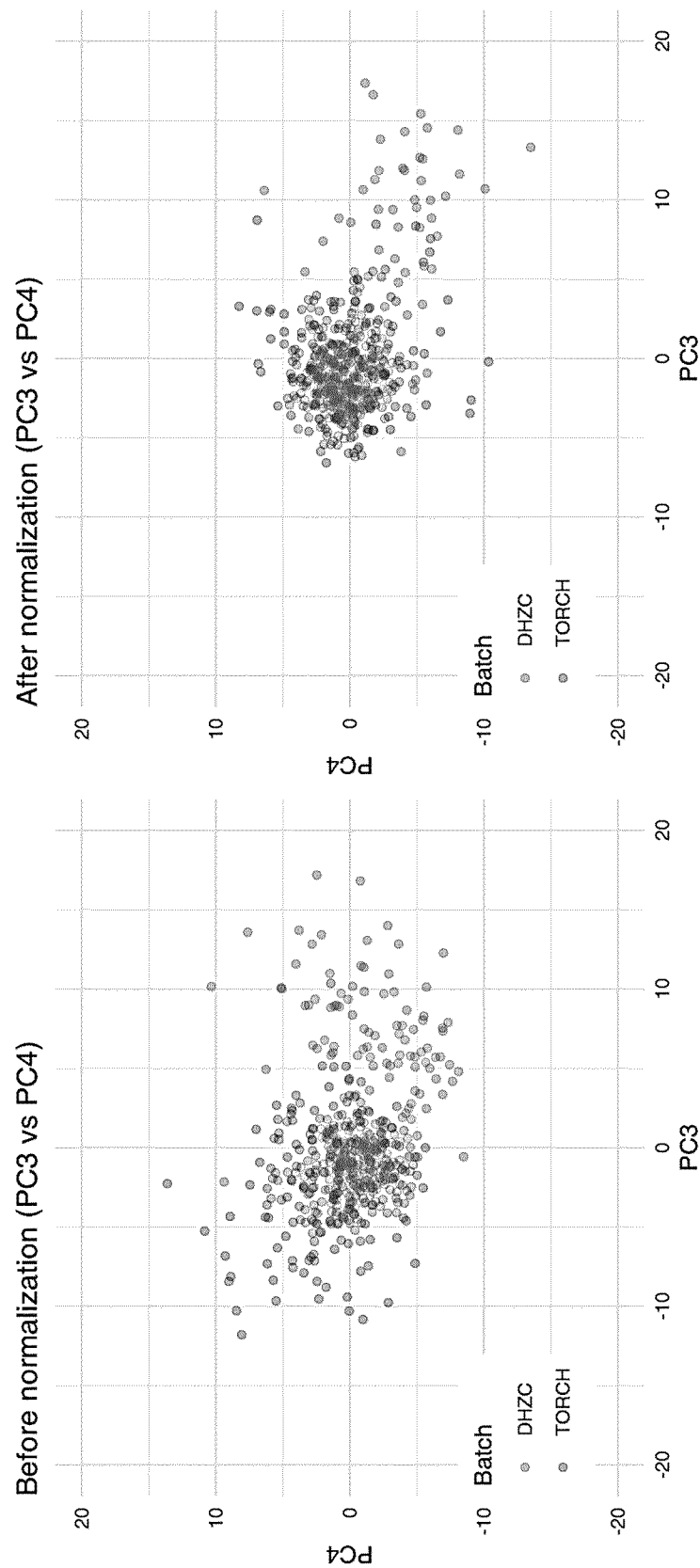


Fig. 8

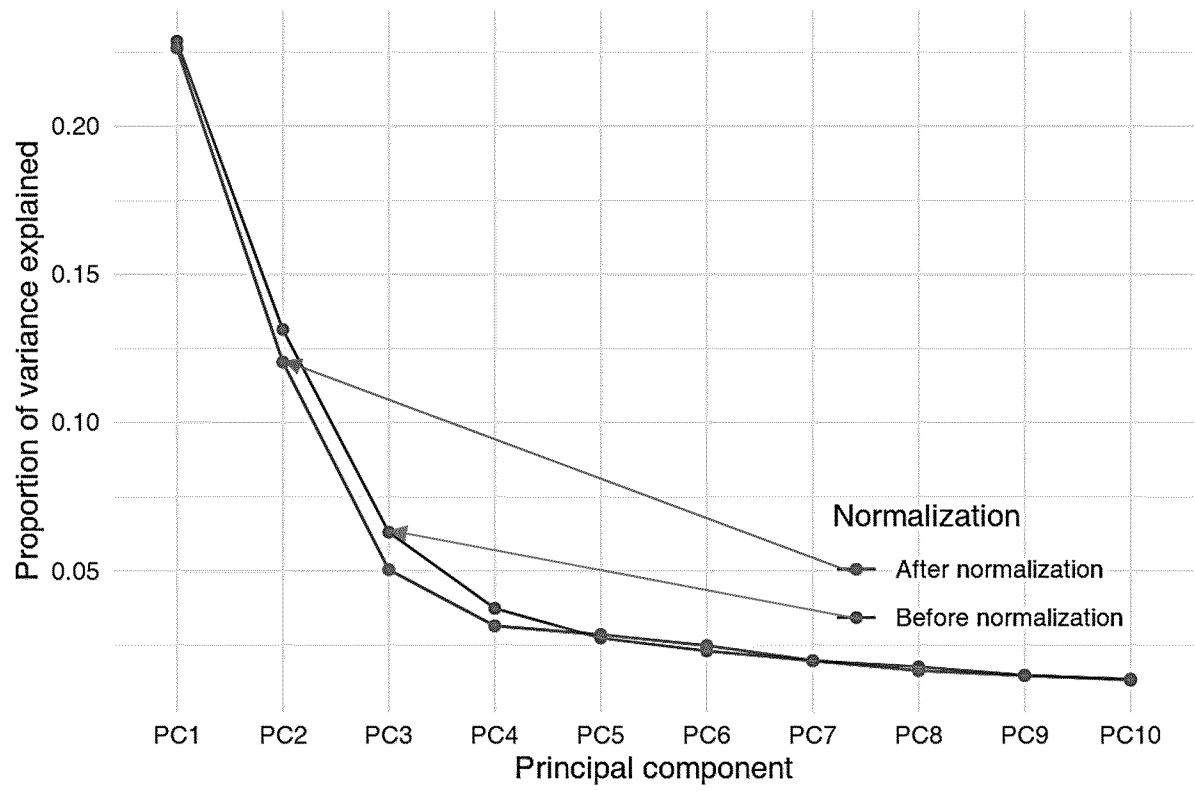


Fig. 9

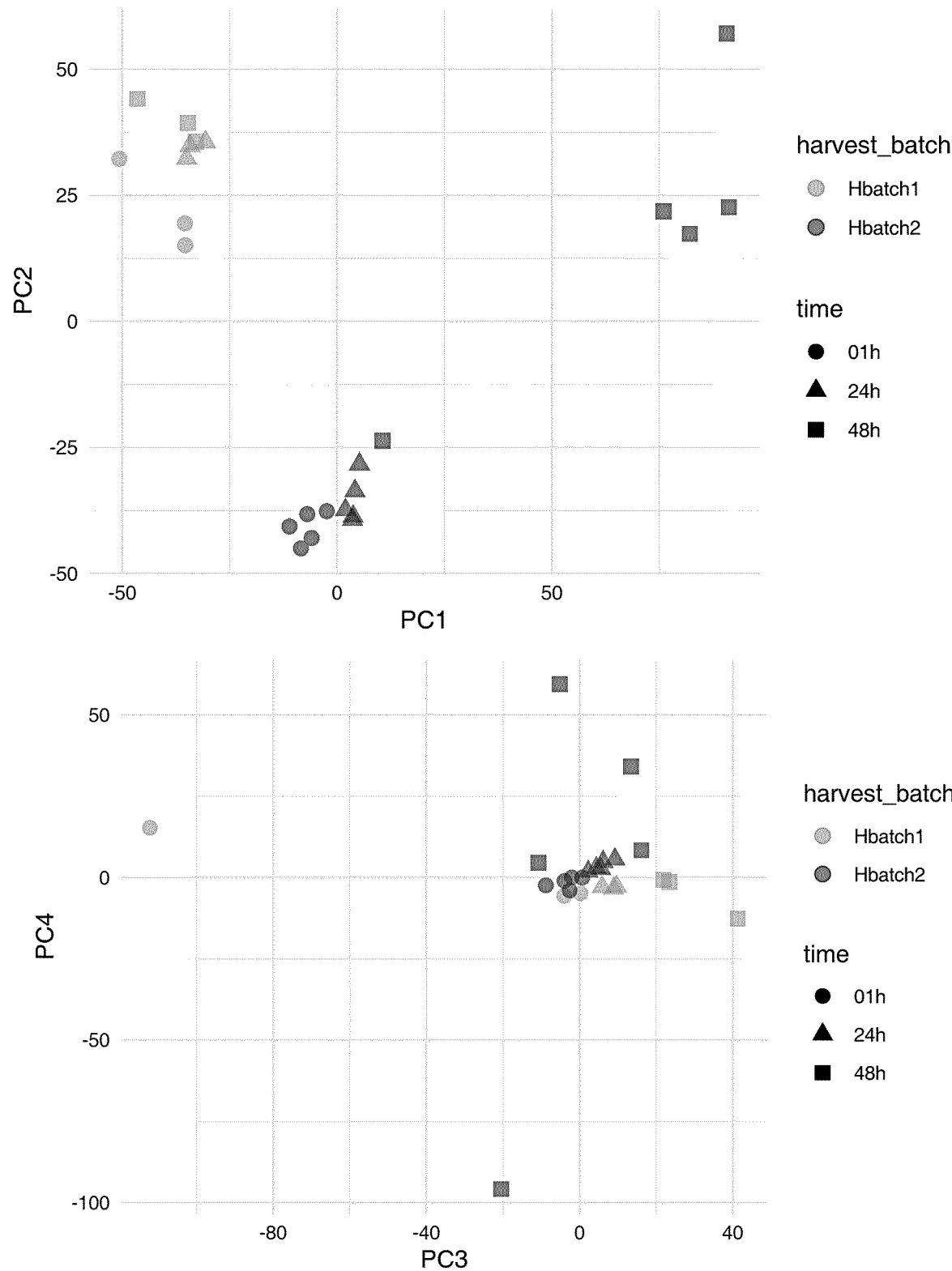


Fig. 10

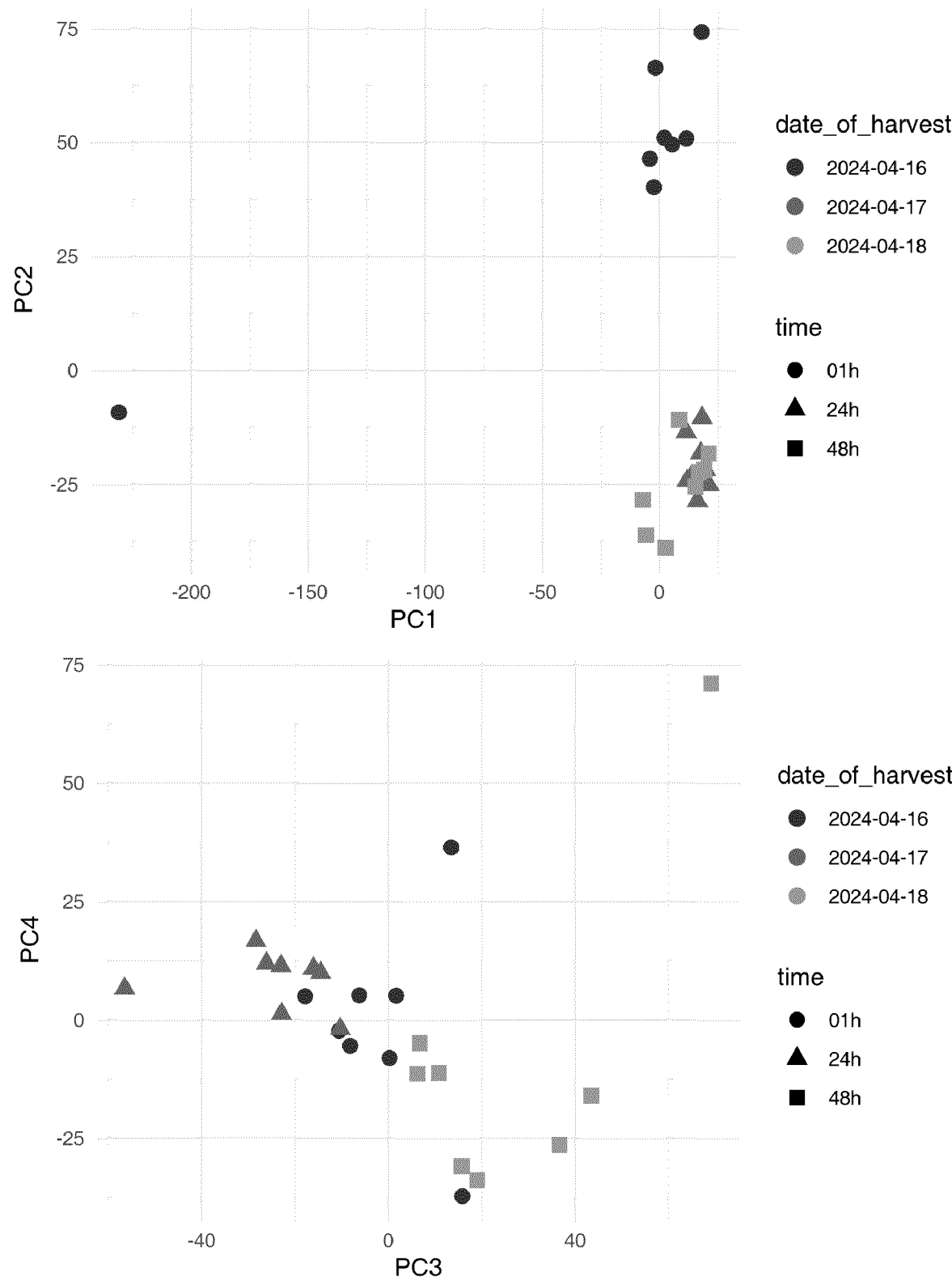


Fig. 11

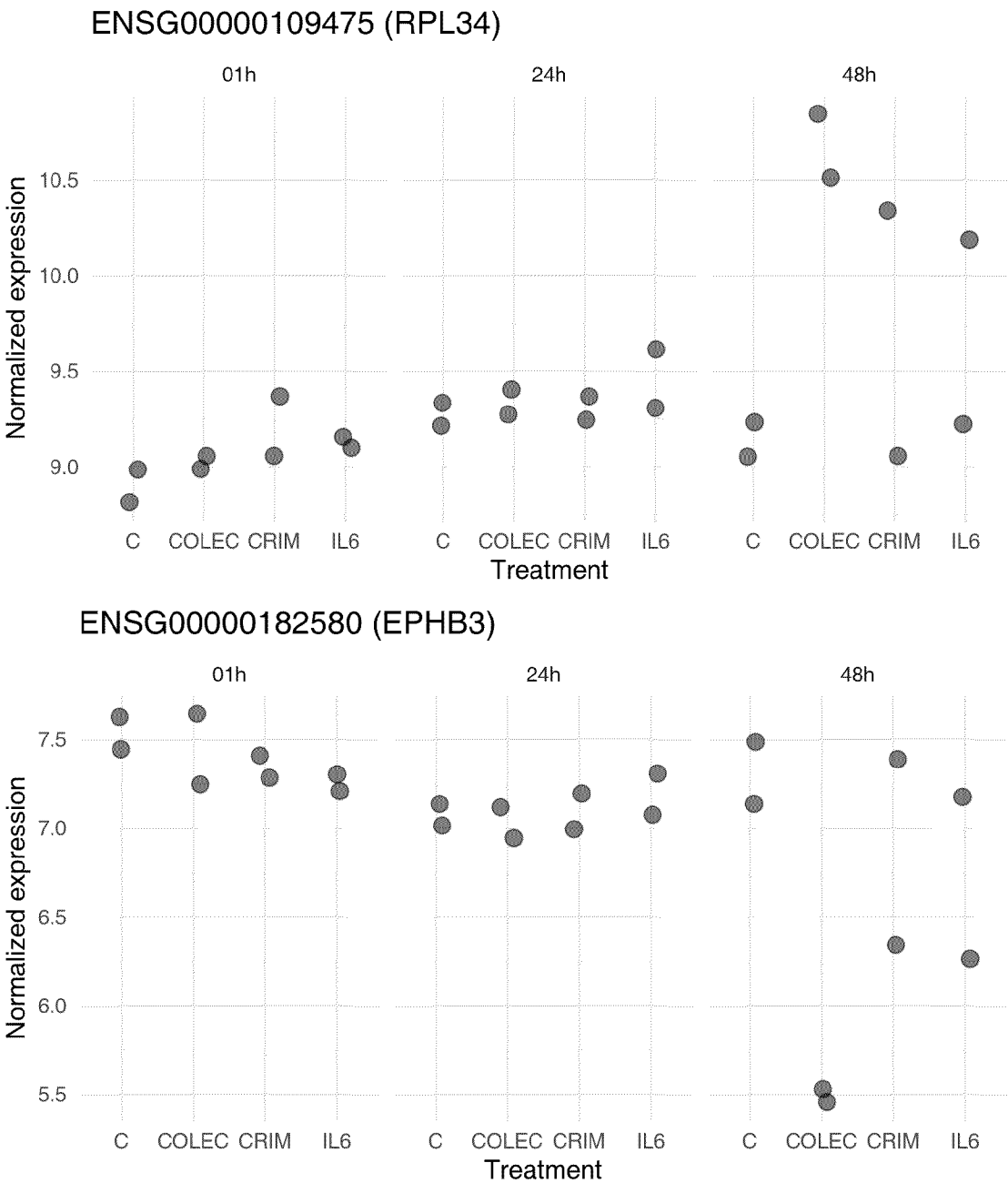


Fig. 11 continued

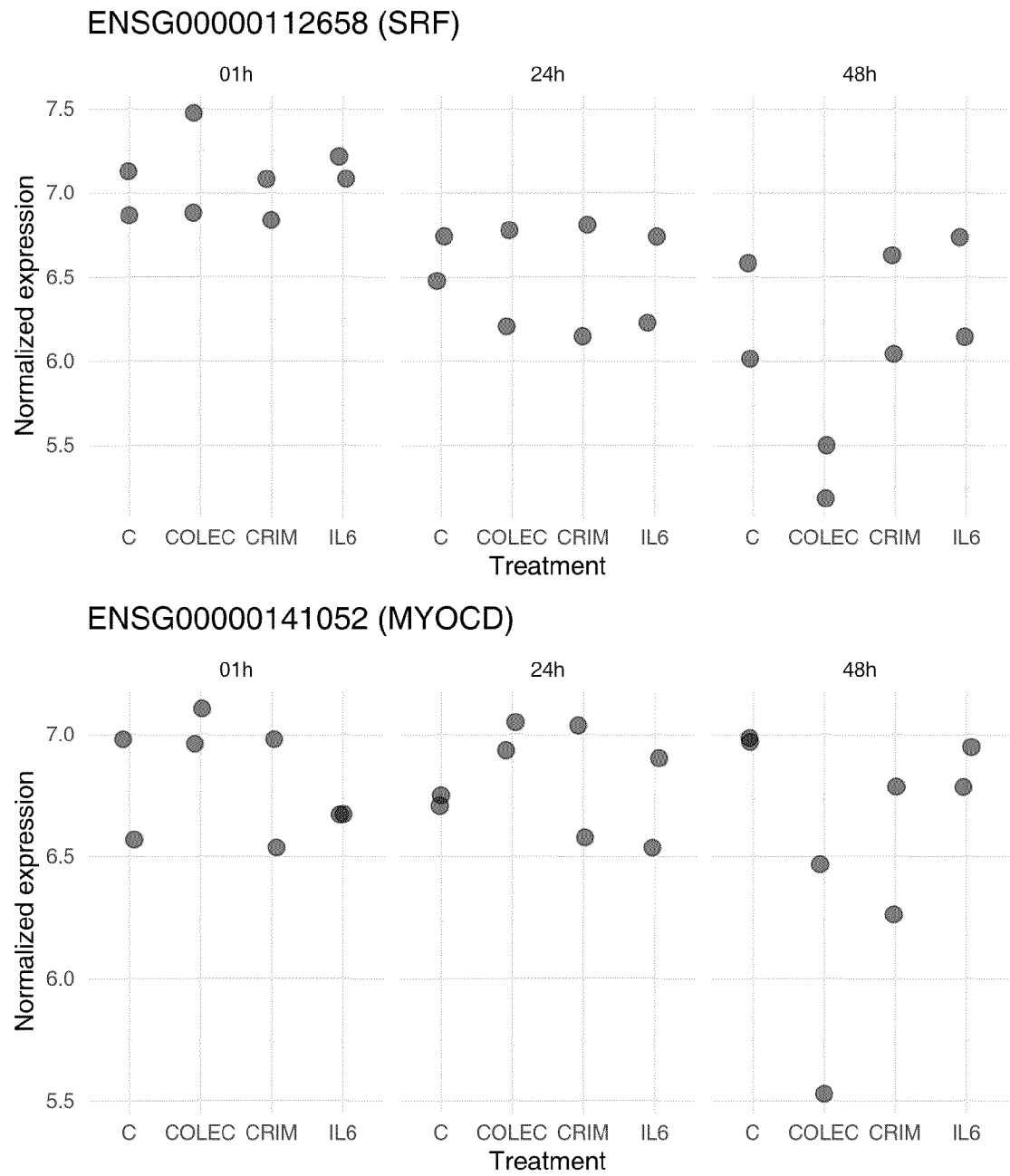


Fig. 12

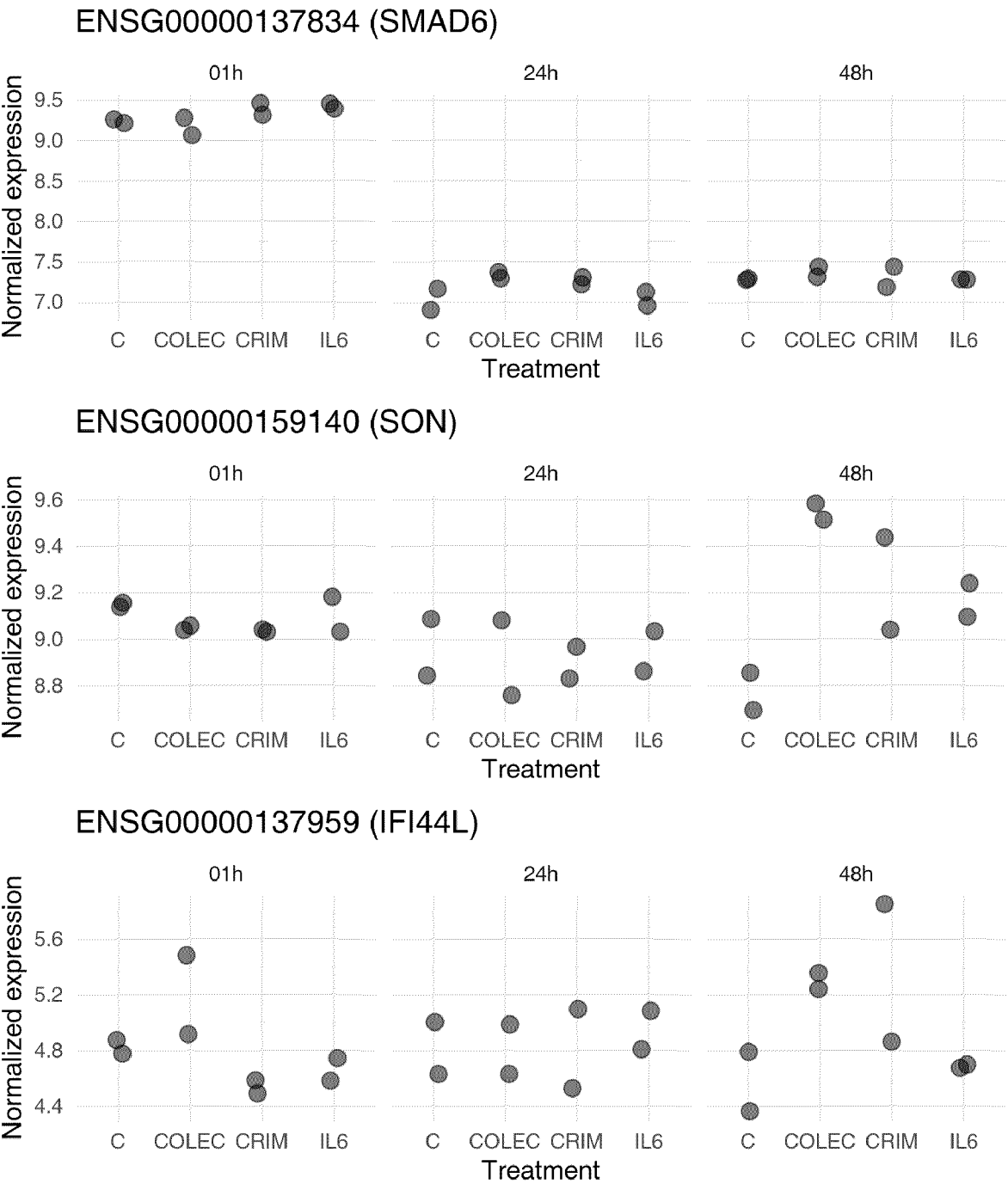


Fig. 12 cont.

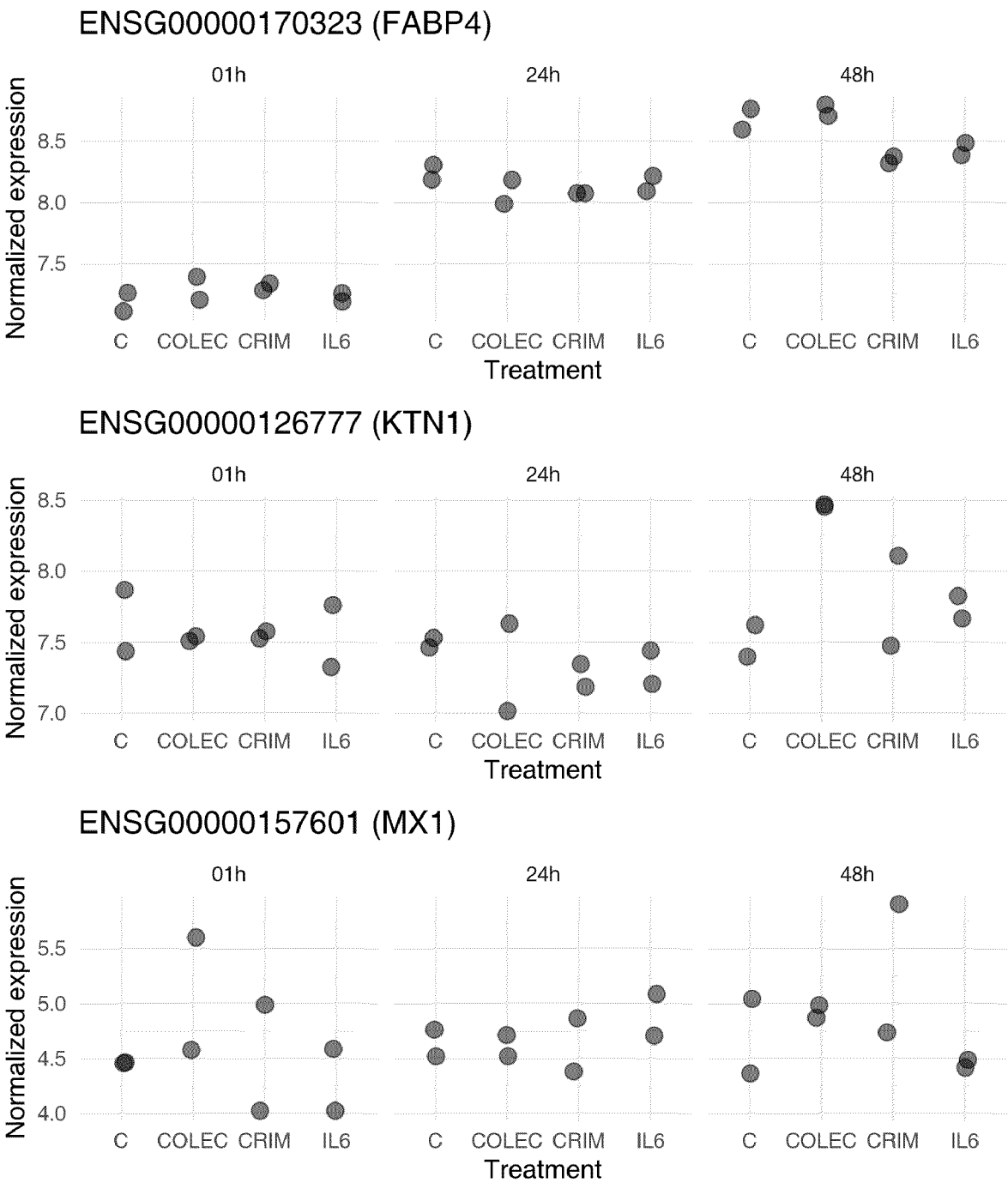
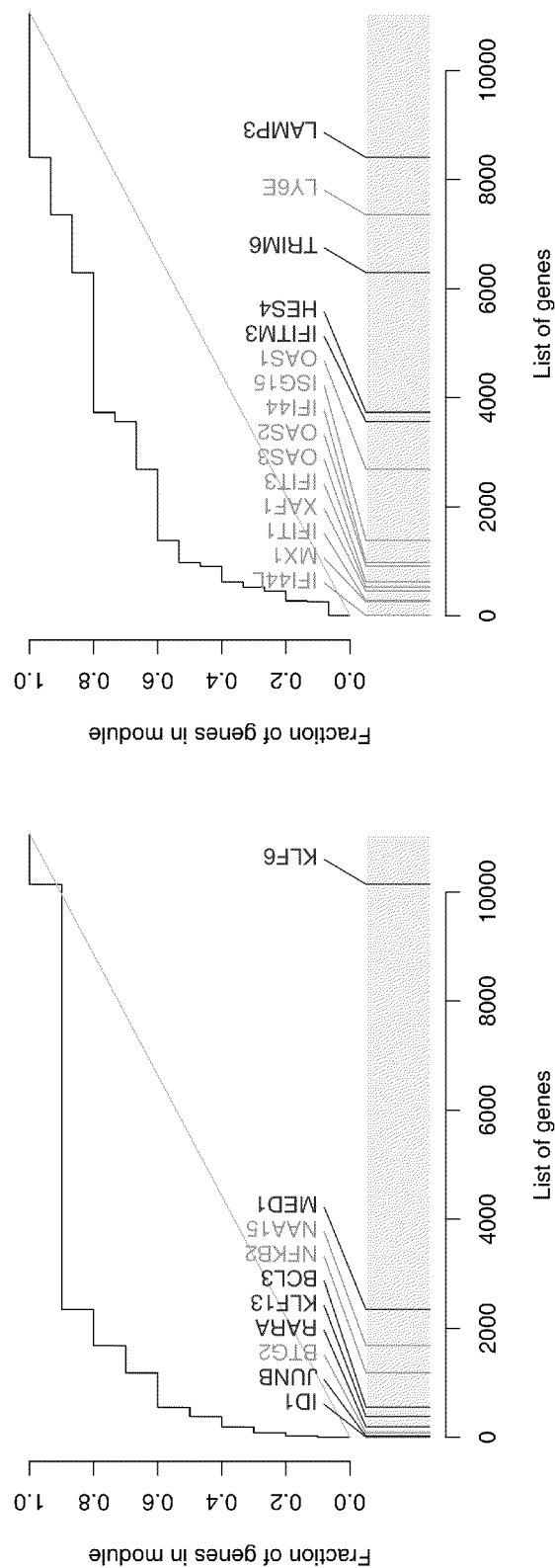


Fig. 13



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/071338

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	A61K31/519	A61K39/395
	A61P9/00	A61P37/02
		A61K45/06
ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
A61K C07K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GOYACK LAURA ET AL: "Rapid effect of benralizumab in fulminant eosinophilic myocarditis in the setting of uncontrolled eosinophilic asthma", JOURNAL OF CARDIOLOGY CASES, ELSEVIER, AMSTERDAM, NL, vol. 28, no. 3, 14 June 2023 (2023-06-14), pages 100-104, XP087388307, ISSN: 1878-5409, DOI: 10.1016/J.JCCASE.2023.04.012 [retrieved on 2023-06-14]	1-6,9-12
Y	pages 4-5 ----- -/-	1-12
☒ Further documents are listed in the continuation of Box C.		
☒ See patent family annex.		
* Special categories of cited documents :		
"A" document defining the general state of the art which is not considered to be of particular relevance		
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"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art		
"&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
4 October 2024		11/10/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Estañol, Inma

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/071338

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	CHEN DONG-YI ET AL: "Cardiovascular toxicity of immune checkpoint inhibitors in cancer patients: A review when cardiology meets immuno-oncology", JOURNAL OF THE FORMOSAN MEDICAL ASSOCIATION, EXCERPTA MEDICA ASIA, HONG KONG, HK, vol. 119, no. 10, 20 August 2019 (2019-08-20), pages 1461-1475, XP086266408, ISSN: 0929-6646, DOI: 10.1016/J.JFMA.2019.07.025 [retrieved on 2019-08-20] Page 1463, Table 2 -----	1-12
X	WO 2022/023490 A1 (HOPITAUX PARIS ASSIST PUBLIQUE [FR]; UNIV SORBONNE [FR] ET AL.) 3 February 2022 (2022-02-03) -----	1,2,4,5, 10-12
Y	example 1 -----	1-12
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; 11 August 2020 (2020-08-11), LIU YUN ET AL: "Tofacitinib for treatment in immune-mediated myocarditis: The first reported cases.", XP002812247, Database accession no. NLM32781887 -----	1-6, 10-12
Y	abstract & LIU YUN ET AL: "Tofacitinib for treatment in immune-mediated myocarditis: The first reported cases.", JOURNAL OF ONCOLOGY PHARMACY PRACTICE : OFFICIAL PUBLICATION OF THE INTERNATIONAL SOCIETY OF ONCOLOGY PHARMACY PRACTITIONERS 11 AUG 2020, 11 August 2020 (2020-08-11), page 1078155220947141, ISSN: 1477-092X ----- -/-	1-12

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/071338

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SINGH VIJAY PRATAP ET AL: "FULMINANT MYOCARDITIS AND MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN, IN THE LIGHT OF CORONA VIRUS", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 77, no. 18, 3 May 2021 (2021-05-03), page 1964, XP086563864, ISSN: 0735-1097, DOI: 10.1016/S0735-1097(21)03320-9 [retrieved on 2021-05-03]	1 - 6, 10 - 12
Y	the whole document -----	1 - 12

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Information on patent family members

International application No
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Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			CA	3190136	A1	03-02-2022
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			KR	20230047130	A	06-04-2023
			US	2023293531	A1	21-09-2023
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