- Research Article -

Improved Outcome up to Ten Years after Intravenous Immunoglobulin Therapy in Patients with Dilated Cardiomyopathy

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Abstract (246/250)

Background and aims: A previous trial of intravenous immunoglobulin therapy (IVIg) added to guideline-directed medical therapy (GDMT) in patients with dilated cardiomyopathy (DCM) and cardiac parvovirus-B19 (B19V) persistence showed no improvement of cardiac function at six months follow-up. We investigated whether IVIg confers long-term clinical benefits and analyzed its molecular and cellular effects in cardiac tissue to elucidate the immunomodulatory mechanisms underlying the observed long-term clinical improvement.

Methods: Fifty patients with DCM and cardiac B19V were blindly randomized to receive either IVIg (n=26; 2g/kg/day over 4 days) or placebo (n=24). The composite clinical endpoint–cardiac death, heart failure hospitalization, and life-threatening arrhythmia–was assessed after a median follow-up of 6.8 [5.6-10.3] years. To investigate IVIg-associated molecular changes in the heart, single-nuclear RNA sequencing (snRNA-seq) sequencing on endomyocardial biopsies (EMB) obtained at baseline (prior to treatment) and at six months after randomization was performed.

Results: The IVIg group experienced fewer composite endpoint events (n=1/26) than the placebo group (8/24; p=0.0075). Comparison of baseline and six-month EMBs by snRNA-seq showed that IVIg treatment reduced cardiac monocyte infiltration and, unlike placebo, prevented the expansion of injured cardiomyocytes. IVIg induced transcriptomic reprogramming across immune cells, fibroblasts, and cardiomyocytes, including diminished myeloid-to-fibroblast signaling with reduced TGFβ activity, decreased pro-fibrotic and pro-inflammatory pathways and metabolic shifts in cardiomyocytes characterized by enhanced oxidative phosphorylation.

Conclusion: In patients with DCM and cardiac B19V persistence, IVIg added to GDMT correlated with improved long-term clinical outcome. IVIg may attenuate myeloid-driven fibrosis formation and mitigate cardiomyocyte deterioration.

INTRODUCTION

Dilated cardiomyopathy (DCM) is characterized by left ventricular dilatation and systolic dysfunction after the exclusion of coronary artery disease and abnormal loading conditions(1). The etiology of DCM is multifactorial, involving a complex interplay between genetic predisposition, environmental factors and immune dysregulation(2). Cardiac inflammation is observed in 15-20% of patients with DCM (3-5), and cardiac parvovirus B19 is detected in up to 80% of DCM cases(6). Despite these findings, no established immunomodulatory or anti-viral therapy for DCM exists so far.

We previously conducted a double-blind, randomized, placebo-controlled trial (RCT) evaluating IVIg on top of guideline-directed medical therapy in patients with DCM and B19V persistence. The trial did not demonstrate a difference in left ventricular ejection fraction (LVEF) - the primary outcome - between IVIg and placebo-treated groups at six months after treatment(7). However, a previous study suggested that IVIg may exert long-term virus-independent immunomodulatory effects such as inhibition of monocyte recruitment(8-10).

Here, we assess the long-term effects of IVIg on the clinical outcome of patients with DCM and B19V persistence as a prespecified analysis. We also studied virus-independent immunomodulatory mechanisms of IVIg using single nucleus RNA (snRNA) sequencing of endomyocardial biopsies (EMB) in an exploratory analysis.

METHODS

Study design and participants

This study was a post-hoc analysis of a previous single-center, prospective, double-blind, randomized, placebo-controlled clinical trial with patients included between the years 2009 and 2018 (NCT00892112 at ClinicalTrials.gov). Adults with idiopathic chronic (>6 months) dilated cardiomyopathy (DCM), left ventricular ejection fraction (LVEF) <45%, and cardiac parvovirus B19 (B19V) persistence—defined as an EMB B19V load >200 copies/µg DNA—were eligible. Participants were on optimal medical therapy for heart failure for at least 6 months before enrolment. The study was conducted in accordance with the declaration of Helsinki and approved by the institutional Medical Ethics Committee. Written informed consent was obtained from all participants.

Randomization and blinding of original study

Fifty patients (39 men; mean age 54 ± 11 years) were randomly assigned in a blinded manner to receive either intravenous immunoglobulin (IVIg, n=26) or placebo (n=24). Randomization followed the minimization method to balance age, gender, baseline LVEF, left ventricular dimensions, and EMB B19V load between groups. Both participants and investigators remained blinded throughout the trial. Study drugs were prepared by study pharmacists according to computer-generated allocation.

Cross-over

One patient crossed over from placebo to IVIg. This participant was excluded from the snRNA sequencing analyses, experienced no study endpoints, and died from cancer 11 years after baseline.

Intervention and original endpoints

Patients in the IVIg group received a total dose of 2 g/kg Nanogam (Sanquin, Prothya Biosolutions, 50 mg/mL) over four days (0.5 g/kg daily, infused over 6 h each day). The placebo group received a plasma volume expander (Albuman 40 g/L, Sanquin, Prothya Biosolutions,) with the same administration schedule and volume as active treatment to mimic the protein load. The prespecified endpoint was change in left ventricle ejection fraction at six months. All adverse events and serious adverse events were recorded from enrollment to the to completion of the study at six months.

Follow-up

The duration of follow-up was a maximum of ten years, during which all patients remained on standard heart failure therapy. The median follow-up duration was 6.8 [IQR 5.6-10.3] years. Long-term follow-up was assessed by comparison of the combined endpoint of heart failure hospitalizations (HFH), life-threatening arrhythmia (LTA), and cardiac death between treatment groups. LTA is defined as non-fatal ventricular fibrillation with or without intracardiac defibrillator shock, sustained ventricular tachycardia with appropriate intracardiac defibrillator shock, or ventricular tachycardia with hemodynamic instability. Cardiac death is defined as death due to heart failure, fatal arrhythmias or sudden cardiac death, or due to other cardiovascular causes.

Endpoint assessments were independently reviewed by two independent investigators who were blinded to treatment allocation.

A comprehensive description of snRNA sequencing and statistical analyses is available in **Suppl. Methods**.

RESULTS

Baseline characteristics

For an overview of baseline characteristics of the original study, we refer to **Table 1.** In brief, the clinical characteristics and HF medication did not significantly differ in both placebo and treatment groups at baseline, six months and at last follow-up (median 6.8 [5.6-10.3] years). Male sex predominated (78%) and mean LVEF was 35 ± 9% at the time of inclusion. There were no differences in the presence of cardiotropic viruses other than parvovirus B19 (Suppl. Table 1).

Long-term follow-up

Over the median follow-up duration of 6.8 [5.6-10.3] years, IVIg-treated patients had a significantly reduced risk of reaching the combined endpoint compared with those receiving placebo (IVIg n=1/26 *versus* placebo n=7/24, Log-rank p=0.0075, IVIg vs. placebo, **Figure 1a,b**.) Details regarding each endpoint can be found in **Suppl. Table 1**. In brief, the endpoint was reached due to a HFH in three patients (n=1/26 *versus* n=2/24), a LTA in two patients (n=0/26 *versus* n=2/24), cardiac death in two patients (n=0/26 *versus* n=2/24), and a combination of cardiac death with a LTA in one patient (n=0/26 *versus* n=1/24). Although LVEF tended to improve over time, it did not differ significantly between treatment groups at last follow-up (median 6.8 [5.6-10.3] years; LVEF in the IVIg-group *versus* placebo-group: 46% [39-53] versus 39% [34-46], p=0.12).

IVIg was associated with improved outcome independent of baseline B19V load (interaction term p=0.35, **Suppl. Table 2**). For more information regarding B19V load characteristics, we refer to the **Suppl. Table 3**.

Cardiac transcriptomic profile at 6 months after treatment.

Cardiac single nuclear RNA profiles from EMBs collected at baseline and six months (i.e., median 6.4 [5.9-7.3] months) after treatment were analyzed in 21 patients (n=10 IVIg, n=11 placebo) of whom we had spare biopsies of both time points (yielding a total of 42 snRNA samples, **Figure 1b**). Clinical characteristics and medication did not significantly differ between patients with and without available spare biopsies at baseline, six months and at last follow-up (**Suppl. Table 4**). After stringent filtering for high quality data (**Suppl. Figure 1**), we annotated 116362 nuclei based on marker gene expression in reference to the latest single cell literature of the human heart (11-13). All major cardiac cell types were detected including cardiomyocytes, endothelial cells, fibroblast, pericytes, vascular smooth muscle cells, myeloid cells, lymphoid cells, neuronal cells and adipocytes (**Figure 2a,b)**. Each major cell type was further divided into previously described subpopulations (**Suppl. Figure 2a**).

To assess whether IVIg may cause transcriptomic alterations in cardiac tissue at six months post-treatment, principal component analysis (PCA) was performed. For this, the snRNA data from each sample was concatenated into so called pseudobulk data. PCA revealed that the first principal component clearly separated IVIg samples from baseline and placebo samples (**Figure 2c**), suggesting a clear molecular effect of IVIg on the heart at six months. PCA was performed after regressing confounders such as donor-effects and the partial overrepresentation of endocardial endothelial cells.

Next, we compared the cell type composition of the EMBs across treatment groups and time points based on major cell type annotations (**Figure 2d**). The number of monocytes was

significantly lower in EMB from IVIg-treated patients compared to the placebo group at 6 months. Expression of FC-receptors – well-established targets of IVIg therapy and modulating the anti-inflammatory properties of monocytes (14-16) – was predominantly detected within this immune cell population in our snRNA dataset (**Figure 2e, Suppl. Figure 2b**).

Despite these compositional changes, differential gene expression analysis within the myeloid cells did not reveal significant differences after correction for multiple testing. To explore potential functional changes of the cardiac myeloid cells, we performed gene set enrichment analysis based on t-value ranking of differential expression results, as well as gene-set-centric differential expression analysis. For the latter approach, we calculated gene set expression scores for pathways closely related to immune cell function and computed log fold changes (logFC) between paired placebo and IVIg samples. Significant alterations in genes associated with *hematopoietic cell lineage commitment*, *cytokine response*, *toll-like receptor signal pathway* and *cytokine mediated signaling pathways* (**Figure 2f**) were present when statistically testing these logFCs. Furthermore, changes in metabolism related gene sets in cardiac myeloid cells following IVIg treatment were found significantly enriched. Specifically, *glycolysis/glucogenesis* and *Insulin signaling* pathway related genes were downregulated, whereas genes involved in *valine, leucine and isoleucine degradation* were upregulated (**Suppl. Figure 2c-e**).

In conclusion, our single nuclear RNA sequencing analysis detected all major cardiac cell types and showed that IVIg treatment was linked to reduced cardiac monocytes, transcriptomic alterations in myeloid cells, and pathway-level changes in immune signaling and metabolism.

IVIg treatment alters cardiac cell-cell communication

We further examined changes in cell-cell communication associated with IVIg treatment. To this end, we used the framework of MultiNicheNet(17), which allows to investigate differences in cell-cell communication between two conditions based on differentially expressed genes. This method predicts the activity of ligand-receptor interactions by integrating the expression of the ligand, receptor and most importantly, the downstream target genes of the ligand. Comparison of ligand-receptor interactions for the most abundant cardiac cell types revealed overall higher sender and receiver scores in the placebo group, except for cardiomyocytes and mural cells, which exhibited higher receiver scores in IVIg-treated hearts (**Figure 3a,b**). Notably, fibroblasts showed strong sender and receiver scores, while endothelial cells were primarily enriched as receiver, and mural cells as senders (**Figure 3b**). In the IVIg samples, cardiomyocytes emerged as predominant receivers of key signaling interactions, including Ephrin A1(EFNA1) to EPH Receptor B1 (EPHB1) signaling from endothelial cells. EPHB1-mediated signaling is important for cardiomyocyte maintenance and disrupted signaling has recently been shown to induce cardiomyocyte stress and hypertrophy(18). Preserving EFNA1-EPHB1 signaling might contribute to the beneficial long-term effects of IVIg. However, the most striking findings was the presence of multiple TGFβ-related interactions exclusively in the placebo group, with endothelial and myeloid cells as key signal sender (TGFβ1- Amyloid Beta Precursor Protein (APP), -Integrin Subunit Beta 8 (ITGB8), -Plexin A4 (PLXNA4), -TGF Beta Receptor 2 (TGFBR2)).

To further explore this observation, we performed signal pathway activation predictions for 14 major pathways using the PROGENy curated database(19). A significantly lower signaling activity of TGFβ in fibroblast from IVIg-treated hearts was observed (**Figure 3c**). Given the central role of TGFβ in immune cell activation and fibroblast-mediated extracellular matrix (ECM) remodeling,

its downregulation might contribute to the long-term benefits of IVIg by limiting fibrotic and pro-inflammatory processes. We therefore specifically compared gene set expression changes for extracellular matrix related genes in fibroblast. Here we observed differences in collagen gene expression (**Suppl. Figure 2e**). An upregulation of *ECM glycoproteins*, *proteoglycans* and *ECM regulatory* genes was present in the placebo group. Inversely the same gene sets tended to shift toward lower expression in fibroblasts after the IVIg treatment. Especially, the *ECM regulatory* gene expression was significantly different between the two groups, further supporting the hypothesis that IVIg alters fibroblast function and cell-cell communication within the cardiac microenvironment.

In conclusion, IVIg treatment was associated with altered cardiac cell–cell communication, with reduced TGFβ-related interactions, enhanced cardiomyocyte-directed signaling, and transcriptional changes in fibroblasts suggestive of decreased pro-fibrotic and pro-inflammatory activity.

IVIg prevents cardiomyocyte deterioration

While overall compositional and cell-cell communication analysis indicate that myeloid cells and fibroblasts are the cell types most affected by IVIg treatment, we also investigated the transcriptional subtypes of cardiomyocytes and a potential impact of the treatment on cardiomyocyte subpopulations. Clustering of cardiomyocytes into different substates is challenging, as the extent to which transcriptional heterogeneity of cardiomyocytes reflects functionally different populations is still unclear. Given that most cardiac snRNA-seq studies define five substates(11-13), we selected our clustering resolution accordingly (**Figure 4a and**

Suppl. Figure 3a,b). Interestingly, while comparing the transcriptomic profiles of the five subpopulations in our snRNA data to profiles defined in independent snRNA-seq studies of HF patients, we found a strong overlap for CM3 with cardiomyocytes annotated as stressed in the public data (**Figure 4b**). Of note, cardiomyocytes vCM3.0 and vCM3.1 from Reichart et al. (11) were not annotated as stressed, however both populations were highly enriched in samples from patients with end stage DCM. In contrast, CM1 demonstrated high similarity to vCM2-Reichart, vCM1-Kuppe, and vCM2-Kanemaru, which can be rather defined as homeostatic or healthy cardiomyocytes compared to the stressed state. As reported in our previous study(7), both treatment groups had a similar improvement in systolic function at six months follow-up. In line with this finding, conventional composition analysis of cardiomyocyte subpopulations did not reveal significant differences between groups (**Suppl. Figure 3c**). However, when assessing the relative changes in cardiomyocyte subtypes within paired patient samples using logFC analysis, a decrease in non-stressed CM1 abundance in the placebo group was observed, whereas stressed CM3 exhibited an increasing trend (**Figure 4c**).

To further characterize potential transcriptomic differences in cardiomyocytes between the two treatment groups, we conducted differential expression analysis followed by gene set enrichment **(Suppl. Figure 3d-f**). Genes of the tricarboxylic acid (TCA) cycle were downregulated in IVIg-treated cardiomyocytes, while genes involved in oxidative phosphorylation were upregulated. In conclusion, IVIg treatment was associated with preservation of non-stressed cardiomyocytes, reduced expansion of stressed cardiomyocytes, and metabolic shifts characterized by downregulation of TCA cycle genes and upregulation of oxidative phosphorylation pathways.

DISCUSSION

This exploratory post-hoc analysis of our double-blind, randomized, placebo-controlled trial associates IVIg with better outcome in patients with DCM over a median follow-up duration of 6.8 [5.6-10.3] years. IVIg treatment was associated with a reduced cardiac myeloid-dependent fibroblast activation, and decreased cardiomyocyte suffering, as revealed by snRNA sequencing of leftover EMBs at baseline and six months.

IVIg associates with improved outcome at ten-year follow-up

One possible explanation for the better event-free survival observed in treated patients in this exploratory post-hoc analysis is the extended follow-up period of 6.8 [5.6-10.3] years. A longer cardiac follow-up enabled the detection of long-term treatment effects on prognosis, which may have been missed in shorter studies (5, 7). Testing the clinical benefit of IVIg therapy may have required a study with greater statistical power than previously attempted (5, 7), either by increasing sample size or extending the follow-up duration. Moreover, high-dosed IVIg therapy may have induced disease-remission by halting a progressive pathogenic immune response (20). In that context, the access to leftover biopsies at baseline and six months, enabled the detection of IVIg's immunomodulatory effects.

Importantly, the suggested beneficial effect of IVIg on event-free survival in DCM appears to be independent of B19V. More specifically, B19V load did not interact with IVIg in the outcome analysis and IVIg did not improve B19V clearance (7). These findings suggests that IVIg's effects are mediated through immunomodulatory pathways independent of B19V load.

snRNA analysis suggests immunomodulation with long lasting effects on fibroblast activation and cardiomyocyte adaptation

IVIg therapy compared to placebo was associated with a reduced cardiac myeloid-dependent fibroblast activation, preservation of non-stressed cardiomyocytes, and metabolic remodeling consistent with attenuated cardiomyocyte stress in EMB obtained at six months after randomization. One of the most prominent findings is a reduction in the cardiac monocyte population following IVIg treatment, in line with attenuating monocyte-driven inflammation upon beneficial IVIg treatment in Kawaski disease (21). Monocyte recruitment plays a central role in cardiac injury, driving inflammation through cytokine secretion, which in turn activates other immune cells and fibroblasts (22). In chronic inflammatory settings, this process can become a self-perpetuating loop, sustained by continuous cytokine signaling and progressive cardiac damage (23). Given that elevated glycolysis is a hallmark for inflammatory myeloid activation (24), the reduced expression of related genes after IVIg treatment suggests that IVIg may modulate immune cell metabolism, potentially reducing monocyte infiltration and promoting a less pro-inflammatory metabolic status. Taken together, our findings of a lower monocyte number, down regulated gene expression related cytokine response and glycolysis in myeloid cells after IVIg, point towards a therapeutic effect that breaks the detrimental pro-inflammatory circle.

Myeloid-derived TGFβ signaling is a central driver of this inflammatory cascade, promoting fibroblast activation and resulting in increased extracellular matrix production and altered matrix composition (25, 26). Our cell-cell communication analysis suggests that IVIg disrupts this signaling axis, potentially reducing chronic and detrimental fibroblast activation. Given that

persistent fibroblast activation contributes to maladaptive cardiac remodeling, the inhibition of this process through IVIg therapy may prevent long-term structural deterioration in the heart.

Beyond its effects on myeloid-fibroblast interactions, IVIg therapy appears to prevent a shift toward stressed/injured cardiomyocyte states and enhances cardiomyocyte metabolic efficiency. In the placebo group, cardiomyocyte subpopulations progressively shifted from a homeostatic state (CM1) to a stress-associated state (CM3) over at six months. IVIg-treatment protected against this shift, preserving a more favorable cardiomyocyte transcriptomic profile over time. Moreover, IVIg improved metabolic efficiency by upregulating oxidative phosphorylation and downregulating TCA cycle, which is particularly relevant given that metabolic remodeling is a hallmark of cardiac diseases (27). The observed metabolic shift in IVIg-treated hearts may indicate a beneficial adaptation favoring a more efficient oxidative metabolic state.

As the LVEF did not improve from baseline to six months (7) and tended -but not significantly- to improve at a median of 6.8 [5.6-10.3] years, these molecular findings suggest that functional recovery does not necessarily equate to molecular recovery. In line, recent snRNA-seq studies on cardiac recovery after left ventricular assist device (LVAD) implantation demonstrated that cardiomyocytes, despite functional recovery, had persistent transcriptional signatures of a failing heart (28). Moreover, fibroblast and macrophage transcriptional states correlated with recovery, while persistent inflammatory signatures were associated with incomplete cardiomyocyte restoration (28). In the context of our data, it is plausible that IVIg therapy modulates the cardiac microenvironment by reducing chronic inflammatory signaling and

fibroblast activation. This, in turn, may facilitate functional recovery while allowing cardiomyocytes to maintain a more favorable and adaptive transcriptional state.

Study strengths and limitations

The strengths of this study include its initial randomized placebo-controlled trial design, which minimizes bias, as well as the post-hoc extended 6.8 [5.6-10.3] years follow-up period. Additionally, the availability of biobank material before and six months after study intervention, including EMBs for snRNA-seq, provides valuable molecular insights into the effects of IVIg treatment on the heart.

The primary limitations of this study are the sample size, statistical uncertainty due to low event numbers, availability of EMBs for snRNA of a subset of the study group, and the post-hoc single center setting, which may limit the generalizability of the clinical findings. Larger, multi-center, randomized placebo-controlled trials are necessary to validate clinical efficacy of IVIg in DCM in B19V independent way. Furthermore, in our snRNA-seq analysis, the detected effect size of IVIg treatment was relatively small relative to patient-to-patient variability, making it challenging to achieve statistical significance for some molecular differences. SnRNA sample number and processing was limited by the availability and size of remaining leftover EMB, as they could be needed for clinical care (e.g. for extra pathology evaluation) or it was deemed unsafe to acquire extra leftover biopsies for biobanking. This highlights the need for larger transcriptomic datasets and complementary functional and mechanistic studies to confirm the observed trends.

CONCLUSION

This exploratory study suggests the addition of IVIg to GDMT in DCM patients -independent of cardiac B19V load- may contribute to an improved event-free survival. Molecular insights from snRNA data indicate that IVIg may attenuate myeloid-driven fibroblast activation and mitigate cardiomyocyte deterioration. These hypothesis-generating findings warrant further investigation on IVIg in DCM.

Conflict of interest

S.R.B.H. receives personal fees for independent scientific advice on early development in the field of heart failure from AstraZeneca and Ribocure and receives research support from AstraZeneca and CSL Behring.

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Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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	Placebo (*n* = 24)	IVIg (*n* = 26)	*P*	Placebo (*n* = 24)	IVIg (*n* = 26)	*P*	Placebo (*n* = 24)	IVIg (*n* = 26)	*P*
	Baseline			***Follow-up at six months***			***Last follow-up*** *6.8 [5.6-10.3] years*		
Age (years)	53±9	54±13	0.76	56±10	55±13	0.99	63±10	62±13	0.99
Female sex	5 (21)	6 (23)	1.00	5 (21)	6 (23)	1.00	5 (21)	6 (23)	1.00
Atrial fibrillation	10 (42)	5 (19)	0.08	12 (50)	6 (23)	0.08	13 (54)	8 (31)	0.15
Acute coronary syndrome	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00
PCI	2 (8)	1 (4)	0.60	2 (8)	1 (4)	0.60	2 (8)	1 (4)	0.60
CABG	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00
Valve repair or implantation	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00	1 (4)	1 (4)	1.00
(Likely) pathogenic mutation	2 (8)	6 (25)	0.13	2 (8)	6 (25)	0.13	2 (8)	6 (25)	0.13
Diabetes mellitus	2 (8)	3 (12)	1.00	2 (8)	3 (12)	1.00	4 (17)	3 (12)	0.70
Beta blocker	22 (92)	24 (92)	1.00	22 (92)	24 (92)	1.00	21 (88)	23 (89)	1.00
ACE-i/ARB/ARNI	23 (96)	25 (96)	1.00	23 (96)	25 (96)	1.00	19 (79)	19 (73)	0.75
MRA	13 (54)	8 (31)	0.15	14 (58)	8 (31)	0.09	15 (63)	17 (65)	1.00
SGLT-2i	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00	5 (22)	3 (12)	0.45
ICD +/- pacemaker	1 (4)	3 (12)	0.61	3 (13)	3 (12)	1.00	4 (17)	4 (15)	1.00
CRT-D	3 (13)	1 (4)	0.34	3 (13)	1 (4)	0.34	4 (17)	6 (23)	0.73
CRT-P	0 (0)	0 (0)	1.00	0 (0)	0 (0)	1.00	1 (0)	1 (0)	1.00

Table 1. Clinical characteristics at baseline and follow-up

1 *Continuous variables are presented as mean* ± standard deviation. Ordinal variables are presented as number (percentages).

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3

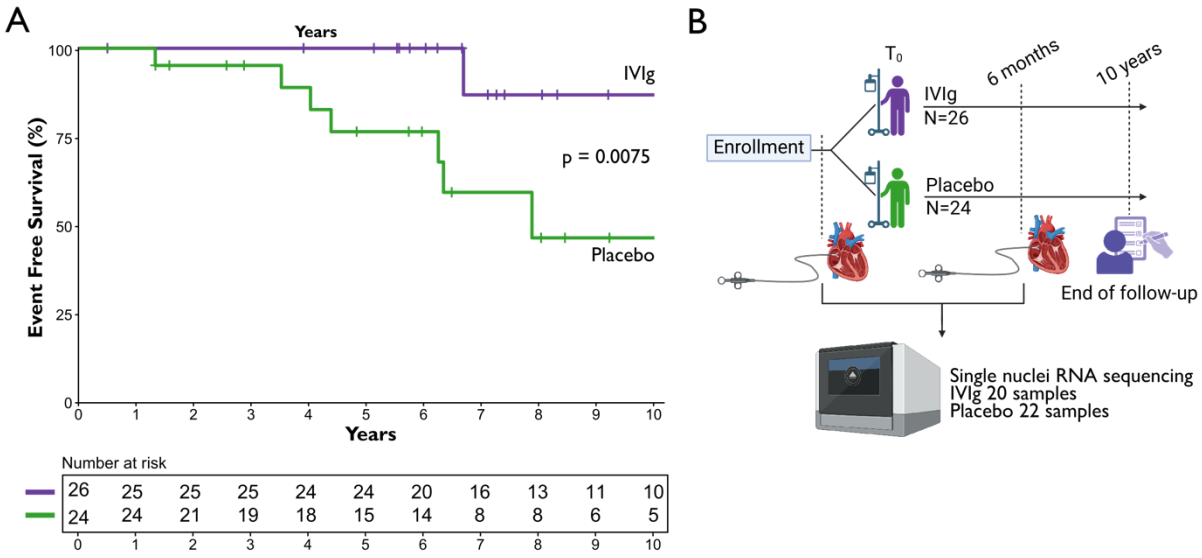


Figure 1: Improved outcome at ten-year follow-up after intravenous immunoglobulin therapy**.** (A) Ten-year follow-up of the combined endpoint of cardiac death, heart failure hospitalization or life-threatening arrhythmia shows improved outcome of patients randomized to intravenous immunoglobulin compared to placebo (Log-rank p=0.0075). (B) Schematic representation of the original study outline and endomyocardial biopsy collection during the study, which were processed for single nuclear sequencing in the present study. Abbreviations: HFH = heart failure hospitalization; LTA = life-threatening arrhythmia; IVIg = intravenous immunoglobulin therapy; GDMT = guideline-directed medical therapy.

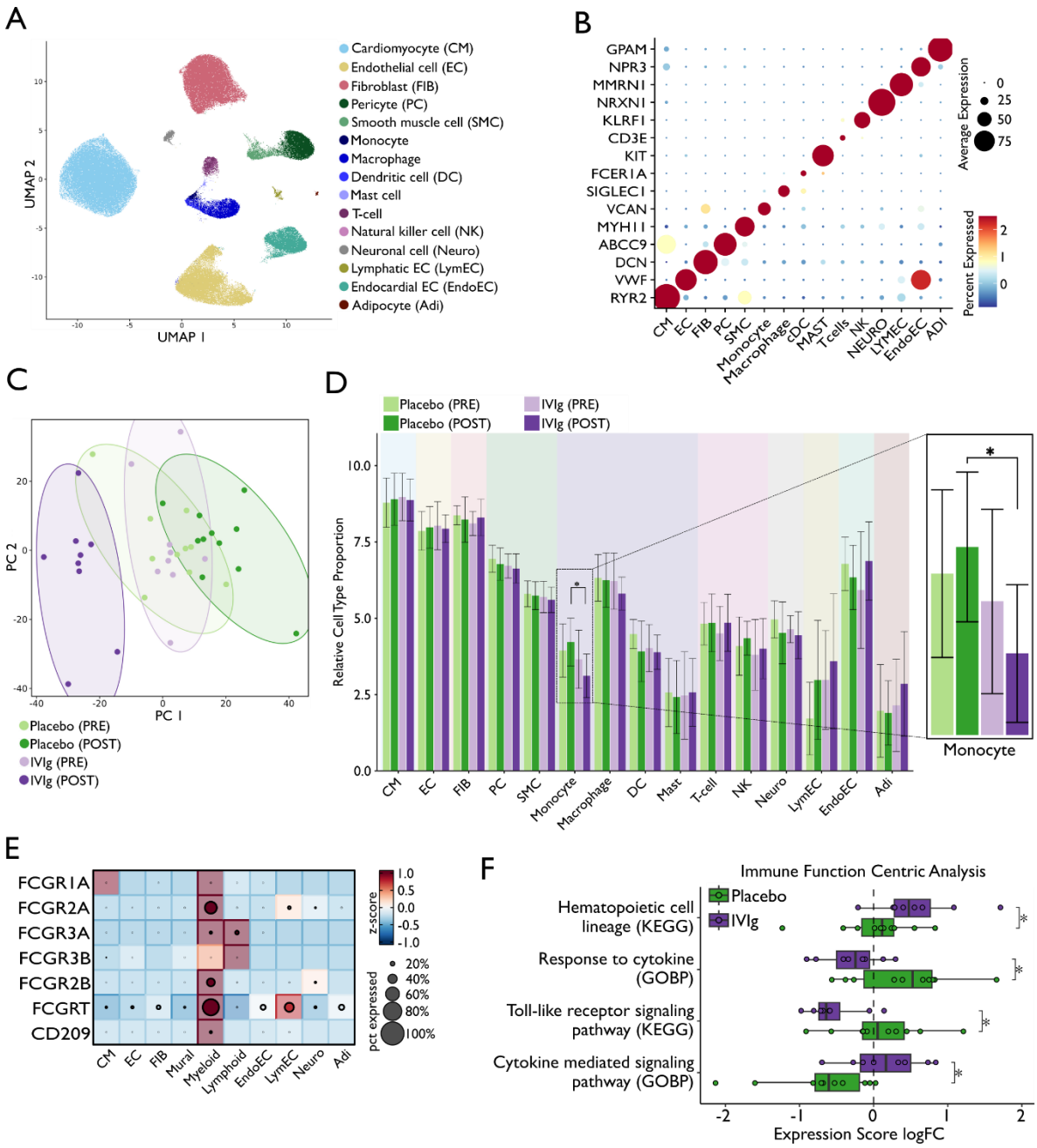


Figure 2: IVIg treatment induces cardiac changes in the transcription level. (A) UMAP representation of the filtered and integrated snRNA data from 39 endomyocardial biopsies with annotation of major cardiac cell types. (B) Dot plot of marker gene expression with established marker genes cell type. (C) Plot of principal component (PC) 1 and 2 after PC analysis based on pseudobulk data per sample, regressed for confounders. (D) Bar graph of endomyocardial biopsy

cell type composition analysis after normalization and logit transformation of proportions per sample. Magnification for monocyte abundance difference. Statistical differences were tested by fitting a linear model with Benjamini-Hochberg correction. Mean ± SD, * = p-value < 0.05. (E) Combined heatmap and dotplot of gene expression for FC-receptor genes per cell type in the snRNA data. (F) Immune function related gene set expression changes in myeloid cells. Log fold change (logFC) was calculated for paired biopsy samples per patient. Boxplots indicate median and interquartile range. Significance was tested with paired Wilcoxon test with Benjamini-Hochberg correction. * = p-value < 0.05. Abbreviations: CM = cardiomyocyte, EC = endothelial cell, FIB = fibroblast, PC = pericyte, SMC = smooth muscle cell, cDC = classic dendritic cell, NK = natural killer cell, NEURO = neuronal cell, LYMEC = lymphatic endothelial cell, EndoEC = endocardial endothelial cell, ADI = adipocyte.

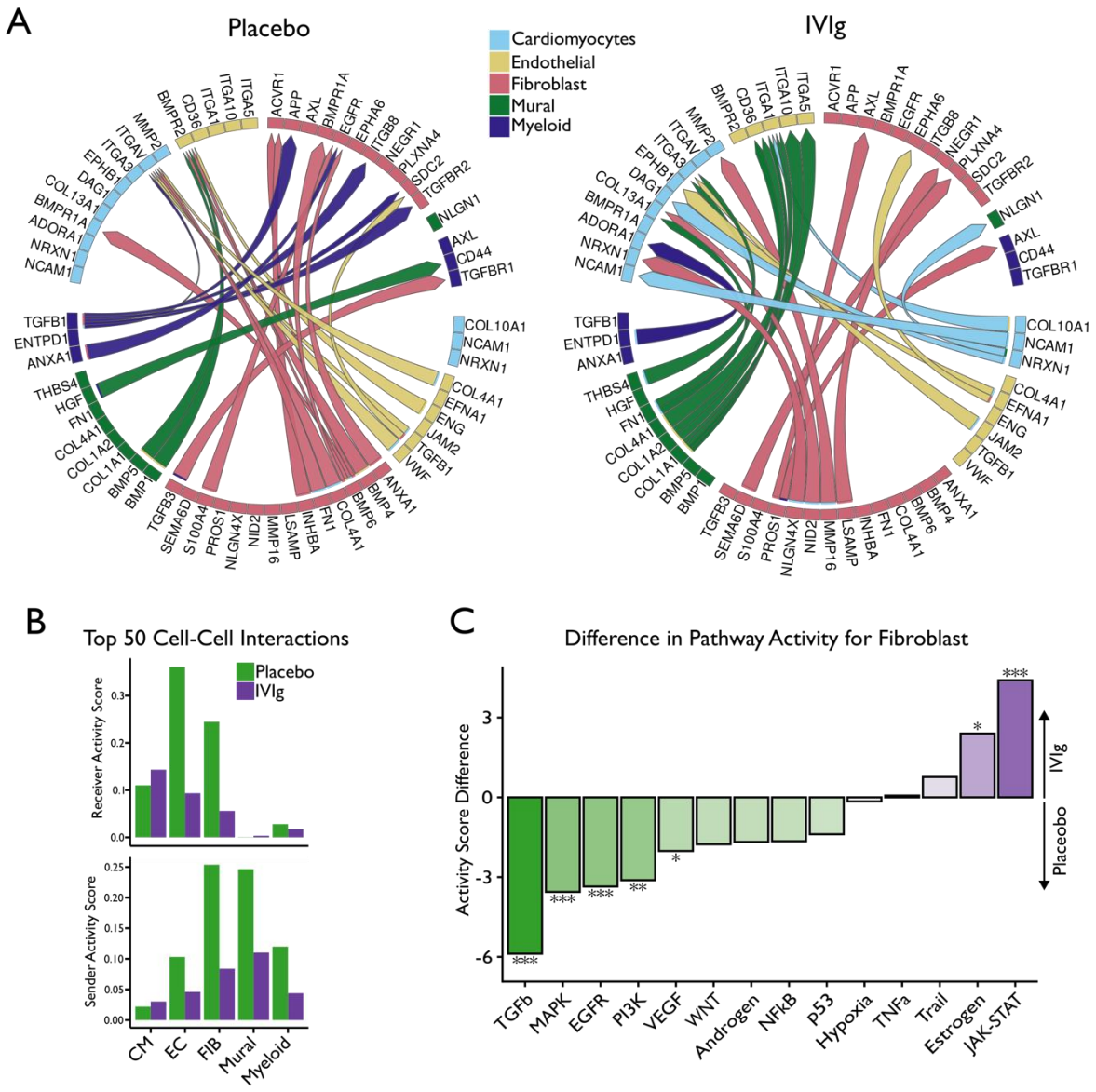


Figure 3: Changes in cell-cell communication of cardiac cells due to IVIg treatment. (A) Chord diagram the top 50 most differential ligand-receptor interactions between placebo and IVIg samples, activity scores predicted by MultiNichNet. Arrows indicate the direction of interaction with senders with the ligand in the lower half of the circle and receiver with the receptor in the upper half. (B) Summarized activity scores per cell type and condition for the top 50 sender and receiver interactions. (C) Differential signal pathway activity between placebo and IVIg in fibroblasts, predicted by a multivariate linear model with PROGENy as pathway reference as

implement in decoupleR. Significance is tested by the multivariate linear model. * = p-value < 0.05, ** = p-value < 0.01, ** = p-value < 0.001.

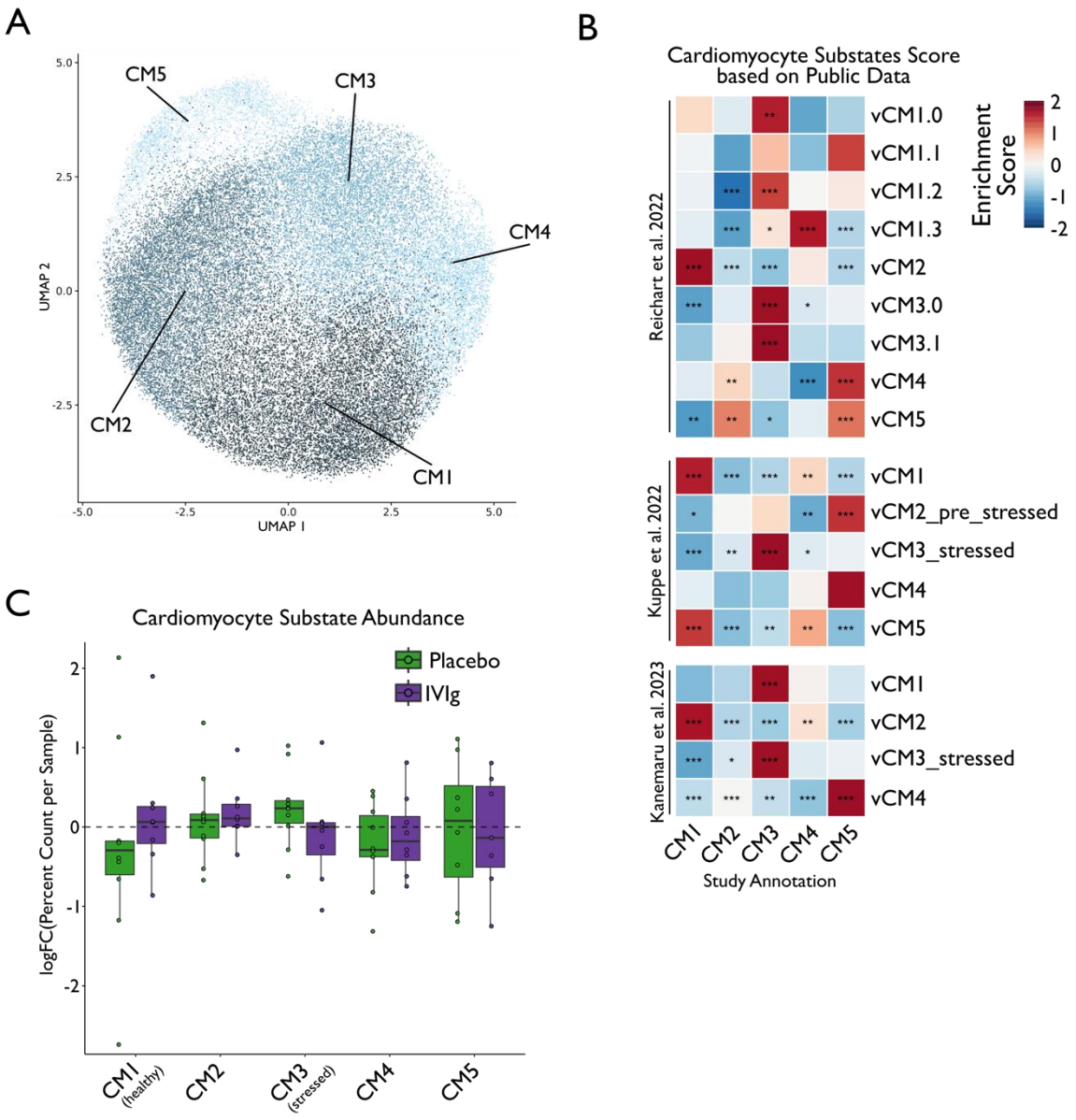


Figure 4: Cardiomyocyte subclustering and transcriptional shift. (A) UMAP representation of cardiomyocyte subclustering (n = 41779 nuclei). (B) Composition analysis of cardiomyocyte substates showing increase or decrease of the relative abundance between the two samples of one patient as log fold change (logFC). All logFC changes were grouped by the treatment conditions for comparison. No significance was found by a paired Wilcoxon test with Benjamini-Hochberg correction. (C) Cardiomyocyte substate signature expression of states described in

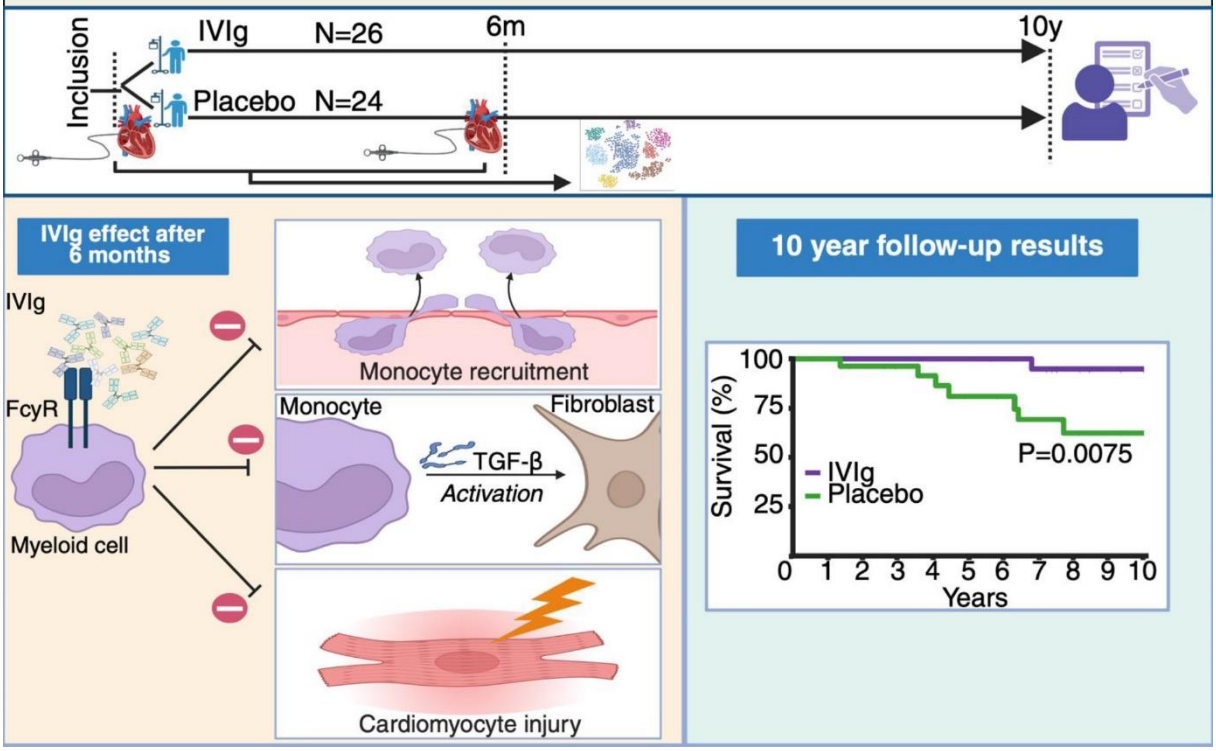
literature. The heatmap shows the expression scores of signature genes in the cardiomyocyte substate of the present study calculated based on marker gene differential expression as input for a multivariate linear model. Three different published studies were used as reference cells (11-13). Significance is tested by the multivariate linear model. * = p-value < 0.05, ** = p-value < 0.01, ** = p-value < 0.001.

The authors do hereby declare that all illustrations and figures in the manuscript are original and not require reprint permission.

Key question
Does intravenous immunoglobulin therapy (IVIg) have a beneficial immunomodulatory effect in dilated cardiomyopathy (DCM) ?

Key finding
IVIg improves long-term cardiac outcome characterized by reduced myeloid-dependent fibroblast activation and decreased cardiomyocyte suffering

Take-home message
This study demonstrates that IVIg therapy may offer clinical benefits for patients with DCM. A large clinical trial is warranted.



Graphical Abstract