

Supplemental Material

Patient inclusion/exclusion criteria

Adult patients (≥ 18 years old at the time of randomization) with DM or PM, as defined by the Bohan and Peter criteria (22, 23) were randomized to sifalimumab or placebo treatment groups. For patients with PM, documentation of a muscle biopsy result that is consistent with the diagnosis of PM was required. Whole blood samples were collected in PAXgene RNA tubes (PreAnalytiX, Hilden, Germany) on study day 0 (before dosing) and on days 28, 56, and 98 postdosing. Muscle specimens were collected at day 0 and day 98. Control whole blood and muscle samples were obtained from 24 and 29 healthy donors, respectively. All healthy donors provided written informed consent.

All patients were required to meet at least two of the following criteria:

- a. Strength in Manual Muscle Test (MMT) $\geq 80/150$ but $\leq 125/150$ using the MMT-8 muscle group testing
- b. Patient Global Activity Assessment by visual analog scale (VAS) ≥ 2.0 cm on a 10-cm scale, which is included as part of CLINHAQ
- c. Physician Global Activity Assessment by VAS ≥ 2.0 cm on a 10 cm scale, which is included as part of MDAAT
- d. CLINHAQ disability index ≥ 0.25
- e. Global extramuscular activity assessment ≥ 1.0 cm on a 10-cm VAS scale (this measure is the physician's composite evaluation and is based on assessments of activity scores on the constitutional, cutaneous, skeletal, gastrointestinal, pulmonary and cardiovascular scales of the MDAAT)

Primary exclusion criteria included: prednisone treatment >35 mg/day, or equivalent dose of other corticosteroids within 14 days before study, hydroxychloroquine > 600 mg/day, mycophenolate mofetil > 3 g/day, methotrexate > 25 mg/week, azathioprine > 3 mg/kg/day, or any dose of cyclophosphamide, cyclosporine, or thalidomide within 28 days before study; on fluctuating doses of antimalarials, mycophenolate mofetil, methotrexate, leflunomide, or

azathioprine within 28 days before study or fluctuating doses of corticosteroids within 14 days before study; received leflunomide > 20 mg/day in the 6 months prior to study; treatment with any investigational drug therapy within 28 days before study or biologic therapies (eg, rituximab) within 30 days or 5 half-lives of the biologic agent, whichever is longer, before study; active or chronic infection within 28 days before study (includes TB); a history or severe viral infection; herpes zoster ≤ 3 months prior to study; infection with Hepatitis A, B or C virus and/or HIV-1 or HIV-2; vaccination with live attenuated viruses 28 days before study; pregnancy; alcohol or drug history <1 year before study; history of cancer, except for basal cell carcinoma or carcinoma in situ of the cervix treated with apparent success with curative therapy more than 1 year prior to study; a history of coagulation disorders; at screening blood tests indicating serum creatinine >4 mg/dL, neutrophils <1,500/mm³, platelet count <50,000/mm³; history of any disease other than DM or PM.

Baseline medications

The six most common immunosuppressants or corticosteroids that patients were on at baseline include the following:

PREDNISONE: 30

METHOTREXATE: 15

IMMUNOGLOBULIN: 7

AZATHIOPRINE: 6

DEXAMETHASONE: 2

MYCOPHENOLIC ACID: 1

Using the two most prevalent baseline medications and their doses, there was no correlation observed between baseline doses of either prednisone or methotrexate and the type I IFN signature (IFNGS; **Figure 1**).

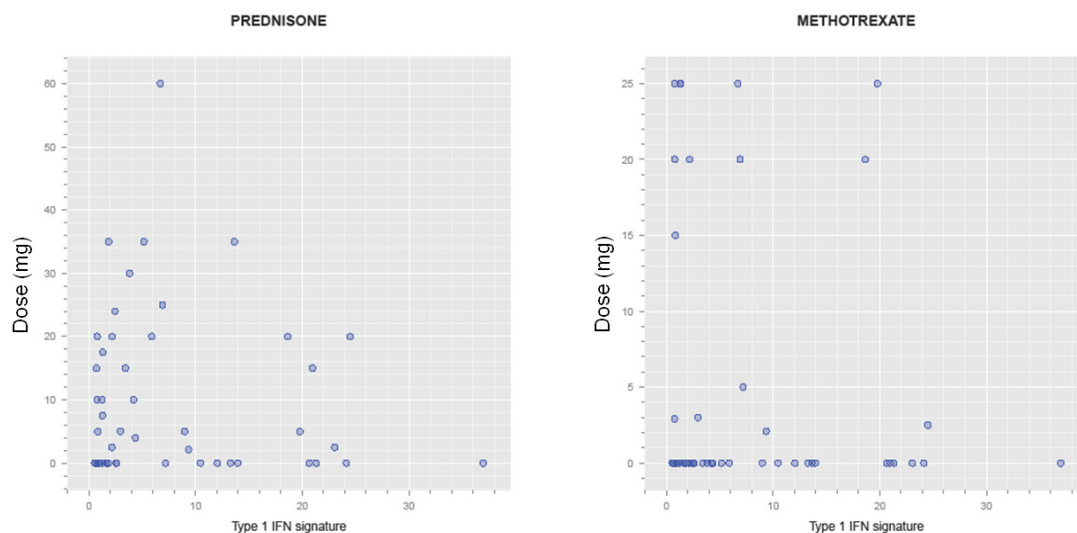


Figure 1: Correlation plots comparing baseline prednisone dose (left) or methotrexate dose (right) with the IFNGS.

Safety assessment

Safety assessments included adverse events (AEs), serious adverse events (SAEs), clinical laboratory evaluations (hematology, serum chemistry, and urinalysis), vital signs, viral cultures and titers, electrocardiograms (ECGs), physical examination, and concomitant medications measured from screening through Day 350.

Before Day 98,

- a total of 49 Treatment-Emergent Adverse Event (TEAEs) occurred in 10/12 subjects (83.3%) in the placebo group and a total of 172 TEAEs occurred in 34/39 subjects (87.2%) in the any sifalimumab group. The frequencies of TEAEs by SOC were generally similar between the 2 treatment groups, with the exception of Blood and Lymphatic System Disorders and Respiratory, Thoracic and Mediastinal Disorders, which occurred at a $\geq 10\%$ higher frequency in the any sifalimumab group compared to the placebo group. Events of the SOCs Investigations and Nervous System Disorders

were the most common in all cohorts. The frequencies of TEAEs by preferred term were generally higher in the any sifalimumab group compared to the placebo group. The most frequently occurring TEAEs (incidence > 10%) in the any sifalimumab group were headache, increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), and decreased lymphocyte count. The most frequently occurring TEAEs (incidence > 10%) in the placebo group were headache, urinary tract infection, hyponatremia, and myalgia. The majority of TEAEs occurring before Day 98 in either treatment group were Grade 1 or Grade 2 in severity

- A total of 2 TESAEs (atrioventricular block and hyponatremia) occurred in 1/12 subjects (8.3%) in the placebo group and a total of 4 TESAEs (coagulopathy, increased international normalized ratio, and two events of musculoskeletal chest pain) occurred in 2/39 subjects (5.1%) in the any sifalimumab group. Before Day 98, the incidences of TESAEs and related TESAEs were similar between the placebo and any sifalimumab groups, with no apparent dose effect across the individual sifalimumab groups.

On or after Day 98,

- a total of 306 TEAEs occurred in 47/51 (92.2%) subjects in all cohorts (78 TEAEs occurred in 11/12 [91.7%] subjects who received placebo before Day 98 and 228 TEAEs occurred in 36/39 [92.3%] subjects in the any sifalimumab group). Events of the SOCs of Infections and Infestations were the most common, followed by Musculoskeletal and Connective Tissue Disorders and Investigations in all cohorts. The most frequently occurring TEAEs (incidence > 10%) in all cohorts were upper respiratory tract infection, decreased lymphocyte count, headache, nasopharyngitis, and arthralgia
- 1 TESAЕ (non-cardiac chest pain) occurred in 1/12 subjects (8.3%) in the placebo before Day 98 group and 18 TESAЕs occurred in 8/39 subjects (20.5%) in the any sifalimumab group. One subject (3.0 mg/kg [3A cohort], sifalimumab group) had a related TESAЕ (Grade 3 pneumonia) that lasted 7 days. The subject completed the study.

IFNGS and target neutralization

The IFNGS score was calculated using 13 type I IFN-inducible genes (IFI27, RSAD2, IFI44L, IFI44, OAS1, IFIT1, ISG15, OAS3, HERC5, MX1, ESPT11, IFIT3, and IFI6) and reported as a median fold change relative to a pool of normal control samples, as described previously (15). Patients were identified as signature positive if their baseline IFNGS score in blood specimens was ≥ 2 or their gene signature in muscle specimens was ≥ 5 . Of the 38 IFNGS positive patients enrolled in the trial, 33 showed the signature above the cutoff in both blood and muscle. Two patients had muscle specimen but blood was not collected at baseline. Four patients were IFNGS positive in muscle while negative in blood.

For both blood and muscle, target modulation for each signature positive patient is defined as the median suppression of the 13 type I IFN-inducible transcripts. The suppression $S_{i,t,d}$ of a transcript t in a subject i at day d is calculated as follows:

$$S_{i,t,d} = \frac{P_{i,t,0} - P_{i,t,d}}{P_{i,t,0} - N_t}, \quad d=0, 28, 56 \text{ or } 98, \text{ where } N_t \text{ is the mean intensity value of the transcript } t \text{ in}$$

the normal samples ($n_{blood}=24$ and $n_{muscle}=29$) and $P_{i,t,d}$ is the intensity value of the transcript t of the sample from the subject i at day d . Therefore, $S_{i,t,0}$ is always equal to 0, that is, no neutralization at the baseline (day 0). There are some adjustments of $S_{i,t,d}$: the baseline signal intensity $P_{i,t,0}$ should be higher than $N_t + 1$ standard deviation of the signal intensities of the normal controls; $S_{i,t,d}$ is coded to null values otherwise, and $S_{i,t,d} = 1$ if $S_{i,t,d} > 1$ and $S_{i,t,d} = -1$ if $S_{i,t,d} < -1$.

Total RNA extraction and microarray processing

The Human Genome U133 Plus 2.0 array platform (Affymetrix, Santa Clara, CA, USA) was used to evaluate the effects of sifalimumab in whole blood and muscle specimens from matched myositis patients. The general procedures for sample processing and data analysis for microarray studies have been described previously.(1-3)

TaqMan assay methods

The TaqMan Low Density Array (TLDA; Applied Biosystems, Foster City, Calif, USA) was used to determine the Delta Cts (Δ Ct) for a panel of 13 genes in WB samples. GAPDH was used as the endogenous reference gene for calculation of delta Cts. Standard procedures for loading the array were followed and the array was run on a 7900HT Real-Time PCR System (Applied Biosystems). Calculation of the resulting Ct values was conducted with SDSv2.2.2 software (Applied Biosystems).

Serum protein profiling

Patient sera were procured both pre and 98 days post administration of placebo or sifalimumab, along with 25 healthy control serum samples. Those samples were frozen at -80°C before shipment to Rules Based Medicine (RBM) (Austin, Texas, USA) for a multiplexed immunoassay based on Luminex xMAP technology. A total of 128 proteins were selected from both the literature as well as a RBM Human MAP panel of markers including chemokines, cytokines, hormones, growth factors, and antigens. Samples were processed and analyzed at RBM according to standard operating procedure. All proteins had an established lowest limit of quantitation (LLOQ), which was used to exclude proteins with serum levels below the LLOQ in more than 70% of all samples.

Protein data analysis

Only those markers for which 30% or more of samples were above the LLOQ were included in subsequent data analysis. Protein concentration was \log_2 -transformed for statistical comparison. Post-treatment changes were assessed using a paired t-test followed by B-H adjustment.

Immunohistochemistry

Each frozen skin sample was embedded in optimum cutting temperature compound and sectioned at 5 μm . The cryo-sections were mounted on charged slides (Mercedes Medical, FL), and fixed in cold acetone. Before staining, sections were fixed in 10% Neutral Buffered Formalin

for 10 seconds, followed by 3 rinses in 1X TBS (Tris-Buffered Saline, pH 7.2). A blocking solution consisting of 3% Normal Goat Serum in 1X TBS was added to each slide for 10 minutes. This was followed by incubation with the designated primary antibodies, which was performed as follows: BDCA-2 (Miltenyl Biotec, Auburn, CA) at a 1:5 dilution for 5 hours; CD83 (Immunotech, Fullerton, CA) at a 1:200 dilution for 1.5 hours; and IP10 (Abcam, Cambridge, MA) at 1:5 dilution for 4 hours; The slides were washed with 1X TBS and incubated with either a peroxidase-labelled polymer conjugated to goat anti-mouse immunoglobulin antibody (Envision+; DakoCytomation, Carpinteria, CA) or a peroxidase-labelled goat anti-rabbit immunoglobulin antibody (Envision+; DakoCytomation, Carpinteria, CA) for 30 minutes, then washed with 1X TBS. Detection was performed using 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, St. Louis, MO) as the chromagen. Slides were washed in dH₂O, counterstained with Mayer's Hematoxylin, dehydrated and mounted with DPX Histology Mounting Medium (Fluka/Sigma-Aldrich, St. Louis, MO).

Identification of pathways downstream of type I IFN suppressed by sifalimumab treatment in muscle specimens from myositis patients

The number of patients displaying a target modulation of >37.5% for each transcript was captured for both sifalimumab and placebo cohorts. This threshold was determined empirically, where values >50% reduced the transcript list to a number too small for an accurate enrichment for pathway analysis. In patient muscle specimens, transcripts with target modulation values greater than 37.5% in at least 5 sifalimumab treated patients and displaying an odds ratio >2 relative to placebo treated patients were retained. Odds ratios were calculated as a function of the number of patients displaying a target modulation >37.5% or ≤37.5% for each transcript. Transcripts were then filtered to obtain transcripts unique to previously identified IFN-inducible genes. These genes were used for pathway enrichment analysis (Ingenuity Systems, Inc. Redwood City, CA, USA) to prioritize those signaling pathways most suppressed by treatment with sifalimumab. To reduce transcripts that are ubiquitous to many pathways in the results, an additional step was taken, where the top enriched pathways using a Benjamini and Hochberg (B-H) false discovery rate p-value adjustment of $p < 0.01$ were re-prioritized based on uniqueness of the transcripts to that pathway. It should be noted that for pathway analysis of transcripts in muscle specimens, only day 98 (the only time point available) was utilized.

IFNGS positive DM or PM patients available for transcript profiling at each time point and within each dose cohort in blood and muscle specimens

Blood					
	0.3 mpk	1.0 mpk	3.0 mpk	10.0 mpk	Placebo
Day-0 (DM)	2	3	7	2	4
Day-28 (DM)	2	3	7	2	4
Day-56 (DM)	2	3	6	1	4
Day-98 (DM)	2	3	6	2	4
Day-0 (PM)	4	5	2	2	5
Day-28 (PM)	4	5	2	2	5
Day-56 (PM)	3	4	2	2	4
Day-98 (PM)	3	5	1	2	4
Muscle					
	0.3 mpk	1.0 mpk	3.0 mpk	10.0 mpk	Placebo
Day-0 (DM)	2	3	9	2	4
Day-98 (DM)	1	3	9	1	3
Day-0 (PM)	4	4	1	2	6
Day-98 (PM)	3	4	1	2	5

Wilcoxon-Mann-Whitney p-values associated with target suppression at each time point in gene signature positive patients in each dose cohort (data plotted in Figure 1)

Dose cohort	Blood day 28	Blood day 56	Blood day 98	Muscle day 98
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0.3 mpk	0.002	0.002	0.002	0.570
1.0 mpk	0.06	0.05	0.05	0.613
3.0 mpk	0.003	0.007	0.01	0.633
10.0 mpk	0.83	0.78	0.28	0.776

Identification of gene signatures indicating leukocyte infiltration and the presence of immunoglobulin and MHC class I molecules (Zhu et al, 2012)

This leukocyte signature, specifically, was developed starting from a simple linear model. In this model, the overall expression level of the gene i in the subject j at a state s is considered as the sum of linear combination of the abundance of each cell type and its cell-type specific expression (represented by $c_{j,k}^s$ and $x_{i,j,k}^s$, respectively). Thus, the overall expression level of the gene i at a state s :

$$y_{i,j}^s = \sum_k c_{j,k}^s \cdot x_{i,j,k}^s \quad (1)$$

where $\sum_k c_{j,k}^s = 1$ for each subject j under the state s over all the cell types, k .

Assuming that there is a gene set I , which are only expressed in a subset of cell types T , i.e.,

$x_{i,j,k}^s \approx 0$ for $k \notin T$ under any state s . Thus,

$$y_{i,j}^s = \sum_{k \in T} c_{j,k}^s \cdot x_{i,j,k}^s = c_{j,t}^s \cdot x_{i,j,t}^s, \quad \text{where } c_{j,t}^s = \sum_{k \in T} c_{j,k}^s \quad \text{and} \quad x_{i,j,t}^s = \frac{\sum_{k \in T} c_{j,k}^s \cdot x_{i,j,k}^s}{\sum_{k \in T} c_{j,k}^s} \quad (2)$$

We may consider the mixture of the subset of cell types T as one special cell type t ,

and its fraction $c_{j,t}^s$ is the sum of all the cell types T and the expression level $x_{i,j,t}^s$ of the gene i in the virtual cell type t is thereby the weighted arithmetic mean of the gene expression within the subset cell types T .

The equation (2) suggests that the expression change as we capture in the transcriptional analysis can be attributed to the two factors: the alteration in the cell proportion and the expression change within the specific cell type.

Myositis muscle biopsies can be considered as normal skeletal muscles infiltrated by lymphocytes, conceptually, in terms of transcriptional analysis under a simplified way. Few leukocyte cells locate in normal muscle. In most of myositis muscles, the leukocyte cells increase dramatically, therefore leukocyte-specific transcripts are “over-expressed” compared to the

normal muscle, which is largely attributed to the change of the cell fraction. With some simple mathematical manipulation, it can further be postulated that all those leukocyte-specific transcripts appear “coherently” overexpressed no matter whether they are under the same transcriptional modulation unit or not. Therefore, the gene signatures developed from the gene clusters (tightly co-expressed genes) should indicate the abundance of the subtypes of infiltrated leukocytes

In a separate independent transcriptional analysis on muscle biopsies from myositis patients ($n=31$) and healthy controls ($n=5$), we identified a number of genes over-expressed in myositis subjects compared to healthy controls.(4) Those over-expressed transcripts formed four robust gene clusters using consensus clustering.(5) We further defined the gene signatures according to the functional annotations of the genes within the cluster, indicating: a type I IFN signature, an immunoglobulin signature, a leukocyte index and an MHC class I signature. Interestingly, the leukocyte index was in strong agreement with the pathological assessment of immune cell infiltration as assessed via hematoxylin and eosin (H&E) staining and accounted for the majority of variation observed in our transcriptional profiling study.

We identified a similar overexpression pattern in these four signatures in myositis muscle specimens from patients in this current clinical study and thereby utilized these gene signatures to better understand the downstream signaling pathways that are suppressed after treatment with an anti-IFN α mAb. This evaluation has demonstrated the robustness of the gene signatures in two independent cohorts of dermatomyositis and polymyositis patients, thus allowing us to better characterize inflammatory myopathies and enhance our understanding of the mechanism of sifalimumab in this disease.

References

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