OPEN ACCESS

TRANSLATIONAL SCIENCE

Combined therapy of prednisone and mTOR inhibitor sirolimus for treating retroperitoneal fibrosis

Hui Gao ,[1] Shibo Liu,[2] Yuanbang Mai,[3,4] Yuying Wang,[5] Xuewu Zhang,[6] Shufen Zheng,[3,4] Chenghua Luo,[2] Cuiping Pan [3]

Handling editor Josef S Smolen

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/ard-2022-223736).

[1]Department of Rheumatology and Immunology, Peking University International Hospital, Beijing, China
[2]Department of Retroperitoneal Tumor Surgery, Peking University International Hospital, Beijing, China
[3]Center for Intelligent Medicine Research, Greater Bay Area Institute of Precision Medicine (Guangzhou), Fudan University, Guangzhou, China
[4]School of Life Sciences, Fudan University, Shanghai, China
[5]Department of Pharmacy, Peking University International Hospital, Beijing, China
[6]Department of Rheumatology, Peking University People's Hospital, Beijing, China

Correspondence to
Dr Cuiping Pan, Greater Bay Area Institute of Precision Medicine (Guangzhou), Guangzhou, China; pancuiping@ipm-gba.org.cn and Chenghua Luo, Peking University International Hospital, Beijing, China; luochenghua@pkuih.edu.cn

HG and SL contributed equally.

Received 9 December 2022
Accepted 19 January 2023
Published Online First 31 January 2023

To cite: Gao H, Liu S, Mai Y, *et al*. *Ann Rheum Dis* 2023;**82**:688–697.

ABSTRACT

Objectives Retroperitoneal fibrosis (RPF) is a rare autoimmune disease with fibrous tissue growth and inflammation in retroperitoneum. Its current treatments involve long-term uptake of glucocorticoids (e.g., prednisone) for controlling inflammation; however, side effects are common. We strived for an improved therapy for fibrosis remission while reducing side effects.

Methods We surveyed gene-disease-drug databases and discovered that mammalian target of rapamycin (mTOR) was a key signalling protein in RPF and the mTOR inhibitor compound sirolimus affected many RPF pathways. We designed a therapy combining a gradual reduction of prednisone with a long-term, stable dosage of sirolimus. We then implemented a single-arm clinical trial and assessed the effects in eight RPF patients at 0, 12 and 48 weeks of treatment by measuring fibrous tissue mass by CT, markers of inflammation and kidney functions by lab tests, immune cell profiles by flow cytometry and plasma inflammatory proteins by Olink proteomics.

Results With the combined therapy, fibrous tissue shrunk about by half, markers of acute inflammation reduced by 70% and most patients with abnormal kidney functions had them restored to normal range. Molecularly, fibrosis-related T cell subsets, including T_H2, T_H17 and circulating T_{FH} cells, were reduced and tumour necrosis factor and related cytokines restored to healthy levels. No severe long-term side effects were observed.

Conclusions Our combined therapy resulted in significant fibrosis remission and an overall regression of the immune system towards healthy states, while achieving good tolerance. We concluded that this new therapy had the potential to replace the steroid monotherapy for treating RPF.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Side effects often accompany the standard glucocorticoids monotherapy for treating retroperitoneal fibrosis (RPF) and attempts to reduce its dosage resulted in frequent disease recurrence.

WHAT THIS STUDY ADDS

⇒ A pathology mechanism that mammalian target of rapamycin (mTOR) is highly activated in the fibrous tissues in RPF.
⇒ A promising new therapy for treating RPF with a gradual reduction of prednisone and a long-term stable dosage of the mTOR inhibitor sirolimus.
⇒ A translational research protocol from disease-gene-drug databases to key proteins in pathology and potential matching drugs as well as from laboratory tests to clinical trials.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The 'cocktail' strategy targeting both inflammation and pathological mechanisms is promising in replacing the standard glucocorticoids monotherapy for treating RPF.
⇒ Bioinformatics-assisted translational research provides precise targets for bedside trials, which may particularly benefit rare disease trials when resources are scarce.

INTRODUCTION

Retroperitoneal fibrosis (RPF), also called Ormond's disease, periureteritis fibrosa, periureteritis plastica, chronic periureteritis and fibrous retroperitonitis, is a rare autoimmune disease characterised by the presence of inflammatory and fibrous tissue in the retroperitoneum. Its prevalence is estimated at 1.4 cases/100 000 inhabitants and incidence at 0.1–1.3 cases/100 000 persons per year.[1] Typical symptoms of RPF include pain in the lower back and abdomen, weight loss, fever, nausea and anaemia. Growth of the fibrous tissue can encase surrounding organs, causing inflammatory abdominal aortic aneurysm and ureteral obstruction.[2] Importantly, ureteral obstruction occurs in about 60%–80% of cases and often causes chronic kidney disease, end-stage renal diseases and kidney atrophy.[3]

More than 70% of the RPF cases developed with no known aetiology, hence idiopathic.[1 3] It can also result from infections, malignancy, drugs, retroperitoneal haemorrhage or various other disorders, termed secondary RPF. Some RPF patients have elevated IgG4 levels, multiorgan inflammation and fibrosis, therefore belonging to IgG4-related diseases (IgG4-RD), whereas others have normal IgG4 levels.[4] Risk factors of RPF include genetic predisposition, for example, the HLA-DRB1*03 allele,[5] and environmental exposures including asbestos and smoking.[6]

Biological mechanisms of RPF have begun to emerge,[3] with abundant $CD4^+$ T cells and B cells recruited in the lesion, releasing interleukins and other cytokines and causing fibroblasts to differentiate into myofibroblasts and produce massive amounts of collagen. Many of these reactions

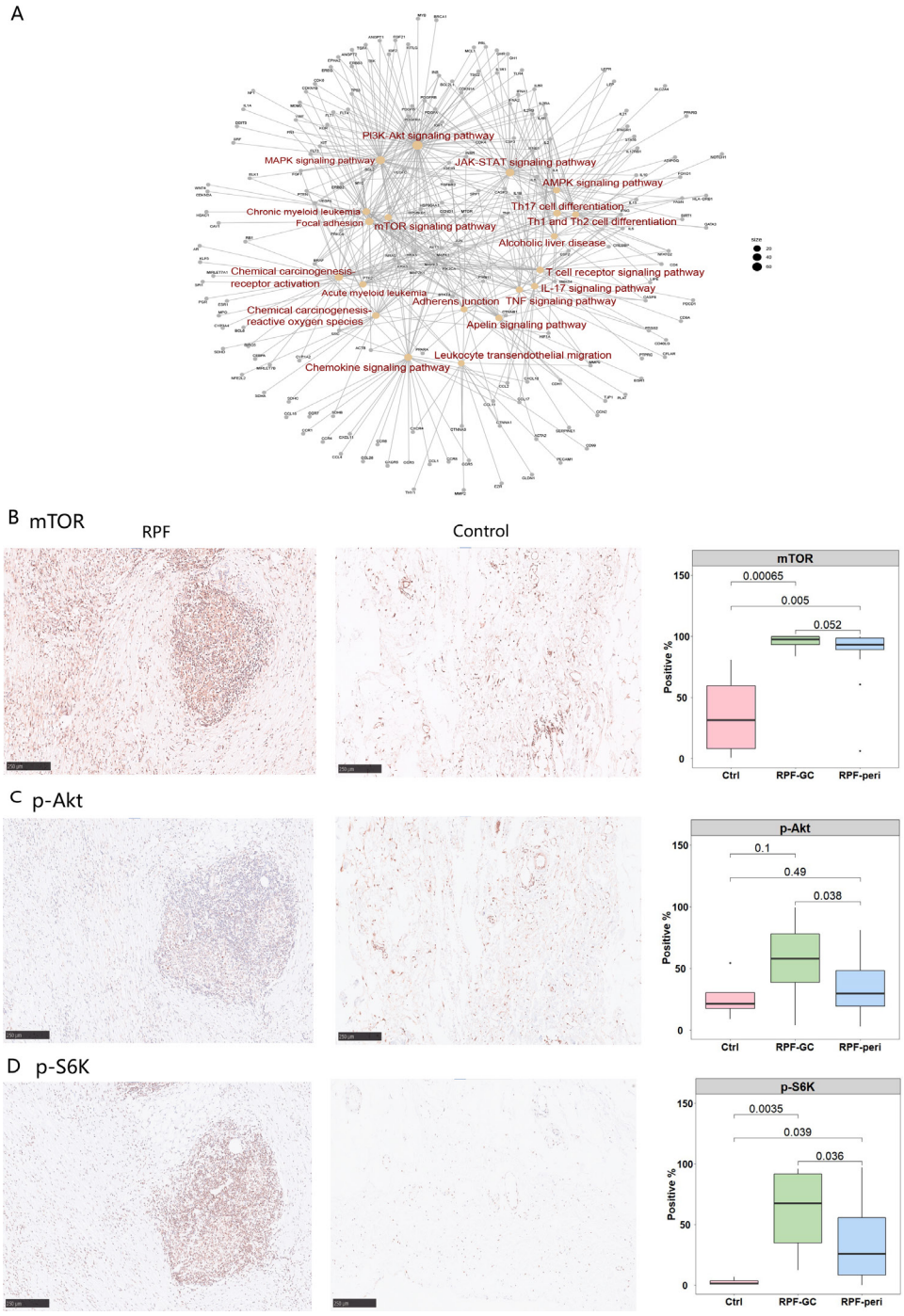


Figure 1 The PI3K-AKT-mTOR pathway was highly activated in RPF tissues. (A) Signalling pathway network enriched in genes related to RPF. (B–D) Immunohistochemical staining of mTOR and its downstream signalling proteins, phospho-Akt and phospho-S6K, with the boxplots on the right displaying the percentages of positive staining in 16 patients for RPF tissues (RPF-GC and RPF-peri) and 4 controls (Ctrl). mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3-kinase; RPF-GC: retroperitoneal fibrosis geminal centre; RPF-peri: RPF peripheral; Ctrl: healthy control.

resemble other autoimmune conditions that involve fibrosis, such as systemic sclerosis (SSc) and interstitial lung disease.[7 8]

While glucocorticoids have been established in the last decade as a standard medication to treat idiopathic RPF,[9] side effects are often observed, especially when used for extended periods.[10] In clinical practices, prednisone is prescribed at a dosage of 0.6–1 mg/kg/day for patients without contraindications, and then tapered to a minimal dosage (generally equivalent to less than 7.5 mg/day) or stopped. However, reducing dosages triggers frequent disease recurrence varying from 17.6% to 72%.[3 11–14] An effective and safe therapy has yet to be defined for RPF.

Here, we leveraged disease-gene and drug-gene databases to identify chemical compounds that potentially impacted RPF *in silico*. As such, we discovered that mammalian target of rapamycin (mTOR) was highly activated in RPF tissues, and the mTOR inhibitor sirolimus (also known as rapamycin) appeared as a good treatment candidate as it targeted most biological pathways of RPF. Next, we designed a single-arm clinical trial of a combined therapy with prednisone and sirolimus, and assessed their effects in a 48-week follow-up study. Our results showed that this combined therapy effectively reduced fibrous mass and restored immune profiles, suggesting it has potential

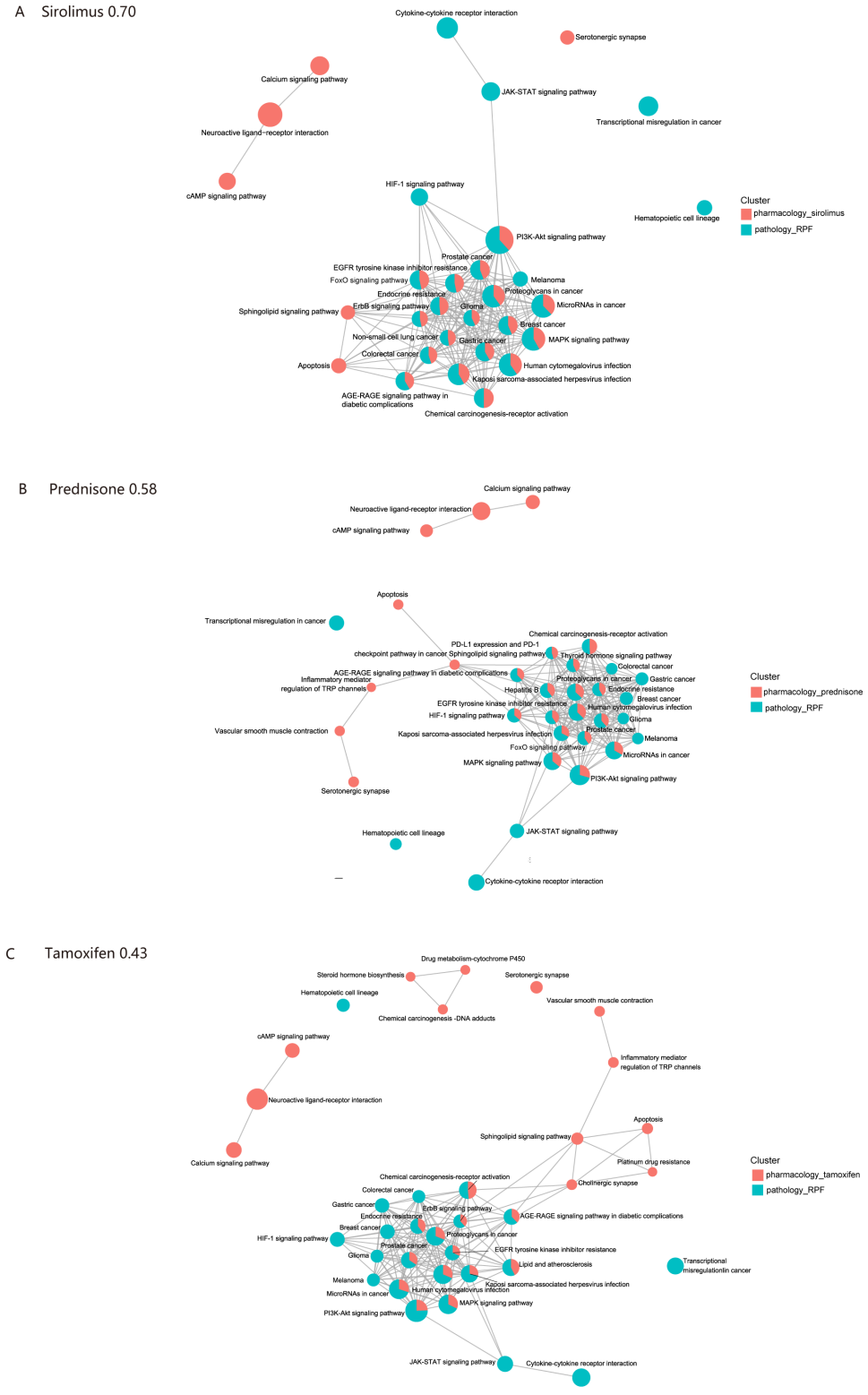
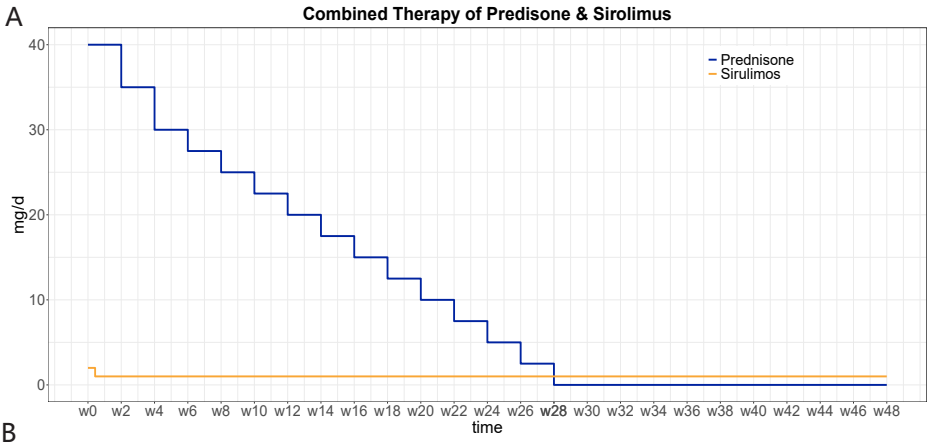


Figure 2 Biological pathways affected by the drugs and by RPF, with those for (A) sirolimus, (B) prednisone and (C) tamoxifen. Pathways affected by the drugs are indicated in red, and the pathways changed in RPF are indicated in cyan. The proportion in the pie chart is based on the number of detected genes in that particular pathway. Pairing scores indicating the match between the drug and the RPF pathology are listed next to the drug names. PI3K, phosphoinositide-3-kinase; RPF, retroperitoneal fibrosis.

A



B

Sample _ID	Age (yr)	Sex	Body weight (kg)	Hydronephrosis	Treatment for Hydronephrosis	Duration of Phase I (weeks)*	Side Effects in Phase I	Duration of Phase II (weeks)**	Side Effects in Phase II
P1	20s	Female	38.5	Bilateral	Ureteral stent implantation; Ureterolysis	24	None	24	None
P2	50s	Female	55.0	No	--	30	Hyperlipidemia	18	None
P3	50s	Female	55.0	No	--	30	Hyperlipidemia; Diabetes was worsened	18	None
P4	60s	Female	60.0	Right	--	32	Hyperlipidemia	16	None
P5	60s	Female	77.0	Bilateral	Ureteral stent implantation	34	Hypertension; Hyperlipidemia	14	Herpes zoster
P6	70s	Male	50.0	Bilateral	Ureteral stent implantation; Pyelostomy	28	Hyperglycaemia; Hyperlipidemia	20	None
P7	50s	Male	70.0	Left	Ureteral stent implantation	34	Hyperglycaemia	14	None
P8	50s	Male	71.0	No	--	34	Hyperlipidemia; acne; Muscle soreness	14	Hyperlipidemia; Muscle soreness

** Duration of the combined prednisone and sirolimus therapy: 30.8 ± 3.5 weeks;
** Duration of the sirolimus monotherapy (weeks) 17.2 ± 3.5 weeks.

Figure 3 Clinical design of a combined prednisone and sirolimus therapy for treating RPF. (A) A prescription of prednisone and sirolimus as a combined therapy for treating RPF in an individual of 50 kg of weight. (B) A summary of the RPF patients (n=8) and their treatments and side effects in this clinical trial. RPF, retroperitoneal fibrosis. *Duration of the combined prednisone and sirolimus therapy: 30.8±3.5 weeks; **Duration of the sirolimus monotherapy (weeks) 17.2±3.5 weeks.

to replace the long-term monotherapy of steroid hormone for treating RPF.

METHODS

Patient recruitment

Idiopathic RPF patients with active disease were enrolled from Peking University International Hospital following the inclusion and exclusion criteria listed in online supplemental table 1. Briefly, participants were required to have not used any glucocorticoid (equivalent to >10 mg per day of prednisone), immunosuppressant or biologic medication within 3 months prior to the enrolment. Besides, those with secondary RPF or contraindication of glucocorticoids/sirolimus were excluded. All patients provided written informed consent. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research. This study was reported under the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) cohort reporting guidelines.[15]

Clinical study design

The combined therapy evaluated in this study consisted of two phases:

1. Prednisone acetate at 0.8 mg/kg/day (maximum dosage 60 mg/day), reduced by 5 mg every 14 days until reaching 30 mg/day, and reduced by 2.5 mg every 2 weeks until discontinuation.
2. Sirolimus at 2 mg/day for the first 3 days and around 1 mg/day thereafter, with plasma drug concentration monitored at 2 weeks, 12 weeks and 48 weeks of treatment to maintain a stable level at 4–15 μg/L.

Laboratory tests

At the time of study enrolment (baseline, i.e., without treatment), 12 weeks and 48 weeks of treatment, the patients underwent physical examination, abdominal CT and peripheral blood sample collection. Laboratory tests were performed on the peripheral blood, including complete blood count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), serum immunoglobulin (Ig) level, IgG4 level, serum creatinine (Scr) and estimated glomerular filtration rate (eGFR), liver function tests, serum electrolytes, fasting glucose, lipid profile and urinalysis.

Side effects were monitored every 1–3 months as appropriate by a checklist of standardised items, blood pressures and the aforementioned laboratory tests.

Table 1 Baseline characteristics of the patients

Clinical features	Counts or lab measurements	Percentage
Sex		
Male	3	37.5
Female	5	62.5
Age (years)	57.0 (52.0, 64.5)	
Time before treatment (months)	2.5 (1.1, 21.0)	
Symptoms		
Back pain	5	62.5
Abdominal pain	2	25.0
Lower limb oedema	1	12.5
Constipation	1	12.5
Hydronephrosis		
Overall	5	62.5
Left	1	20.0
Right	1	20.0
Bilateral	3	60.0
Baseline laboratory tests		
CRP (mg/L)	8.93 (4.30, 27.17)	
ESR (mm/h)	39 (27, 50)	
eGFR (mL/min/1.73m^2)	74.63 (24.87, 102.76)	
Scr (μmol/L)	87 (60, 237)	
IgG4 (g/L)	0.46 (0.41, 1.04)	
IgG (g/L)	15.61 (12.25, 17.16)	
IgM (g/L)	0.80 (0.43, 1.06)	
IgE (IU/mL)	37 (8, 64)	
IgA (g/L)	2.36 (1.79, 3.76)	
Haemoglobin (g/L)	118 (104, 134)	
Thickness of RPF mass (mm)	29 (23, 31)	
Craniocaudal RPF length (mm)	93 (75, 114)	
Pathological features (n=5)		
<10 IgG4$^+$ plasma cells/HPF	1	20.0
10–50 IgG4$^+$ plasma cells/HPF	2	40.0
>50 IgG4$^+$ plasma cells/HPF	2	40.0
IgG4$^+$/IgG$^+$ ≤40%	4	80.0
IgG4$^+$/IgG$^+$ >40%	1	20.0

Here refers to the number of IgG4$^+$ plasma cells under the high-power field microscopy.
Lab measurements are presented in median values, followed by 25th percentile and 75th percentile values in parenthesis. The category of 'pathological features' refers to IgG4$^+$ status.
CRP, C reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; HPF, high power field microscopy; RPF, retroperitoneal fibrosis; Scr, serum creatinine.

Immunohistochemical assays

Fibrous mass was derived by puncture biopsy or surgery from 16 patients with active RPF. Control samples were the mesenteric root tissue biopsies obtained from four early diagnosed colon cancer patients who were confirmed by pathological examination to have no lymphatic metastasis at the time of biopsy. Tissue samples were fixed and cut into slices of 4 μm thick. After antigen retrieval, non-specific antigen sites were blocked and tissue sections were incubated with mTOR antibodies (Abcam, dilution 1:400), P-AKT (S473) antibodies (Abcam, dilution 1:500) and P-S6K1 (T389+T412) antibodies (Abcam, dilution 1:100). Peroxidase activity was revealed by 3–30-diamino -benzidine-tetrahydrochloride. The fibrotic area was manually outlined, and the software Visiopharm Integrator System was used for quantification.

Immune profiling by flow cytometry

PBMCs were isolated from peripheral blood of 8 RPF patients by the Percoll gradient density centrifugation. Cells were stained with fluorescence conjugated antibodies against cell surface markers at room temperature for 30–40 min. All antibodies for flow cytometry in this study are listed in online supplemental table 4. Proportions of regulatory T cells (T_{REG}), Mucosal-associated invariant T cells (MAIT), T follicular regulatory cells (T_{FR}), T helper 1 (T_H1), T helper 2 (T_H2), T helper 17 (T_H17) and circulating T follicular helper (cT_{FH}) cells were acquired on a Beckman Coulter Cytoflex LX flow cytometer and analysed by FlowJo (V.10.8.1). The cell type markers were listed in online supplemental figure 3C.

Olink proteomics assays

Cytokines and chemokines in intravenous blood were profiled using the proximity extension assays in a 96-plex inflammatory panel developed by Olink Proteomics (Sweden) and serviced by the Shanghai Biotechnology Corporation. Standard protocols for quality control and data normalisation by referencing internal and external controls were carried out in the Olink normalised protein expression (NPX) Manager software (V.3.3.2.434). The NPX values, as a relative quantification method, was used for comparing expression levels of individual proteins in different conditions.

Bioinformatic analysis

Genes affected in RPF were obtained by querying 'retroperitoneal fibrosis' in the databases of MalaCards, DisGenet and GeneCards. Genes affected by the drugs sirolimus, prednisone and tamoxifen, respectively, were obtained by querying the drug names in the databases of SwissTargetPrediction, DrugBank and ChEMBL. Gene sets or pathway enrichment analysis was performed via ClusterProfiler (V.4.4.4).

For comparing the enriched terms between the pathological and pharmacological gene sets, a pairing score was developed, in which the top 20 enriched terms from one gene set were matched with the top 50 enriched terms from the other gene set. The matching was classified into two tiers. Tier 1 refers to matching the top 20 terms in the other gene set, with each match given full weight of 1. Tier 2 refers to matching the top 21–50 terms in the other gene set, with each match granted the weight of 0.5. The overall paring score was the sum of the tier 1 and tier 2 scores, generalised as: paring score = $\Sigma_{mutual}(m_{mutual}{*}w_{tier1})/n + \Sigma_{pathology}(m_{path}{*}w_{tier2})/n + \Sigma_{pharmacology}(m_{phar}{*}w_{tier2})/n$, where m denotes a matching event between pathology terms and pharmacology terms; n is the number of terms classified as tier 1, set as 20 in our analysis; w_{tier1} is the weight for any matching in tier 1 terms, set as 1; w_{tier2} is the weight for any matching in tier 2 terms, set as 0.5.

Statistical analysis

CT scan, lab tests and flow cytometry quantification results were assessed by wilcoxon rank-sum tests. CT scan and lab test results were derived from all eight patients at various time points, and flow cytometry results were recorded for minimally five individuals for each time point. Cytokine and chemokine expression levels measured by Olink were assessed by t-test, paired t-test and equivalence test. Briefly, RPF case group (n=22) was compared with the healthy control group (n=21) by group-level

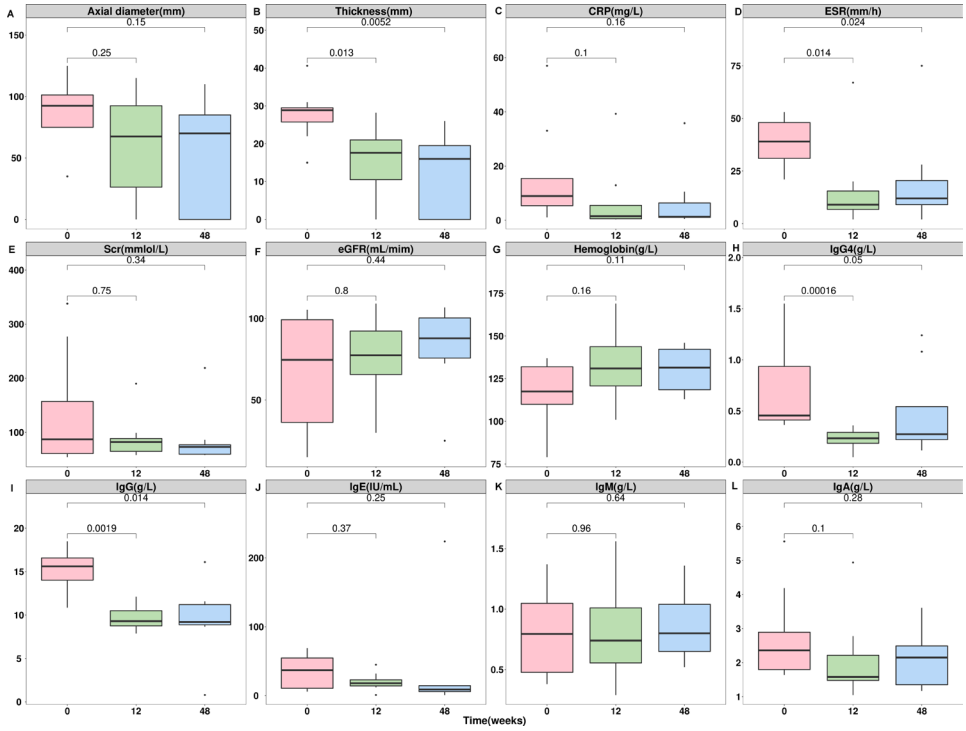


Figure 4 Lab tests to assess the treatment outcomes of the prednisone and sirolimus combined therapy for RPF. Displayed are (A, B) changes of fibrous tissue mass, (C, D) inflammatory markers, (E, F) kidney functions, (G) haemoglobin, (H) IgG4 and (I–L) immunoglobulin (Ig) major types, as measured in all eight patients with the combined therapy. CRP, C reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; RPF, retroperitoneal fibrosis; Scr, serum creatinine; 0, baseline; 12, 12 weeks of treatment; 48, 48 weeks of treatment.

t-test (R software); patients at 48-week treatment (n=8) were compared with the healthy controls (n=8) by equivalence test with the power set at 0.7 (R package Toster); and treatment responsiveness was assessed (n=8 patients) between 0–12 weeks, 12–48 weeks, 0–48 weeks by paired t-test (R software). No missing data were filled. In all comparisons, controls were age-matched and sex-matched healthy individuals. FDR adjusted p value threshold was set at 0.05 for the t-test and equivalence test, and 0.1 for the paired t-test.

RESULTS

mTOR pathway is highly activated in RPF

Searching the disease-gene databases MalaCards,[16] DisGenet[17] and GeneCards[18] resulted in over 500 genes related to RPF (online supplemental figure 1). MalaCards and DisGenet identified a few genes enriched for cytotoxicity/kinase pathways and cytokine/chemokine pathways (adjusted $p<0.05$), respectively. GeneCards uncovered many more genes and covered all the biological terms by MalaCards and DisGenet, therefore, corroborating the diverse disease biology. A pooled analysis of all genes revealed key signalling pathways, including the mammalian target of rapamycin (mTOR) pathway and its associated phosphoinositide-3-kinase (PI3K)- Akt pathway (figure 1A). For validation, we stained mTOR and its two immediate downstream signalling molecules, phospho-S6K (p-S6K) in mTOR-complex 1 and phospho-AKT (p-Akt) in mTOR-complex 2, at three locations of the fibrous tissues: the germinal centres (GCs) where the fibrosis was most dense with a high infiltration of T and B cells, the peripheral which was proximal to the GCs, and non-fibrosis controls (figure 1B–D). We observed >90% of cells stained for mTOR in the GCs or peripheral. p-S6K activity was low in the controls (1.6%) and activated by 40 folds in the GCs (67.4%); p-Akt increased by a few folds in the GCs (58.0%) compared with controls (21.3%). Therefore, we concluded that mTOR-complex 1 was highly activated in the fibrous tissues.

The mTOR inhibitor sirolimus affects most of the RPF pathways

Learning the high mTOR activity in RPF, we analysed *in silico* if the mTOR inhibitor sirolimus, an immunosuppressive drug, could target the RPF pathways. We searched in three drug databases, SwissTargetPrediction,[19] DrugBank[20] and ChEMBL,[21] and identified over 400 genes affected by sirolimus, including key pathway genes mTOR and PI3K-Akt (online supplemental figure 2). For systematically comparing the overlap between the pharmacological terms of drugs and the pathological terms in diseases, we developed a paring score, which measures how well the top 20 terms from either dataset matched to the top 50 terms in the other dataset, and assigned weights according to their ranks. The match between the sirolimus pharmacology and RPF pathology resulted in a pairing score of 0.70 (figure 2A, online supplemental table 2). We also constructed the matching scores for prednisone and tamoxifen, the current and the previous generations of treatment for RPF, respectively. Prednisone had fewer overlaps, resulting in a pairing score of 0.58 (figure 2B). Among the three drugs, tamoxifen had the least overlap, with a pairing score of 0.43 (figure 2C). We concluded from this pathway analysis that sirolimus had a high potential for treating RPF.

Design of a combined therapy with prednisone and sirolimus to treat RPF

We next implemented a clinical trial to evaluate the actual effects of combining prednisone and sirolimus in treating RPF, in which prednisone was prescribed at a standard dosage, followed by a

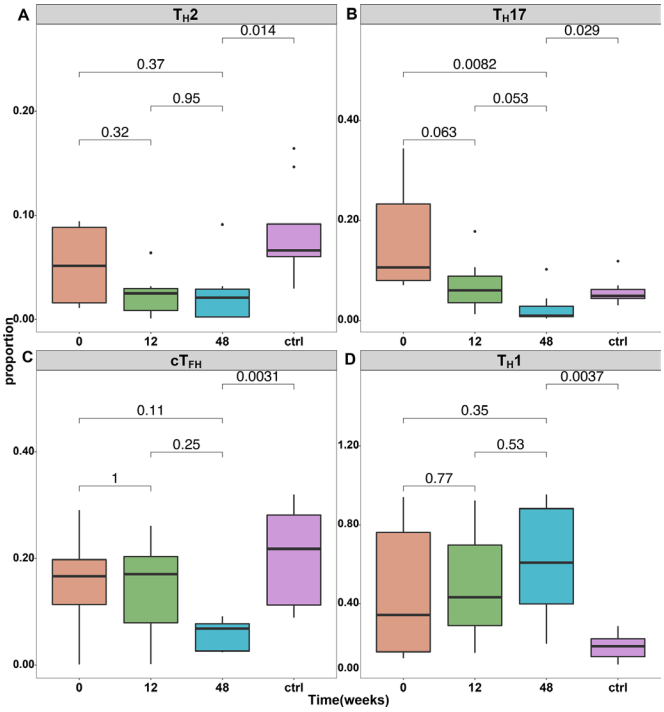


Figure 5 Immunoprofiling by flow cytometry indicated that specific T cell subsets were regulated with the therapy. Changes were in proportions for (A) T_H2 cells, a type of IL-4 producing T cells, with the markers of $CD3^+CD4^+CD8^-CXCR3^+CCR6^-CCR4^+CCR7^{low}$; (B) T_H17 cells, a type of IL-17 producing T cells, with the markers of $CD3^+CD4^+CD8^-CXCR3^-CCR6^+CCR4^+CCR7^{lo}$; (C) cT_{FH} cells, that is, circulating T follicular helper cells, with the markers of $CD3^+CD4^+CD8^-CD45RA^-CXCR5^+CCR7^{low}PD\text{-}1^{high}$; (D) T_H1 cells, a type of IFN-γ, TNF and IL-2 secreting T cells, with the markers of $CD3^+CD4^+CD8^-CXCR3^+CCR6^-CCR4^-CCR7^{low}$. 0: baseline; 12: 12 weeks of treatment; 48: 48 weeks of treatment; ctrl: age-matched and sex-matched healthy controls.

gradual reduction to zero, and sirolimus was given as a boost in the first 3 days, followed by a reduced and stable dosage for long term. An illustrative prescription protocol is given in figure 3A for an individual of body weight of 50 kg. Assessments were collected at the baseline (i.e., onset of the treatment), 12 weeks and 48 weeks of treatment, including contrast-enhanced CT to determine sizes of the fibrous tissues, various lab tests on markers for inflammation and functions of kidney and liver, assessment of side effects, profiling cell types and abundance by flow cytometry, and measuring plasma inflammatory proteins by proteomics.

Patient characteristics

In total, we recruited 12 RPF patients for the combined therapy. Recruitment criteria were listed in online supplemental table 1. Eight patients, including five females and three males, completed the 48-week assessments (figure 3B). The rest four people dropped out due to the reason for not following the prescriptions strictly. Patient characteristics at baseline were documented in table 1. In each patient, one fibrous tissue locus was observed, with the median thickness of 28.85 mm and the craniocaudal length of 92.50 mm. Five patients developed hydronephrosis and four of them had ureteral stents implanted to alleviate the symptom. On average, the combined intake of prednisone and sirolimus lasted for 30.8±3.5 weeks, followed by the sirolimus monotherapy for 17.2±3.5 weeks.

RPF symptoms were improved with the combined therapy

With the combined therapy, most of the RPF symptoms displayed improvement (figure 4). CT scan showed that the fibrous tissue shrunk nearly by half in both axial diameter and thickness at both 12-week and 48-week treatments. Two markers of acute inflammation, CRP and ESR, were reduced by 70%–80%. Two markers of kidney functions, Scr and the eGFR, displayed constant improvement along the treatment—while five out of the eight patients had either marker being abnormal before treatment, only one patient remained distorted after the treatment. The combined therapy also corrected for anaemia in RPF patients, with haemoglobin concentrations raised by about 12%. Interestingly, the circulating levels of IgG4 displayed a 40%–50% reduction with the treatment. We also profiled the major isotypes of immunoglobulins and found that IgG was reduced along the treatment while IgE, IgM and IgA did not show significant changes.

Profiles of immune cell types and abundances

We performed comprehensive immunophenotyping by flow cytometry on seven subsets of T cells in peripheral blood (online supplemental figure 3). Over the course of the therapy, a trend of reduction in cell abundance was observed for T_H2, T_H17 and cT_{FH} cells, but not for T_H1 (figure 5). T_H2 and T_H17 cells are IL-4-producing and IL-17-producing $CD4^+$ T cells, respectively. cT_{FH} cells are known to play important roles in the development of GCs as well as antibody production. Their upregulation has been reported in other autoimmune conditions such as systemic lupus erythematosus (SLE).[22 23]

Profiles of circulating inflammation proteins

We leveraged the Olink technology to quantify circulating inflammatory proteins along the treatment, in relation to the age-matched and sex-matched healthy controls.[24] In total 75 proteins were quantified and their responses to treatment were categorised to nine groups in online supplemental table 3. Twenty-five proteins displayed changes in diseased state relative to the controls, including families of tumour necrosis factor (TNF), interleukins and chemokines (t-test, adjusted $p<0.05$, (online supplemental figure 4). After treatment, six of them regressed to healthy levels (group 1), namely CCL23, OSM, TNF, TNFRSF9, TRAIL and VEGFA (figure 6A). IL6 responded but still deviated from the healthy level. The rest abnormal proteins displayed trends of change (group 3) but did not pass statistical tests likely due to the limited sample size. Note that most proteins at healthy levels in RPF remained stable at the end of the treatment (equivalence test, adjusted $p<0.05$). However, a careful examination did reveal that among the uncertain proteins, AXIN1, CCL20, CXCL1, CXCL5, FGF-2 and SIRT2 appeared as normal in the diseased state but deviated from normal after the treatment (online supplemental figure 5), suggesting these perturbations were likely attributed to drug side effects (6 out of 75, 8%). We performed KEGG pathway enrichment on all the 25 treatment-responsive inflammatory proteins, and found they were mostly enriched in the cytokine, chemokine and other immune-related signalling pathways (figure 6B).

Side effects of the combined therapy

We recorded a few adverse reactions during phase I of the therapy, when both prednisone and sirolimus were prescribed: six patients with hyperlipidaemia, two patients with hyperglycaemia, one patient with hypertension and one patient with muscle soreness (figure 3B). Entering phase II, when prednisone

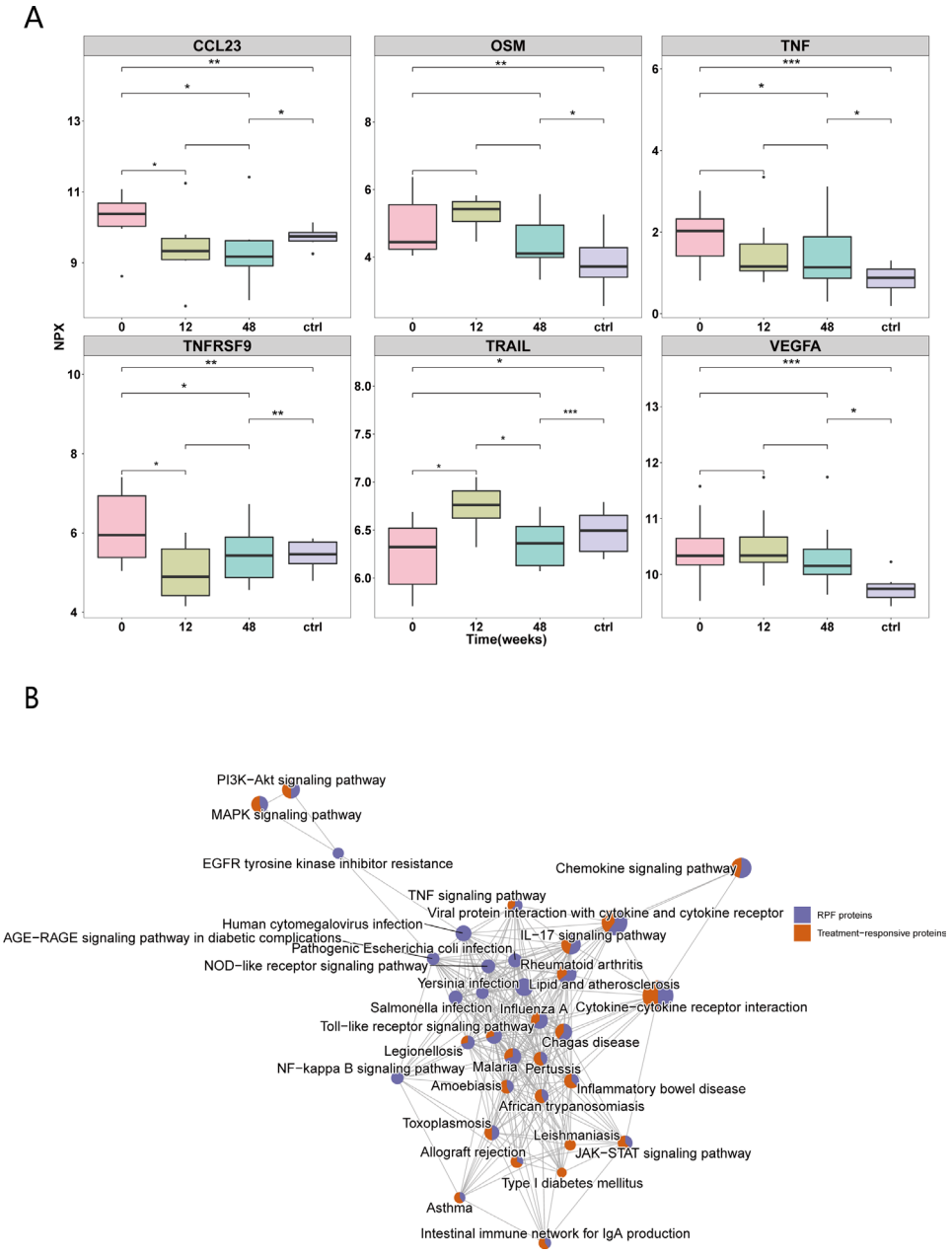


Figure 6 Cytokine profiling via Olink Target 96 inflammation panel. (A) Abnormal proteins of RPF regressed towards healthy levels after the treatment. 0: baseline; 12: 12 weeks of treatment; 48: 48 weeks of treatment; ctrl: age-matched and sex-matched healthy controls. (B) Signalling pathways enriched in the inflammation proteins that were characteristic of RPF (purple) and responsive to the treatment (red). *p<0.05, **p<0.01, ***p<0.001. RPF, retroperitoneal fibrosis.

was removed, most of these adverse effects disappeared, except for a female patient who developed Herpes Zoster and a male patient whose hyperlipidaemia and muscle soreness remained. Overall, the combined therapy was tolerant, and it was evident that side effects of the steroid hormone were much reduced in phase II, when prednisone was removed and sirolimus became the single drug to maintain the treatment.

DISCUSSION

In this study, we presented a combined therapy of prednisone and sirolimus for treating idiopathic RPF. This was opposed to the classic steroid hormone monotherapy, and leveraged the discovery that mTOR was highly activated in RPF tissues and the biological pathways affected by sirolimus had a high degree of match with that in RPF (paring score of 0.70). Our strategy was to use both prednisone and sirolimus in phase I, in which a regular dosage of prednisone was used to boost start the fibrosis reduction, followed by a gradual reduction to zero, while maintaining a stable plasma concentration of sirolimus. In phase II, sirolimus remained the only drug to sustain the fibrous remission. In our 48-week clinical trial in RPF patients, we observed that fibrous tissues shrunk, inflammation weakened and kidney functions were improved already in phase I and sustained in phase II, suggesting sirolimus was able to maintain the treatment effects. It is interesting to note that all patients displayed much reduced IgG4 with the treatment, suggesting it was also potent for treating the $IgG4^+$ RPF. Furthermore, most side effects induced in phase I, such as hyperlipidemia and hyperglycaemia, disappeared in phase II, suggesting side effects of the steroid hormone were controlled in this therapy. Overall, our

treatment strategy of using gradient reduction of prednisone and a long-term stable dosage of sirolimus demonstrated as effective for treating idiopathic RPF with tolerant side effects. As current treatments of RPF involving long-term steroid hormones often result in severe side effects, our therapy potentially alleviates this issue, casting hope for a new treatment direction.

Treatments to idiopathic RPF rely on medication to suppress inflammation and relief of ureteral obstruction via stent implantation, percutaneous nephrostomy and ureterolysis. For medication, the first-generation drug was tamoxifen, which has long been used as an anti-oestrogen compound for treating early-stage oestrogen-sensitive breast cancer and, in the recent three decades, for treating immune disorders.[25] Vaglio *et al* reported in a randomised clinical trial that glucocorticoids monotherapy achieved better treatment outcomes over tamoxifen,[9] and has since become the mainstay therapy for RPF. Since then, further improvement of RPF therapies have not been much discussed in medical literature, except for a few clinical trials with empirical designs.[26–29] Here, we leveraged bioinformatic analysis to systematically interrogate the biological mechanisms of RPF and inferred that sirolimus was a potential drug for treating it. We quantified the match of the drugs with the pathological pathways, with the former recorded in drug-gene databases and the latter in disease-gene databases. We found that the pairing scores were 0.43 for tamoxifen-RPF, 0.58 for prednisone-RPF and 0.70 for sirolimus-RPF, displaying a trend of continuous improvement. This indicated that the mTOR inhibitor sirolimus more specifically captured the altered biological pathways in RPF.

Our study suggested mTOR as a critical player in RPF. Indeed, mTOR emerged as an important signalling molecule in fibrosis, which promoted fibroblast proliferation and strengthened proinflammatory responses via proliferating T_H1, T_H17 and $CD4^-CD8^-$ T cells. mTOR inhibition by sirolimus was demonstrated to improve numerous autoimmune diseases, including SLE, juvenile idiopathic arthritis and primary antiphospholipid syndrome.[30–33] However, sirolimus has not been reported to treat RPF. Our clinical trial expanded the list of autoimmune conditions that could benefit from mTOR inhibition. It is worth noting that not all autoimmune conditions involving fibrosis are targets of sirolimus. A previous follow-up study on SSc suggested sirolimus had a limited treatment effect, although mTOR activation was evident.[34] Clinical trials and careful examinations are needed to evaluate the drug effects.

Fibrosis is a common complication of diseases and accounts for up to 45% of death in industrialised countries.[35] Although no effective medicine exists yet to completely revert the fibrous process, it is known as highly dynamic and therefore presents opportunities for correction and cure. As mTOR signalling is general in fibrosis, the strategy of combining a much-reduced dosage of steroid hormones and a stable dosage of mTOR inhibitor sirolimus may have a broad application to other fibrosis-related diseases.

There are several limitations in the current study. First, the patient cohort size was small, with only eight patients completing the assessment. As RPF is a rare condition with a low prevalence rate, that is, about 1.4 cases/100 000 inhabitants,[1] patient recruitment has been challenging. Second, randomised clinical trials are desired to evaluate treatment differences between our proposed combined therapy and the standard prednisone monotherapy. For this we are carrying out a randomised clinical trial (NCT04047576) and in the process of recruiting patients and collecting data. This will provide a more rigorous examination on the benefits of the 2-phase sirolimus-prednisone combined therapy versus the prednisone monotherapy. Third, our plasma proteomics assayed only 75 inflammation-related proteins and some of the key players including IL-4, IL-13 and IL-21 were not detected, limiting the power of the immune proteome profiling. A more comprehensive treatment assessment will be revealed by including a larger number of proteins. Future studies with more RPF patients, longer follow-up periods and more inclusive measurements will provide richer information and stronger analytical power for assessing our new treatment strategy of combining prednisone and sirolimus.

Twitter Cuiping Pan @cuipingpan

Acknowledgements We thank Drs. Shengguang Li, Feng Yu, Tong Zhang, Jing Li, Xiaoying Zhang and Yuanchun Feng from Peking University International Hospital, Dr. Jinxia Zhao from Peking University Third Hospital, Dr. Xiaoying Zhang from Peking University People's Hospital for their assistance in referring study participants, collecting peripheral blood samples, performing immunohistochemical experiments, pathological analysis and imaging analysis. We also thank members of the Laboratory of Intelligent Computing in Biomedicine in the Greater Bay Area Institute of Precision Medicine (Guangzhou) for insightful discussions and suggestions.

Contributors HG, CP and CL designed the study. CP and HG drafted the manuscript. HG, SL and CL assembled the study cohort, consented the patients, followed up with the patients and performed laboratory tests. XZ performed flow cytometry. CP, YM, YW and SZ performed bioinformatic and statistical analysis, Olink proteomic assays, and generated the figures and tables. All authors contributed to result interpretation and discussions. CP, HG and CL critically reviewed the manuscript. HG and CP are guarantors of this work.

Funding This study was supported by the Greater Bay Area Research Institute of Precision Medicine (Guangzhou) Research Grant (I0005), National Natural Science Foundation of China (81771678) and Peking University International Hospital Research Grant (YN2019QN02).

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Research Ethics Committee at Peking University International Hospital. The ethics approval number: 2019-031 (BMR). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iDs
Hui Gao http://orcid.org/0000-0002-3303-9767
Cuiping Pan http://orcid.org/0000-0002-8152-2489

REFERENCES

1 Vaglio A, Maritati F. Idiopathic retroperitoneal fibrosis. *J Am Soc Nephrol* 2016;27:1880–9.
2 van Bommel EFH, Jansen I, Hendriksz TR, *et al*. Idiopathic retroperitoneal fibrosis: prospective evaluation of incidence and clinicoradiologic presentation. *Medicine (Baltimore)* 2009;88:193–201.
3 Vaglio A, Salvarani C, Buzio C. Retroperitoneal fibrosis. *Lancet* 2006;367:241–51.
4 Tanaka T, Masumori N. Current approach to diagnosis and management of retroperitoneal fibrosis. *Int J Urol* 2020;27:387–94.
5 Martorana D, Vaglio A, Greco P, *et al*. Chronic periaortitis and HLA-DRB1*03: another clue to an autoimmune origin. *Arthritis Rheum* 2006;55:126–30.

6 Goldoni M, Bonini S, Urban ML, *et al*. Asbestos and smoking as risk factors for idiopathic retroperitoneal fibrosis: a case-control study. *Ann Intern Med* 2014;161:181–8.
7 Distler JHW, Györfi A-H, Ramanujam M, *et al*. Shared and distinct mechanisms of fibrosis. *Nat Rev Rheumatol* 2019;15:705–30.
8 Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 2012;18:1028–40.
9 Vaglio A, Palmisano A, Alberici F, *et al*. Prednisone versus tamoxifen in patients with idiopathic retroperitoneal fibrosis: an open-label randomised controlled trial. *Lancet* 2011;378:338–46.
10 Yasir M, Goyal A, Sonthalia S. Corticosteroid adverse effects. In: *StatPearls*. Treasure Island (FL), 2022.
11 Moriconi D, Giannese D, Capecchi R, *et al*. Risk factors for relapse and long-term outcome of idiopathic retroperitoneal fibrosis. *Clin Exp Nephrol* 2019;23:1147–53.
12 Raffiotta F, da Silva Escoli R, Quaglini S, *et al*. Idiopathic retroperitoneal fibrosis: long-term risk and predictors of relapse. *Am J Kidney Dis* 2019;74:742–50.
13 Zhao J, Li J, Zhang Z. Long-Term outcomes and predictors of a large cohort of idiopathic retroperitoneal fibrosis patients: a retrospective study. *Scand J Rheumatol* 2019;48:239–45.
14 Razanamahery J, Bouldoires B, Humbert S, *et al*. Characteristics of adult patients with idiopathic retroperitoneal fibrosis and assessment of risk of relapse at diagnosis. *J Clin Med* 2021;10:1380.
15 von Elm E, Altman DG, Egger M, *et al*. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008;61:344–9.
16 Rappaport N, Twik M, Plaschkes I, *et al*. MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. *Nucleic Acids Res* 2017;45:D877–87.
17 Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, *et al*. The disgenet knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res* 2020;48:D845–55.
18 Stelzer G, Rosen N, Plaschkes I, *et al*. The genecards suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinformatics* 2016;54:1.
19 Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res* 2019;47:W357–64.
20 Wishart DS, Feunang YD, Guo AC, *et al*. DrugBank 5.0: a major update to the drugbank database for 2018. *Nucleic Acids Res* 2018;46:D1074–82.
21 Gaulton A, Hersey A, Nowotka M, *et al*. The chembl database in 2017. *Nucleic Acids Res* 2017;45:D945–54.
22 Gensous N, Charrier M, Duluc D, *et al*. T follicular helper cells in autoimmune disorders. *Front Immunol* 2018;9:1637.
23 Muhammad Yusoff F, Wong KK, Mohd Redzwan N. Th1, Th2, and Th17 cytokines in systemic lupus erythematosus. *Autoimmunity* 2020;53:8–20.
24 Assarsson E, Lundberg M, Holmquist G, *et al*. Homogenous 96-plex pea immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014;9:e95192.
25 Behjati S, Frank MH. The effects of tamoxifen on immunity. *Curr Med Chem* 2009;16:3076–80.
26 Alberici F, Palmisano A, Urban ML, *et al*. Methotrexate plus prednisone in patients with relapsing idiopathic retroperitoneal fibrosis. *Ann Rheum Dis* 2013;72:1584–6.
27 Binder M, Uhl M, Wiech T, *et al*. Cyclophosphamide is a highly effective and safe induction therapy in chronic periaortitis: a long-term follow-up of 35 patients with chronic periaortitis. *Ann Rheum Dis* 2012;71:311–2.
28 Scheel PJ, Feeley N, Sozio SM. Combined prednisone and mycophenolate mofetil treatment for retroperitoneal fibrosis: a case series. *Ann Intern Med* 2011;154:31–6.
29 Urban ML, Maritati F, Palmisano A, *et al*. Rituximab for chronic periaortitis without evidence of IgG4-related disease: a long-term follow-up study of 20 patients. *Ann Rheum Dis* 2020;79:433–4.
30 Foroncewicz B, Mucha K, Paczek L, *et al*. Efficacy of rapamycin in patient with juvenile rheumatoid arthritis. *Transpl Int* 2005;18:366–8.
31 Su T-IK, Khanna D, Furst DE, *et al*. Rapamycin versus methotrexate in early diffuse systemic sclerosis: results from a randomized, single-blind pilot study. *Arthritis Rheum* 2009;60:3821–30.
32 Lai Z-W, Kelly R, Winans T, *et al*. Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. *Lancet* 2018;391:1186–96.
33 Xie W, Ji L, Zhang Z. Sirolimus monotherapy for thrombocytopenia in primary antiphospholipid syndrome: a pilot study from a tertiary referral center. *Front Immunol* 2022;13:857424.
34 Shah M, Edman MC, Janga SR, *et al*. A rapamycin-binding protein polymer nanoparticle shows potent therapeutic activity in suppressing autoimmune dacryoadenitis in A mouse model of sjögren's syndrome. *J Control Release* 2013;171:269–79.
35 Henderson NC, Rieder F, Wynn TA. Fibrosis: from mechanisms to medicines. *Nature* 2020;587:555–66.

Correction: *Combined therapy of prednisone and mTOR inhibitor sirolimus for treating retroperitoneal fibrosis*

Gao H, Liu S, Mai Y, *et al*. Combined therapy of prednisone and mTOR inhibitor sirolimus for treating retroperitoneal fibrosis. *Ann Rheum Dis* 2023;82:688-697.

Figure 4 is incorrect and contains the following labeling errors in sub-figures E and F: cr(mmlol/L), eGFR (mL/min)

The correct version should read: Scr(μmlol/L), eGFR (ml/min/1.73 m^2)

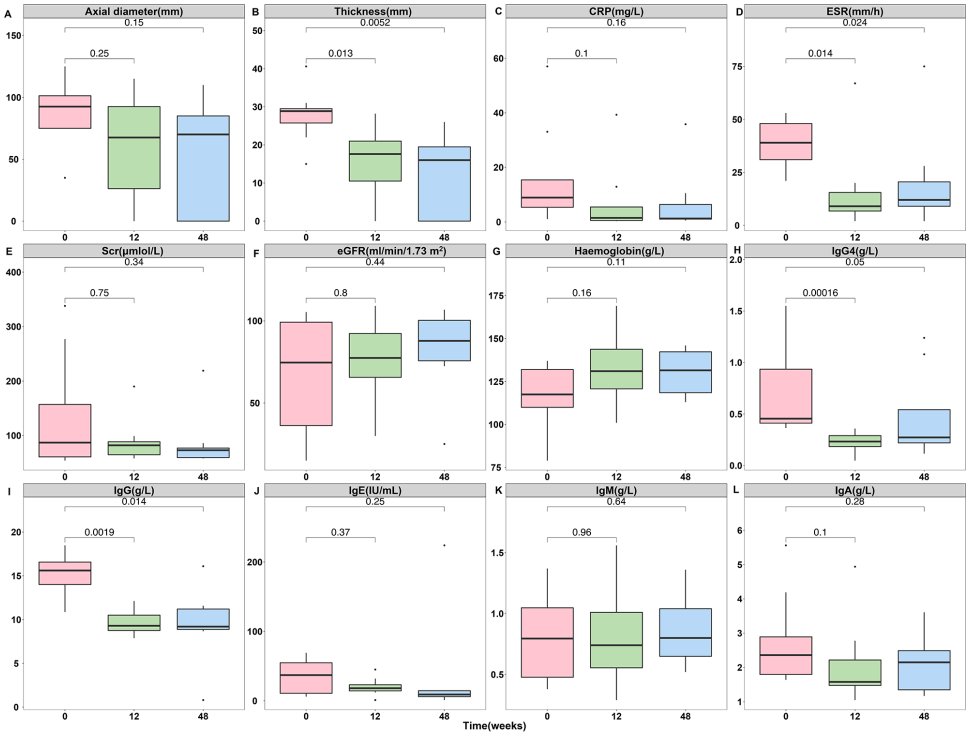
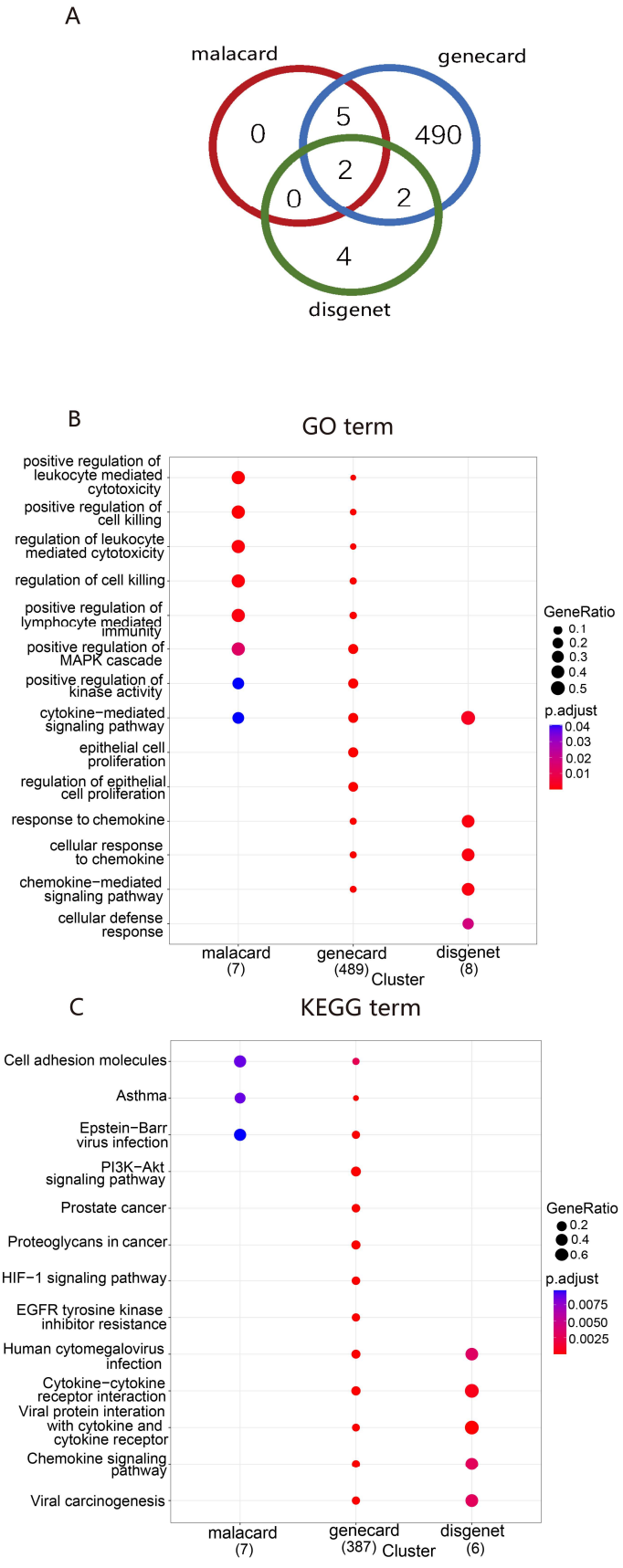


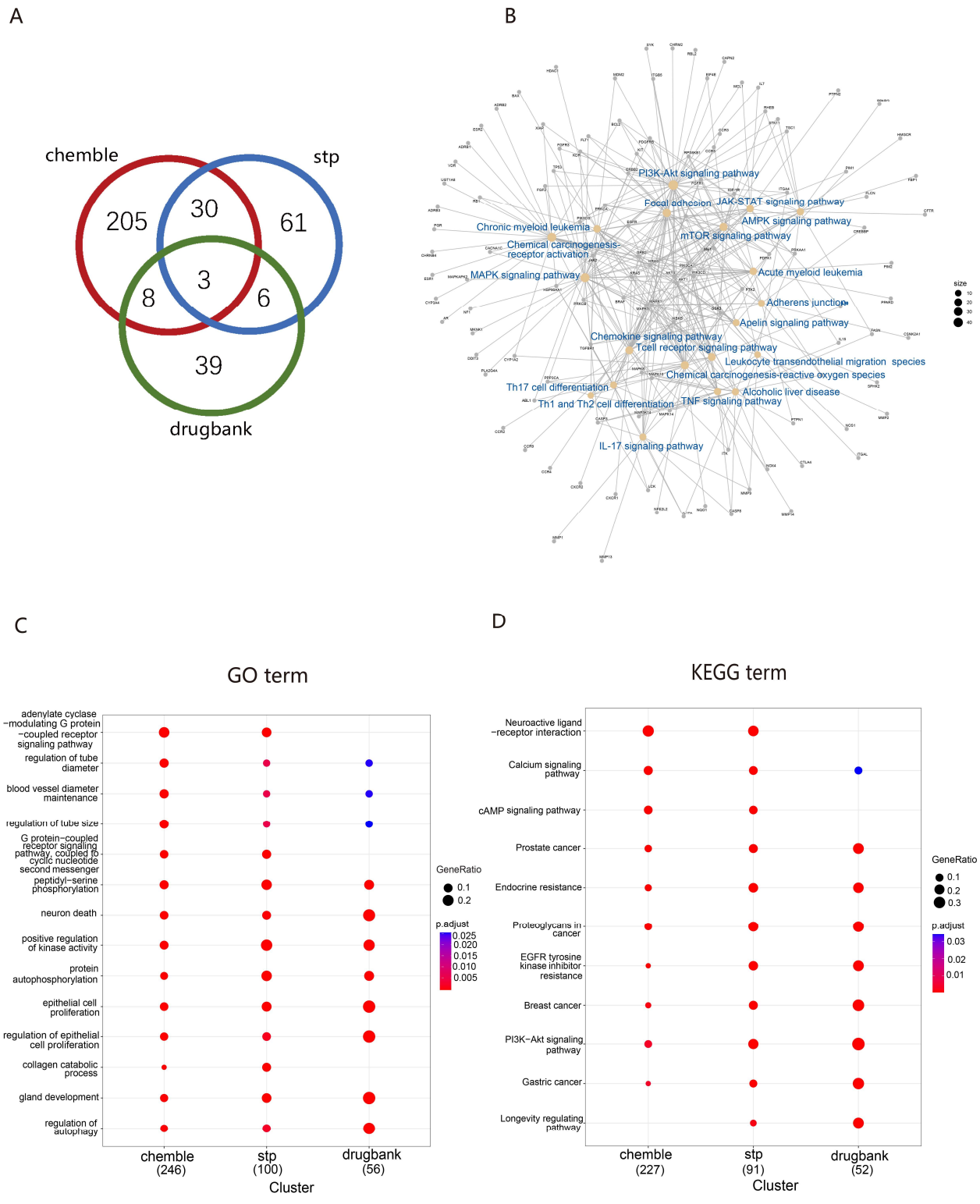
Figure 4

doi:10.1136/ard-2022-223736

Ann Rheum Dis 2023;**82**:e220. doi:10.1136/ard-2022-223736corr1

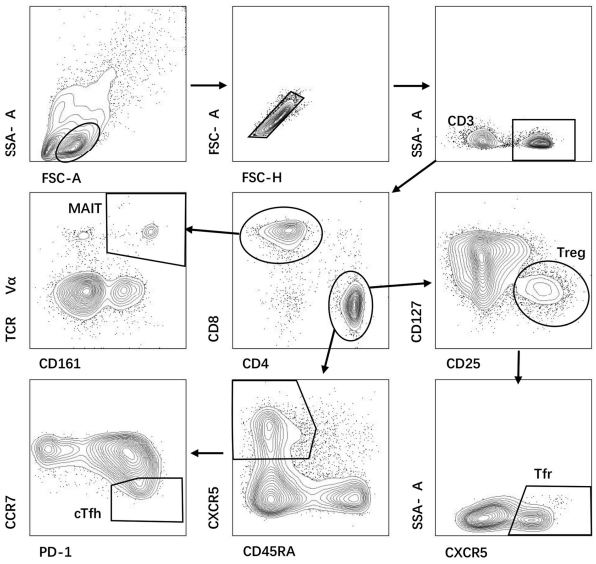


Supplemental Figure 1. Pathology pathway enrichments for RPF. (A) Overlap of genes queried from the three gene-disease databases; (B) Gene Ontology (GO) enrichment for the identified genes in each database; (C) Pathway enrichment using Kyoto Encyclopedia of Genes and Genomes (KEGG) database for the identified genes in each database.

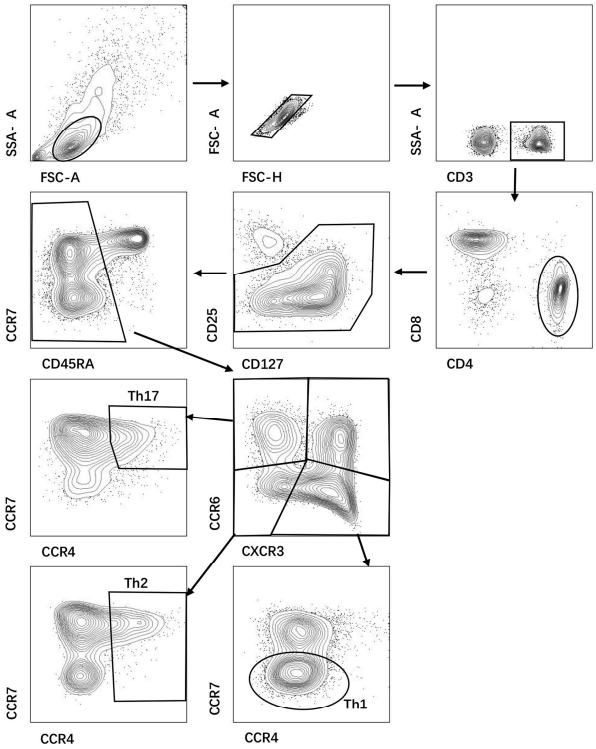


Supplemental Figure 2. Target genes and pathways of sirolimus. (A) Overlap of genes queried by sirolimus from the three drug-gene databases; (B) signaling pathway network enriched from genes affected by sirolimus. (C) GO enrichment for the identified genes in each database; (D) Pathway enrichment using KEGG database for the identified genes in each database.

A



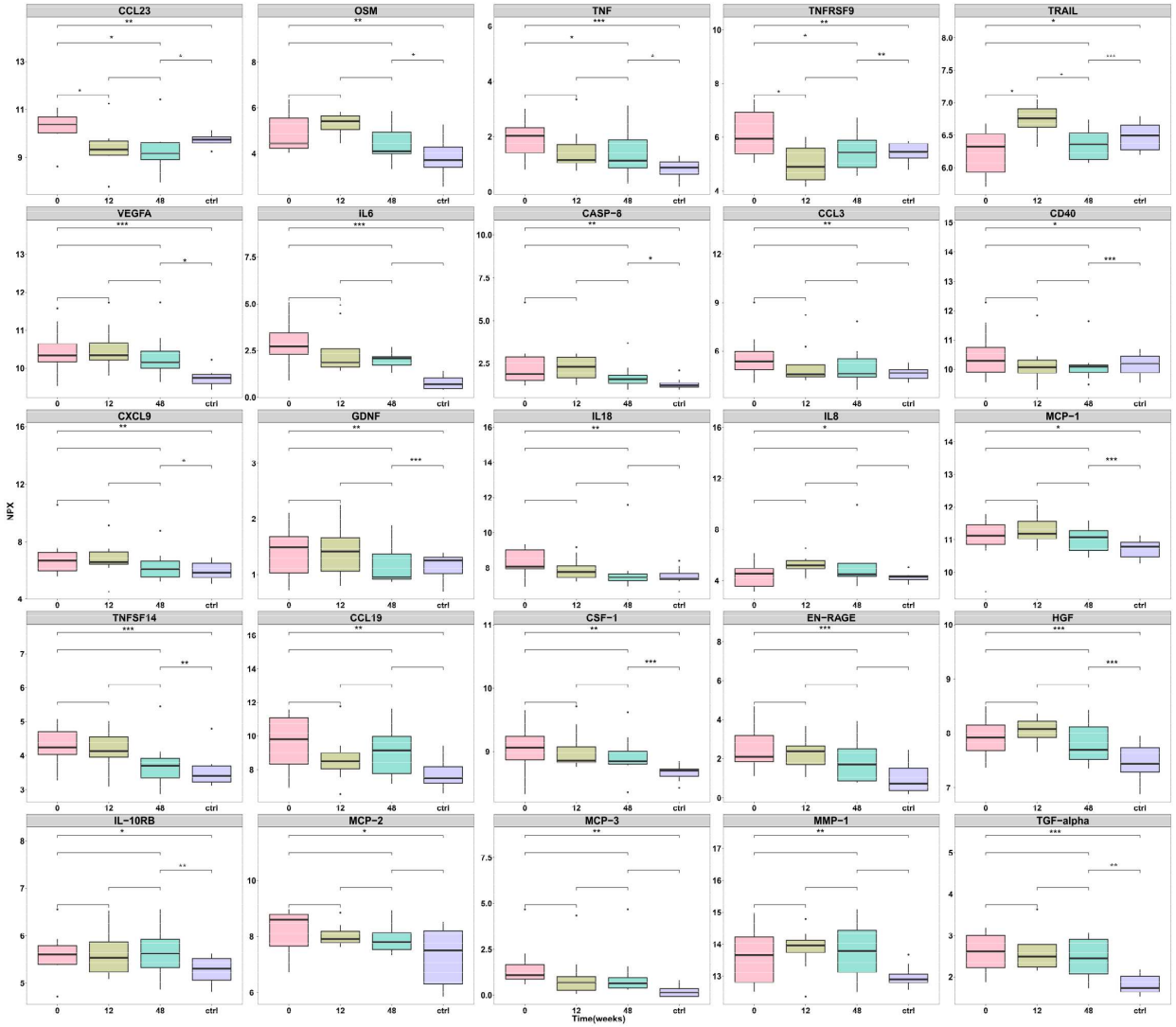
B



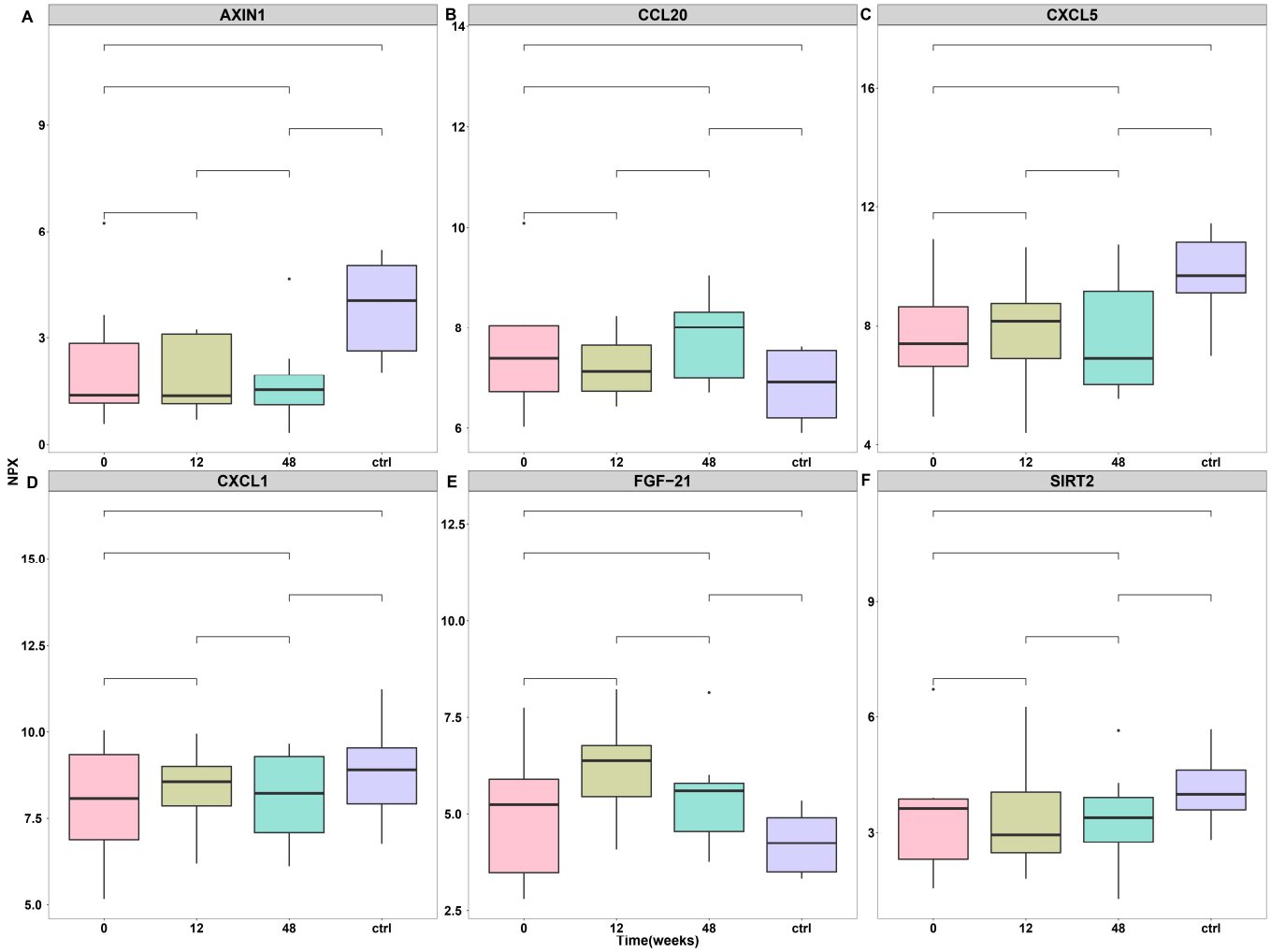
C

T_H1	$CD3^+CD4^+$ $CD8^-CXCR3^+CCR6^-CCR4^-CCR7^{low}$
T_H2	$CD3^+CD4^+CD8^-CXCR3^+CCR6^-CCR4^+CCR7^{low}$
T_H17	$CD3^+CD4^+CD8^-CXCR3^-CCR6^+CCR4^+CCR7^{low}$
T_{REG}	$CD3^+CD4^+$ $CD8^-CD25^{high}CD127^{low}$
MAIT	$CD3^+CD4^-CD8^+TCRV\alpha^+CD161^{high}$
cT_{FH}	$CD3^+CD4^+CD8^-CD45RA^-CXCR5^+CCR7^{low}PD\text{-}1^{high}$
T_{FR}	$CD3^+CD4^+CD8^-CD25^{high}CD127^{low}CXCR5^+$

Supplemental Figure 3. Phenotypic characterization of T-cell subsets by Flow cytometry.Illustrative examples of the lymzphocyte gating strategy were provided for identifying T cell subset (A) T_{FR} and (B) T_H1; (C) a complete marker list for each T cell subset. T_H1: T helper 1 cells; T_H2: T helper 2 cells; T_H17:Thelper17cells; T_{REG}: regulatory T cells; MAIT: mucosal-associated invariant T cells; cT_{FH}: circulating follicular helper T cells; T_{FR}: T follicular regulatory cells.



Supplemental Figure 4. Inflammation-related proteins displaying significant changes between RPF patients (n=8) at and age- and sex- matched healthy controls (n=8). Shown are their protein quantification levels (NPX) in four assessments: 0: baseline, before treatment; 12: 12 weeks of the treatment; 48: 48 weeks of the treatment; ctrl: age- and sex- matched healthy controls. Statistical tests were employed as follows: 0-ctrl, t-test; 48-ctrl: equivalence test; 0-12,12-48,0-48: paired t-test. *: adjusted P < 0.05; **: adjusted P < 0.005; ***: adjusted P < 0.0005.



Supplemental Figure 5. Inflammation-related proteins with likely normal plasma levels at the baseline but distinct levels after 48 weeks of treatment with the combined therapy. 8 patients with RPF and 8 age- and sex- matched controls were assessed. (A-F): AXIN1, CCL20, CXCL5, CXCL1, FGF-21 and SIRT2. NPX: protein quantification levels in the Olink platform. 0: baseline, before treatment; 12: 12 weeks of treatment; 48: 48 weeks of treatment; ctrl: age- and sex- matched healthy controls. Statistical tests were employed as follows: 0-ctrl, t-test; 48-ctrl: equivalence test; 0-12,12-48,0-48: paired t-test. *: adjusted P < 0.05; **: adjusted P < 0.005; ***: adjusted P < 0.0005.

Supplemental Tables

Supplemental Table 1. Recruitment criteria of patients in the RPF combined therapy trial.

Inclusion criteria (all of them):

1) 18-75 years old;
2) Diagnosed as idiopathic RPF by CT or MRI - for patients suspected to have secondary RPF or atypical idiopathic RPF, puncture biopsy was conducted for clarification;
3) Increased ESR and CRP levels caused by RPF or active lesions detected by imaging.

Exclusion criteria (either one of them):

1) Secondary retroperitoneal fibrosis;
2) Usage of any glucocorticoid (equivalent to >10 mg per day of prednisone), immunosuppressant, or biologic medication within 3 months prior to the enrollment;
3) Having any contraindication of glucocorticoid or sirolimus, allergic to sirolimus, or experienced serious adverse reactions from previous use of any of the above drugs;
4) Massive proteinuria (24-hour urine protein quantitation ≥3 g), moderate-to-severe anemia (hemoglobin <90 g/L), agranulocytosis (white blood cell count $<1.5\times10^9$/L or neutrophil count $<0.5\times10^9$/L), platelet count < 50,000, or interstitial pneumonia;
5) Uncontrollable diabetes, hypertension, hyperlipidemia, infection, or heart failure;
6) Any malignant tumor;
7) Other serious complications or general conditions that do not permit study enrollment;
8) Pregnancy or plan for pregnancy in the near future;
9) Unable to adhere to follow-up or refuses to provide consent.

Supplemental Table 2. Pairing scores for evaluating matching between disease pathology and drug pharmacology.

Overlap of Enrichment Terms						
Category	Pharmacology_sirolumus	Pathology	Pharmacology_prednisone	Pathology	Pharmacology_tamoxifen	Pathology
Input	50	50	50	50	50	50
Tier-1 overlap	10	10	7	7	4	4
Tier-2 overlap	4	4	5	4	3	6
Paring Scores						
Category	Sirolimus vs RPF		Prednisone vs RPF		Tamoxifen vs RPF	
Tire-1	0.50		0.35		0.20	
Tier-2 pharmacology	0.10		0.13		0.08	
Tier-2 pathology	0.10		0.10		0.15	
Total	0.70		0.58		0.43	

Supplemental Table 3. Olink quantified plasma inflammatory proteins in response to the combined therapy.

Abnormal level inflammatory proteins in RPF (n=25) ①			
Normal at 48W (n=6)	Group 1 ①+⑤+⑦	CCL23, OSM, TNF, TNFRSF9, TRAIL, VEGFA	
Abnormal at 48W (n=1)	Group 2 ①+④	IL6	
Uncertain at 48W(n=18)	Group 3 ①+⑥, ①+⑤+⑧	Group 3.1 Likely Normal (n=10)	CASP-8, CCL3, CD40, CXCL9, GDNF, IL18, IL8, MCP-1, TNFSF14
		Group 3.2 Likely Abnormal (n=8)	CCL19, CSF-1, EN-RAGE, HGF, IL-10RB, MCP-2, MCP-3, MMP-1, TGF-alpha
Normal level inflammatory proteins in RPF (n=35) ②			
Normal at 48W (n=34)	Group 4 ②+⑤	4E-BP1, ADA, CCL11, CCL25, CCL28, CCL4, CD244, CD5, CD6, CD8A, CDCP1, CST5, CX3CL1, DNER, FGF-19, FGF-5, Flt3L, IFN-gamma, IL-15RA, IL-17A, IL-18R1, IL7, LAP TGF-beta, LIF-R, MMP-10, NT-3, OPG, PD-L1, SCF, SLAMF1, TNFB, TRANCE, TWEAK, uPA	
Abnormal at 48W (n=0)	Group 5 ②+④	-	
Uncertain at 48W (n=1)	Group 6 ②+⑥	IL-17C	
Uncertain in RPF (n=15) ③			
Normal at 48W (n=9)	Group 7 ③+⑤	CXCL10, CXCL11, CXCL6, FGF-23, IL-12B, IL10, MCP-4, ST1A1, STAMBP	
Abnormal at 48W (n=0)	Group 8 ③+④	-	
Uncertain at 48W (n=6)	Group 9 ③+⑥	AXIN1, CCL20, CXCL1, CXCL5, FGF-21, SIRT2	

Classification was based on stringent criteria:

① Case : Control - Abnormal: t.test, Padjust <= 0.05
② Case : Control - Normal: t.test, Padjust > 0.05 & equivalence test, Padjust <= 0.05
③ Case : Control - Uncertain: t.test, Padjust > 0.05 & equivalence test, Padjust > 0.05
④ 48W : Control - Abnormal: t.test, Padjust <= 0.05
⑤ 48W : Control - Normal: t.test, Padjust > 0.05 & equivalence test, Padjust <= 0.05
⑥ 48W : Control - Uncertain: t.test, Padjust > 0.05 & equivalence test, Padjust > 0.05
⑦ Treatment-responsive: paired t.test, Padjust <= 0.1
⑧ Treatment-responsive: paired t.test, Padjust > 0.1

Supplemental Table 4. Antibodies used in flow cytometric analysis.

Antibodies	Source	Identifier
Alexa Fluor® 700 anti-human CD3 Antibody	Biolegend	Cat:317340; RRID: AB_2563408
FITC anti-human CD4 Antibody	Biolegend	Cat:357406; RRID: AB_2562357
PerCP anti-human CD8 Antibody	Biolegend	Cat:344708; RRID: AB_1967149
PE anti-human CD25 Antibody	Biolegend	Cat:302606; RRID: AB_314276
Brilliant Violet 510™ anti-human CD45RA Antibody	Biolegend	Cat:304142; RRID: AB_2561947
Brilliant Violet 605™ anti-human CD127 (IL-7Rα) Antibody	Biolegend	Cat:351334; RRID: AB_2562022
Brilliant Violet 421™ anti-human CD197 (CCR7) Antibody	Biolegend	Cat:353208; RRID: AB_11203894
Brilliant Violet 711™ anti-human TCR Vα7.2 Antibody	Biolegend	Cat:351732; RRID: AB_2629680
PE/Dazzle™ 594 anti-human CD161 Antibody	Biolegend	Cat:339940; RRID: AB_2565868
Brilliant Violet 650™ anti-human CD196 (CCR6) Antibody	Biolegend	Cat:353426; RRID: AB_2563869
Alexa Fluor® 647 Rat Anti-Human CXCR5 (CD185)	BD Biosciences	Cat:558113; RRID: AB_2737606
CD279 (PD-1) Monoclonal Antibody (eBioJ105 (J105)), Biotin, eBioscience™	Thermo Fisher	Cat:13-2799-82; RRID: AB_837120
CD194 (CCR4) Monoclonal Antibody (D8SEE), APC, eBioscience™	Thermo Fisher	Cat:17-1949-42; RRID: AB_2573176
PE-Cy™7 Streptavidin (SA)	BD Biosciences	Cat:557598; RRID: AB_10049577
PE-CF594 Mouse Anti-Human CD183 (CXCR3)	BD Biosciences	Cat:562451; RRID: AB_11153118