

## Original Article

# Tocilizumab infusion therapy normalizes inflammation in sporadic ALS patients

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**Abstract:** Patients with sporadic amyotrophic lateral sclerosis (sALS) show inflammation in the spinal cord and peripheral blood. The inflammation is driven by stimulation of macrophages by aggregated superoxide dismutase 1 (SOD1) through caspase1, interleukin 1 (IL1), IL6 and chemokine signaling. Inflammatory gene activation is inhibited in vitro by tocilizumab, a humanized antibody to IL6 receptor (IL6R). Tocilizumab inhibits global interleukin-6 (IL6) signaling, a key mechanism in chronic rheumatoid disorders. Here we studied in vivo baseline inflammatory gene transcription in peripheral blood mononuclear cells (PBMCs) of 10 sALS patients, and the effects of tocilizumab (Actemra<sup>®</sup>) infusions. At baseline, one half of ALS subjects had strong inflammatory activation (Group 1) (8 genes up regulated >4-fold, P<0.05 vs. controls) and the other half (Group 2) had weak activation. All patients showed greater than four-fold up regulation of MMP1, CCL7, CCL13 and CCL24. Tocilizumab infusions in the Group 1 patients resulted in down regulation of inflammatory genes (in particular IL1 $\beta$ ), whereas in the Group 2 patients in up regulation of inflammatory genes. Post-infusion serum and CSF concentrations of tocilizumab inhibited caspase1 activation in vitro. Three of 5 patients receiving tocilizumab infusions showed time-limited attenuation of clinical progression. In conclusion, inflammation of sALS patients at baseline is up- or down-regulated in comparison to controls, but is partially normalized by tocilizumab infusions.

**Keywords:** Amyotrophic lateral sclerosis, tocilizumab, Actemra<sup>®</sup>, macrophage, superoxide dismutase1, caspase1, interleukin1, interleukin6, CCL24, CCL20

## Introduction

ALS is a tragic incurable disease and new therapeutic approaches are urgently needed. Patients with sporadic amyotrophic lateral sclerosis (sALS) have evidence of chronic peripheral and central nervous system inflammation with infiltration of the ALS spinal cord by inflammatory macrophages, IL17A-positive T cells, and mast cells [1, 2]. Inflammatory macrophages appear to have a central role in the demise of motor neurons in the ALS spinal cord because 19% motor neurons in post-mortem ALS spinal cords, including both healthy-appearing and apoptotic neurons, exhibited evidence of phagocytosis by interleukin-6 (IL6)- and tumor necrosis factor- $\alpha$  (TNF $\alpha$ )-positive macrophages [3]. The inflammation is present

in the peripheral blood as inflammatory genes for cytokines (interleukin-1 $\beta$  (IL1 $\beta$ ), IL6, TNF $\alpha$ ) and the chemokines (CCL3, CCL20, CXCL2, CXCL3, CXCL5) are highly up regulated in peripheral blood mononuclear cells (PBMCs) of sALS patients in Group 1, and weakly up regulated in PBMCs of patients in Group 2 [4]. When stimulated by fibrillar or demetallated wild type or demetallated mutant SOD-1, PBMCs of sALS patients up regulate the transcription and secretion of inflammatory cytokines, in particular IL1, IL6, and IL23A [2]. Some sALS patients intermittently display IL17A serum levels [2]. ALS patients with more rapidly progressing disease have decreased numbers of regulatory T lymphocytes [5], suggestive of an impact of unbridled inflammation on disease progression. In mutant SOD1 mice, astrocytes express-

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**Table 1.** ALS patients in the study: Demographics, ALS type, ALS Group, Actemra<sup>R</sup> therapy

#*	Age, Sex	ALS Type	ALS Group**	ALS duration (months)***	FRS-R change before Actemra <sup>R</sup> or first visit (points/mo)	FRS-R change after Actemra <sup>R</sup> (points/mo)****
1	59, M	Spinal	Group 1	24	-2.6	-0.4
2	55, F	Spinal	Group 1	38	-1.3	-0.75
3	52, F	Spinal	Group 1	20	-0.75	ND
4	51, M	Spinal and bulbar	Group 1	28	-1.0	ND
5	50, F	Spinal	Group 1	23	-0.59	ND
6	65, M	Spinal	Group 2	11	-0.7	+1.0
7	72, M	Spinal	Group 2	16	-0.37	-2.0
8	55, M	Spinal	Group 2	48	-0.47	ND
9	26, M	Spinal	Group 2	35	-0.37	ND
10	58, M	Spinal and bulbar	Group 2	11	-0.3	ND
11	63, M	Spinal	Group unknown	8	-3.5	-0.5

\*Patient #. \*\*Group 1=strong inflammation; Group 2=weak inflammation. \*\*\*at first blood test. \*\*\*\*decrease or increase of FRS-R points per month after Actemra.

ing mutant SOD-1 together with microglia produce toxic factors, such as cytokines, nitric oxide, peroxynitrite and superoxide, that decrease survival of neurons [6]. A positive role of microglia was demonstrated by ablation of proliferating microglia in mutant SOD1 mice, which led to decreased survival accompanied by decreased transcription of IL1 and TGF- $\beta$  but increased transcription of IL6 [7].

Inflammation is a current therapeutic target in sALS patients, but previous anti-inflammatory approach with the cyclooxygenase-2 (COX-2) inhibitor celecoxib [8] failed in clinical trials, and celecoxib did not show biological effect on prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels in the spinal fluid. COX-2 is an enzyme responsible for production of inflammatory mediators called prostanooids from arachidonic acid. When acetylated by aspirin, however, COX-2 stimulates production of pro-resolving and anti-inflammatory lipid mediators called resolvins (e.g. resolvin D1 (RvD1)) from omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [9]. RvD1 attenuates the transcription and secretion of inflammatory cytokines induced by aggregated SOD1 in ALS PBMCs and macrophages [3]. Thus COX-2 inhibition by celecoxib could have adverse effect on the production of resolvins, which are produced by prostanoid class switching [10]. On the other hand, ALS patients could benefit from fish oil supplements with DHA.

Interleukin-6 (IL6) signaling is a key mechanism targeted in chronic inflammatory diseases, such as rheumatoid arthritis, and, as suggested here, in ALS. Tocilizumab (Actemra<sup>R</sup>), a humanized antibody to IL6 receptor (IL6R) [11], inhibits global IL6 signaling, i.e. IL6R/gp130 membrane-associated signaling and IL6/sIL6 trans-signaling [12]. Tocilizumab, like resolvin D1, attenuated in vitro the pro-inflammatory effects of mutant SOD1 [4]. Actemra<sup>R</sup> is approved for therapy of rheumatoid arthritis and systemic juvenile idiopathic arthritis. Actemra<sup>R</sup> therapy produced clinical response and attenuation of IL6 signaling in a patient with neuromyelitis optica [13].

Tocilizumab was previously shown to attenuate inflammation in sALS patients in vitro [4]. The objective of this study was to analyze the in vivo effects of Actemra<sup>R</sup> infusions on peripheral blood inflammation at mRNA and protein level in order to advance the development of tocilizumab as an anti-inflammatory ALS approach.

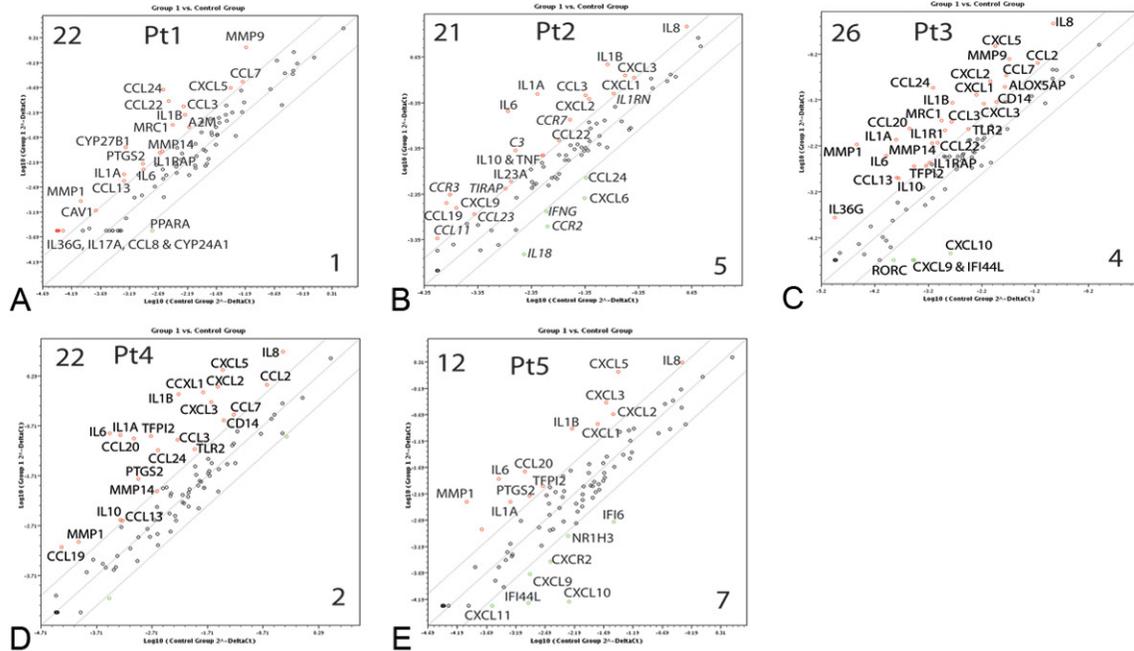
### Materials and methods

#### Study population

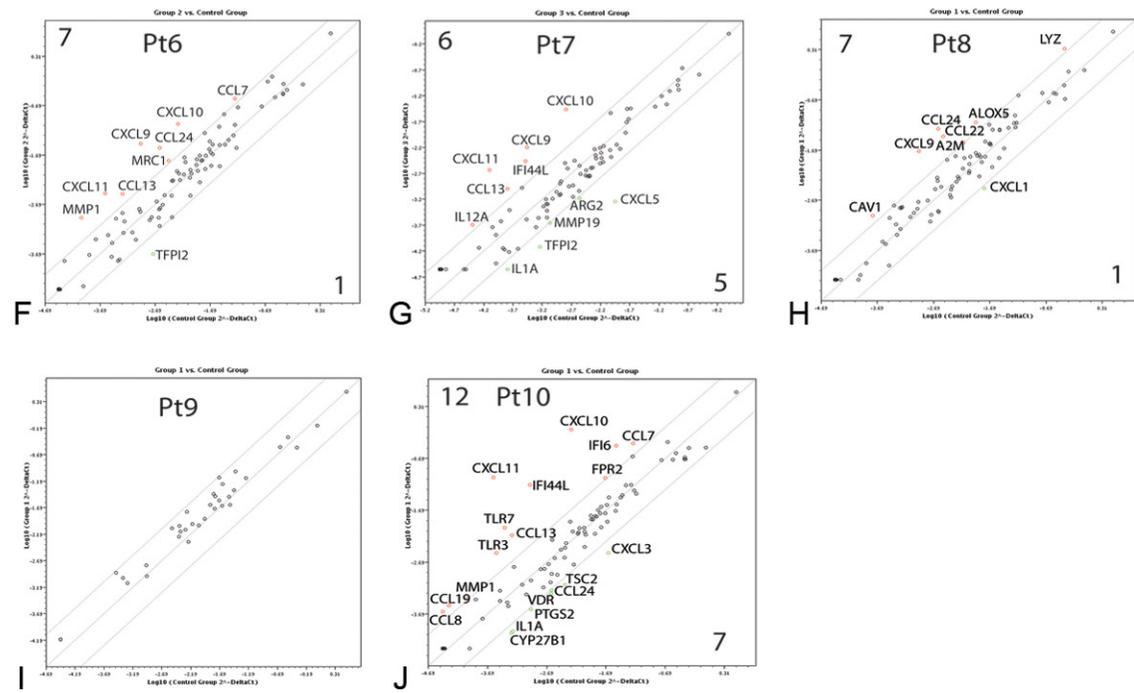
Eleven patients with sporadic ALS (#1-11) (mean age 55 years) were referred by their physicians for the study (Table 1). Four normal controls (mean age 66 years) were recruited from the UCLA personnel. All patients except patient #11 provided peripheral blood specimens (20

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## Group 1 ALS Patients (High Basal Inflammation) Baseline Patient/ Controls



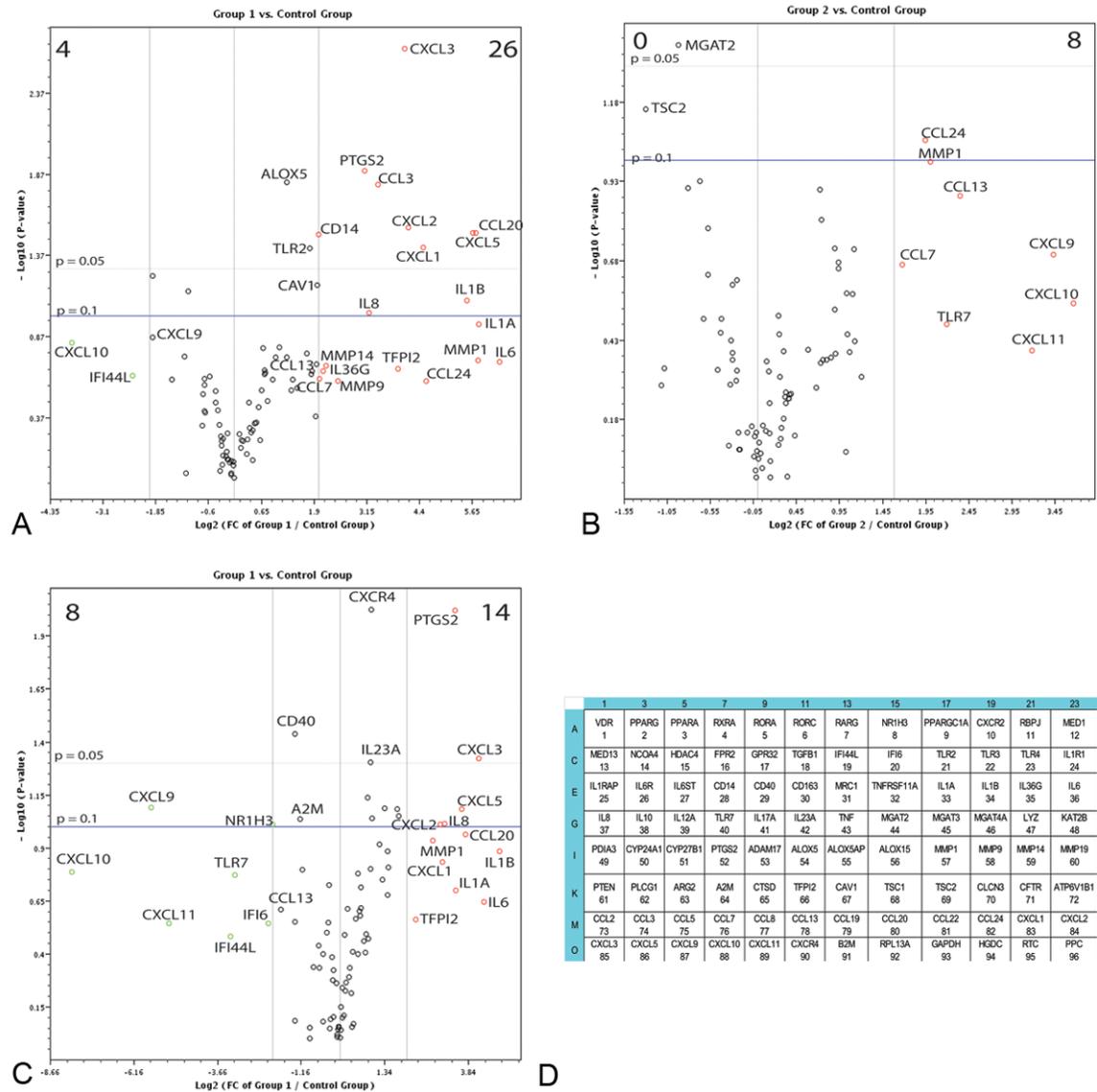
## Group 2 ALS Patients (Low Basal Inflammation) Baseline Patient/ Controls



**Figure 1.** Baseline mRNA expression of ALS patients compared to controls. In all panels the baseline mRNA expression in five million PBMCs isolated from ten different ALS patients is compared to four grouped controls to produce the scatter plots. All mRNA levels observed to be upregulated more than 4-fold are indicated by red, open circles and labeled with their gene symbol. Those that are more than 4-fold down regulated are indicated by a green, open circle and labeled with their gene symbol. The total number of genes deregulated is indicated. Panels (A-E) highlight the ALS patients that showed strong inflammatory activation in Group 1 (e.g. over 4-fold increased expression of cytokines, chemokines and matrixmetalloproteinases). It is noted that panel B results were obtained using a dif-

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ferent RT-PCR profiler array (PAHS077, version 3), while all other plots were generated by adding cDNA mixtures to a custom profiler array (see Figure 1D). Panels (F-J) highlight the ALS patients in Group 2 that showed low relative baseline inflammation when compared to controls. These patients did not show upregulation of cytokine expression when compared to controls and also showed lower up regulation of many chemokines when compared to the Group 1 samples. It is noted that patient 9 results were obtained with a custom eight sample, 48-gene array that contained nearly all the genes assayed in the other patients.



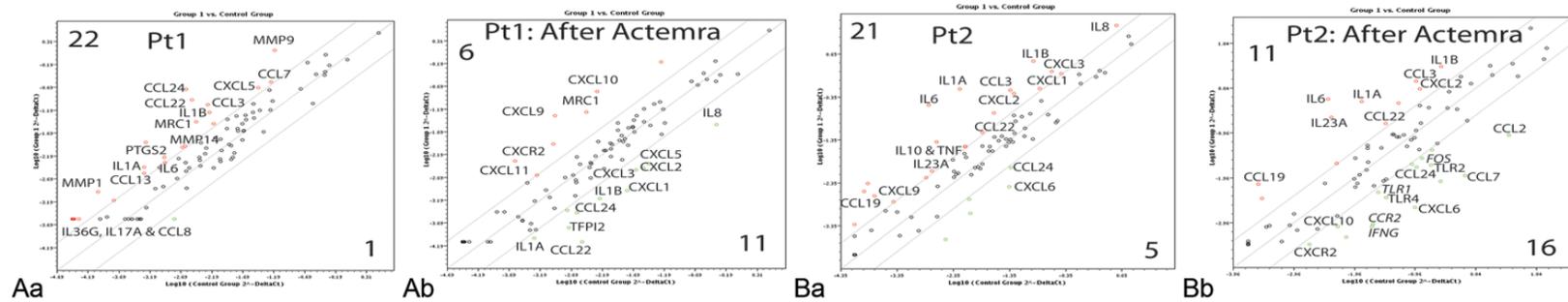
**Figure 2.** Statistical analysis of transcription in Group 1 and 2 patients. A: Inflammatory gene transcription in Group 1 (high inflammation) patients (n=5) vs. controls (n=4); B) Inflammatory gene transcription in Group 2 (low inflammation) patients (n=5) vs. controls (n=4); C) Inflammatory gene transcription in Group 1 vs. Group 2; D) Genes in the array. All >4-fold up regulated mRNA levels are indicated by red, open circles and their gene symbol; all >4-fold downregulated are indicated by green, open circle and their gene symbol. The total number of up regulated genes is in the left upper corner and the number of down regulated genes is in the right lower corner. The results on x-axis indicate four-fold up- or down-regulation, and on the y-axis significance (P<0.05).

cc) after signing the Informed Consent approved by the UCLA IRB. Patient #11 was followed according FRS-R score. The patients were diag-

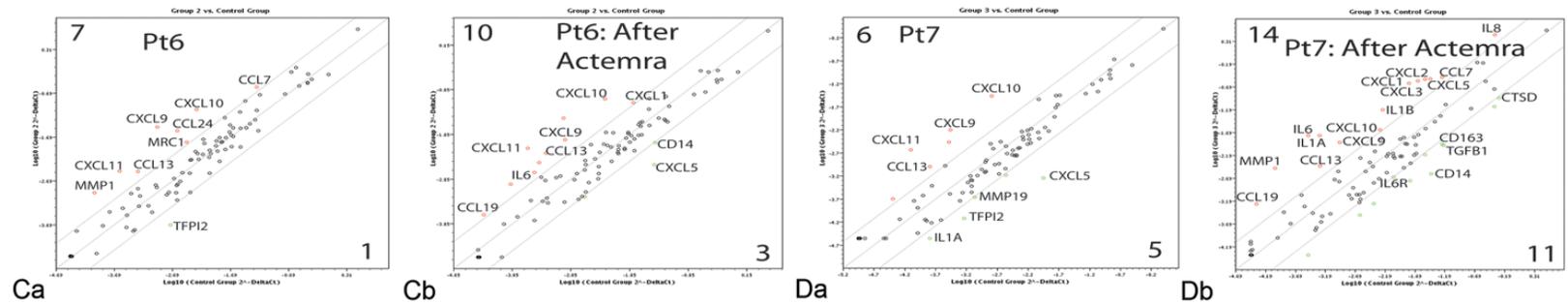
nosed as probable or definitive ALS by the revised El Escorial criteria [14]. Five patients (#1, 2, 6, 7, 11) received tocilizumab (Actemra<sup>®</sup>)

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## Group 1 ALS Patients (High Basal Inflammation)

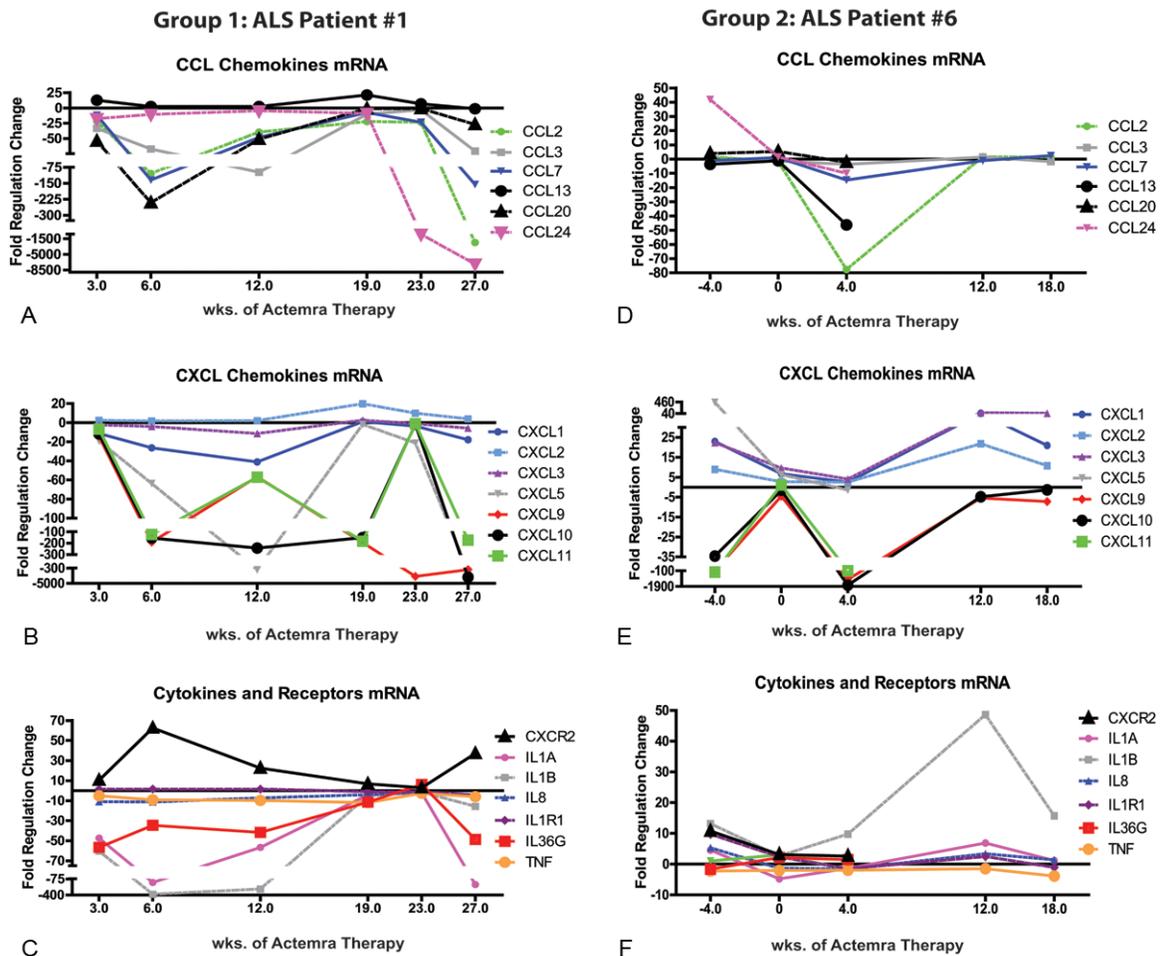


## Group 2 ALS Patients (Low Basal Inflammation)



**Figure 3.** mRNA transcription in sALS patients at baseline and after Actemra infusions A) to E) mRNA transcription in Group 1 (high inflammation) patients; Aa) Ba) baseline transcription in patients #1 and #2; Ab) Bb) transcription in patients 1 and 2 after Actemra infusion; F) to J) mRNA transcription in Group 2 (low inflammation) patients; Fa) Ga) baseline transcription in patients #6 and #7; Fb) Gb) transcription in patients #6 and #7 after Actemra infusion. In all panels, the transcription in each patient is compared to four grouped controls. All >4-fold up regulated mRNA levels are indicated by red, open circle and their gene symbol; all >4-fold down regulated mRNA levels are indicated by green, open circle and their gene symbol. The total number of up regulated genes is in the left upper corner and the number of down regulated genes is in the right lower corner.

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**Figure 4.** Longitudinal effects of Actemra<sup>R</sup> infusions on cytokine and chemokine mRNAs. A-C: Chemokine and cytokine mRNA transcription in PBMCs of patient #1 before and after the start of Actemra infusion therapy. D-F: Chemokine and cytokine mRNA transcription in PBMCs of patient #6 before and after the start of Actemra infusion therapy.

infusions from their physicians and four provided blood specimens obtained before and within 1 hour after the infusion (within approximately two hour interval) and one provided spinal fluid. The dosage and infusion schedule of Actemra<sup>R</sup> were the same as those used to treat patients with rheumatoid arthritis (4 mg/kg followed by an increase to 8 mg/kg every 4 weeks). Patients were free to take nutritional supplements, including fish oil as Smartfish<sup>R</sup> drink containing omega-3 fatty acids stabilized against oxidative degradation by botanical additives. Patients in the study self-reported the FRS-R scores [15].

### *Tocilizumab concentrations in the serum and spinal fluid*

Tocilizumab concentrations were tested using an enzyme-linked immunosorbent assay (ELISA), as previously described [16, 17].

**Blood cells:** Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by the Ficoll-Hypaque technique, and macrophages were prepared in 8-chamber slides with Iscove's Modified Dulbecco's Medium (IMDM) and 10% autologous human serum, as previously described [4].

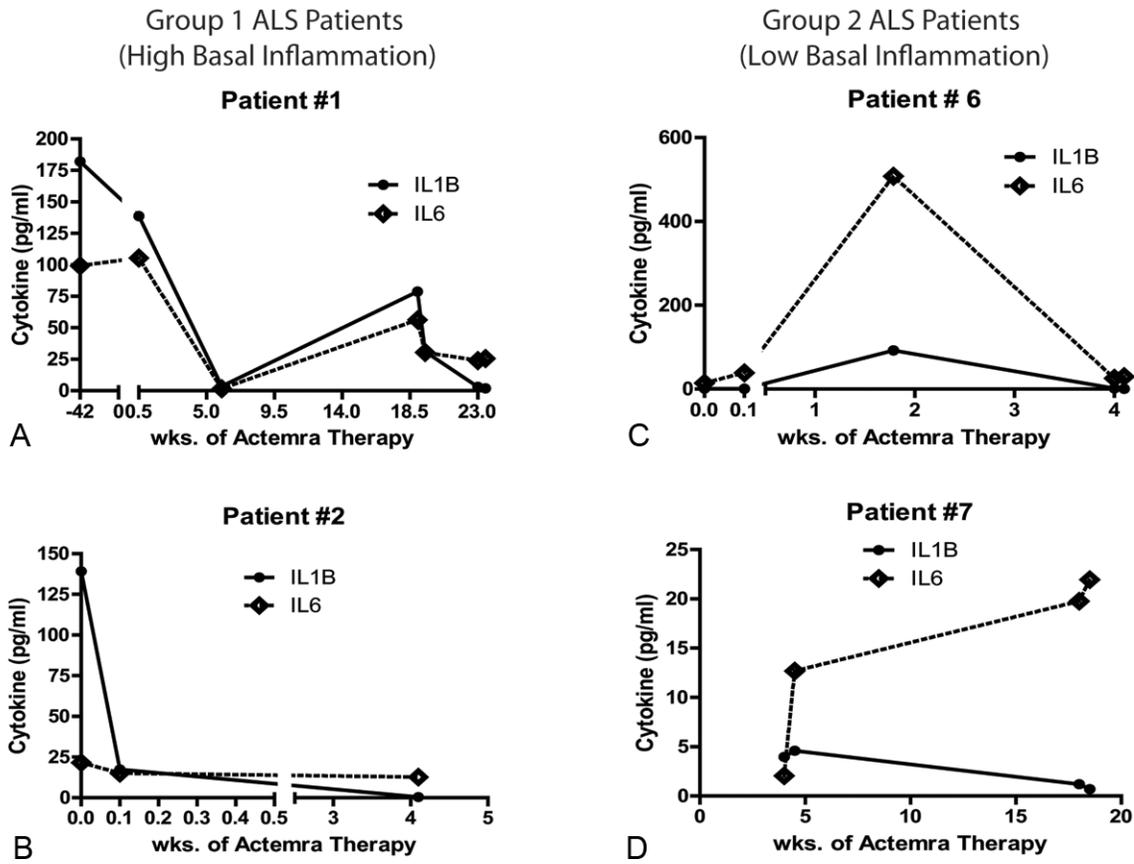
### *RT PCR assay of inflammatory and autoimmune gene mRNAs*

The assay was done using a custom array of 90 inflammatory genes (**Figure 2D**) on the Roche LightCycler 480 using the  $\Delta\Delta C_t$  method as described previously [4].

### *ELISA assay of IL1 $\beta$ and IL6*

IL-1 $\beta$  and IL-6 cytokines in the serum were assayed using the bead based multiplex

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**Figure 5.** Longitudinal effects of Actemra<sup>®</sup> infusions on cytokine serum levels. A and B: IL1 $\beta$  and IL6 serum levels in patients #1 and #2 before and after the start of Actemra therapy. C and D: IL1 $\beta$  and IL6 serum levels in patient #6 before and after the start of Actemra therapy. Acute effects are shown by the points, which are closely-spaced in time.

Fluorikine MAP<sup>®</sup> Inflammation kit following manufacturer protocol (R&D Systems, Minneapolis, MN, USA) and was analyzed using the Bio-RAD<sup>®</sup> - BioPlex<sup>®</sup> analyzer.

### Statistical analyses

The significance of gene up- or down regulation was determined by proprietary software by SABiosciences PCR Data Analysis Web Portal (Qiagen, Valencia, CA). The results indicate on x-axis four-fold up- or down-regulation, and on the y-axis significance ( $P < 0.05$ ).

### Results

#### Transcription of inflammatory gene mRNAs in Group 1 and 2 patients

We examined inflammatory gene mRNAs in PBMCs of ten sporadic ALS patients and compared them to four controls. Five patients showed strong inflammation at baseline (pre-

viously described as “Group 1” patients [4]), while five others had weak inflammation at baseline (“Group 2” patients [4]) (Figure 1). The inflammatory activation involved CC- and CXC-chemokines, cytokines, and metalloproteinases, as described previously [2, 3]. In all patients ( $n=10$ ), PBMCs showed a common “ALS signature”, i.e. greater than four-fold up regulation of MMP1, CCL7, CCL13 and CCL24 (Figure 2A). The RT PCR results were analyzed by statistical software (Qiagen, Valencia, CA) that provides significance (on y-axis) and fold upregulation (on x-axis). In Group 1 patients, 26 genes were four-fold upregulated (8 of these genes,  $P < 0.05$ ) and 4 genes (N.S.) were four-fold down regulated in comparison to controls (Figure 2A). In Group 2 patients, 8 genes were 4-fold upregulated (N.S) and no genes were down regulated (N.S.) (Figure 2B). In comparison to Group 2, Group 1 patients had a greater up regulation of inflammatory cytokines (IL1, IL6 and IL23A), enzymes (PTGS2 and MMP1), and some CC-

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**Table 2.** Effect Actemra<sup>R</sup> on the levels of active caspase-1 in macrophages of a control subject and ALS patient #6

Treatment	Control macrophages IOD/cell	Patient #6 macrophages IOD/cell
Untreated	624.6 ± 358.1	1903.6 ± 208.1
2 µg/ml apo-G37R OD1	1813.2 ± 618.7*	2243.2 ± 383.6
apo-G37R + 10 µg/ml Tcz	928.7 ± 533.3	1284.7 ± 366.1*
apo-G37R + 1.0 µg/ml Tcz	ND	803.8 ± 160.5*
apo-G37R + 0.1 µg/ml Tcz	ND	806.8 ± 558.9*
apo-G37R + 0.01 µg/ml Tcz	ND	1466.4 ± 737.2

Macrophages from a control subject and ALS patient #6 were untreated, treated with apo-G37R SOD1 alone or in combination with 0.01 to 10 µg/ml tocilizumab (Tcz) overnight, fixed with 4% PFA and stained for active caspase-1 (GeneTex). IOD/cell in the table represent averaged mean intensity values obtained from three separate experiments. Significant changes ( $p \leq 0.05$ ) in expression of active caspase-1 in macrophages treated with SOD-1 and different concentrations of tocilizumab in comparison to SOD-1-treated macrophages are indicated by (\*).

(e.g. CCL20) and CXC-chemokines (CXCL3, CXCL5, CXCR4), and a greater down regulation of CXCL9, 10 and 11 mRNA levels (**Figure 2C**).

### Acute effects of Actemra<sup>R</sup> infusions on inflammatory gene mRNAs

The in vitro effects of tocilizumab on the inflammatory gene mRNAs and proteins differ in Group 1 and Group 2 patients [4]. We tested gene transcription in two Group 1 and two Group 2 patients who were treated with Actemra<sup>R</sup> for 4-8 months. To test acute Actemra<sup>R</sup> effects, PBMCs were obtained immediately before and ~1 hr after the first full infusion and the effects were analyzed in relation to the pre-infusion sample. In addition, serum was obtained from the blood sample before and/or after each infusion for testing the cytokines.

Two Group 1 patients (#1 and #2) were treated with Actemra<sup>R</sup> and both showed after the infusion an acute, greater than four-fold down regulation of some of the inflammatory gene mRNAs that were up regulated at baseline. In patient #1 22 genes were >4-fold up regulated before Actemra therapy but only 6 genes after Actemra therapy; in patient #2, 21 genes were up regulated before therapy but only 11 after the therapy (**Figure 3Aa** and **3Ab**). In patient #1, the down regulated mRNAs included the cytokines

IL1 and IL6, and the chemokines CCL7, CCL22, CCL24 and CXCL5. In patient #2, the down regulated mRNAs included only the chemokines CCL7, CCL24 and CXCL6, but not the cytokines IL1 and IL6 (**Figure 3Ba** and **3Bb**).

Two Group 2 patients treated with Actemra<sup>R</sup> showed after the infusion an acute stimulatory effect on inflammatory markers: in patient #6, 7 genes were up regulated before Actemra<sup>R</sup> and 10 after Actemra<sup>R</sup> therapy (**Figure 3Ca** and **Cb**); in patient #7, 6 genes were up regulated before Actemra<sup>R</sup> and 14 after Actemra<sup>R</sup> therapy (**Figure 3Da** and **Db**). The up regulated gene mRNAs in both patients

included IL1 $\alpha$ , IL6, CXCL1 and CXCL2, but not IL1 $\beta$ .

### Longitudinal Actemra<sup>R</sup> effects on inflammatory gene mRNAs and serum cytokines

During Actemra<sup>R</sup> therapy, cytokine mRNAs were tested in patient #1 (Group 1) and patient #6 (Group 2). In patient #1 the mRNAs of inflammatory cytokines and chemokines were reduced for first 12 weeks; however, following week 12 and up to week 23, an inflammatory spike of most cytokines and chemokines was observed, which again decreased at week 27 (**Figure 4A-C**). IL1 $\beta$  and IL6 serum protein concentrations were high 15 months before and at the time of the initial 4 mg/kg Actemra<sup>R</sup> infusion. In the first 6 weeks of Actemra<sup>R</sup> therapy these levels plummeted, then increased in conjunction with the inflammatory spike, and again decreased at week 23 (**Figure 5A**). In Patient #2, serum IL1 $\beta$  and IL6 levels also decreased during the first four weeks of therapy, although the mRNAs were not reduced (**Figure 5B**).

In Group 2 patient #6 at 4 weeks after the initial infusion the chemokine and cytokine mRNA responses were variable but at 12 weeks all mRNAs increased or remained stable (**Figure 4D-F**). IL1 $\beta$  and IL6 serum concentrations were low prior to the first infusion; IL1 $\beta$  and IL6 levels increased after the first infusion and then

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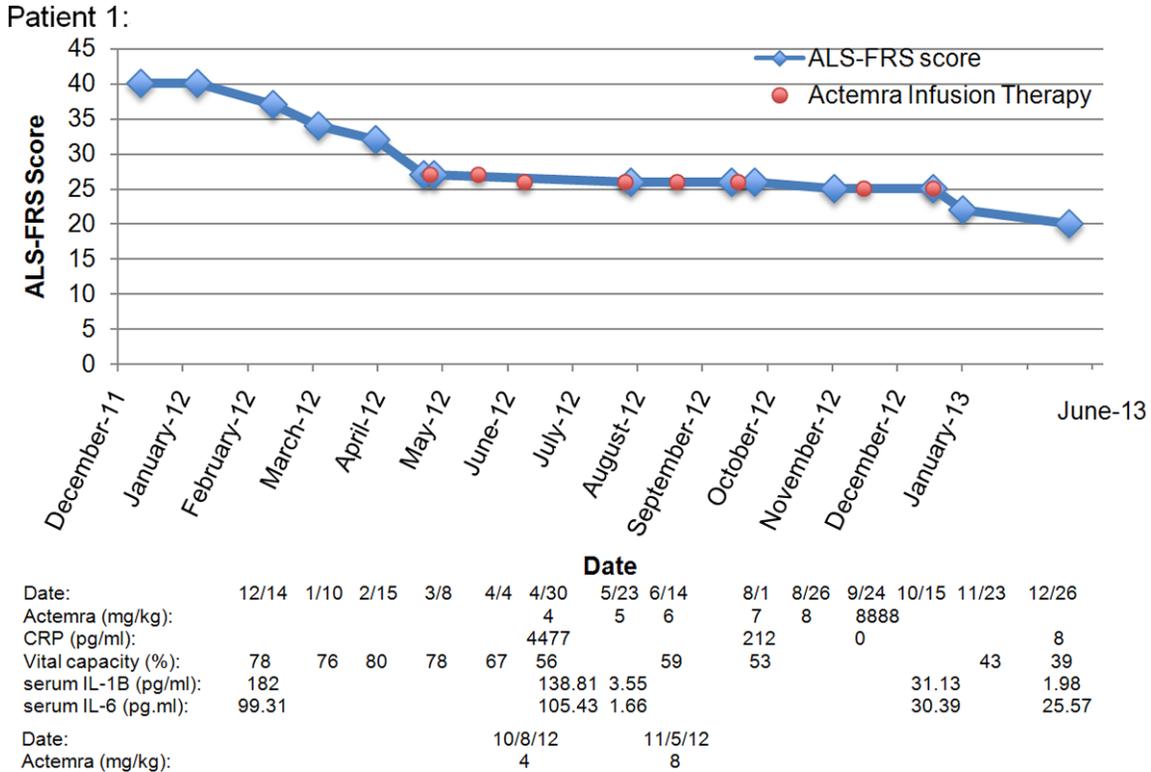


Figure 6. Neurological and laboratory progression in patient #1.

decreased (Figure 5C). In patient #7, IL6 level increased while IL1 $\beta$  level remained low (Figure 5D).

Thus, during Actemra<sup>R</sup> infusions, Group 1 patients experienced down regulation of the IL1 $\beta$  protein and mRNA levels, whereas Group 2 patients experienced up regulation of the IL1 $\beta$  mRNA and increased IL6 serum levels.

### Actemra<sup>R</sup> serum and spinal fluid concentrations and effect on activation of caspase-1

Tocilizumab concentrations in the serum of patient #1 at the 6<sup>th</sup> infusion were 12.3  $\mu$ g/mL before the infusion and 109  $\mu$ g/mL after the infusion; the concentration in the cerebrospinal fluid was 0.18  $\mu$ g/ml 2.5 hr after the infusion. Tocilizumab (10  $\mu$ g/ml) blocks in ALS macrophages inflammation [4], which can be induced by aggregated SOD1 through caspase-1 activation [3]. To determine if levels measured in the CSF of patient #1 following Actemra<sup>R</sup> infusion could impact caspase-1 activation in ALS macrophages, we tested different concentrations of tocilizumab (0.01  $\mu$ g/ml to 10  $\mu$ g/ml) against activation of caspase-1 in ALS macrophages.

Tocilizumab showed inhibitory effect at concentrations 0.1 to 10  $\mu$ g/ml (Table 2).

### Progression of ALS disability in ALS patients before and after Actemra<sup>R</sup> therapy

A comparison of the FRS-R scores before Actemra<sup>R</sup> therapy in all patients in Group 1 (high inflammation) and Group 2 (low inflammation) suggests that the decline per month in Group 1 was higher (range 0.59 to 2.6) than in Group 2 (range 0.3 to 0.7) (Table 1).

Five patients have been treated with Actemra, #1 for 8 months, #2 for 2 months, #6 for 4 months, #7 for 5 months, and #11 for 4 months (Table 1). Three patients showed strong attenuation of the loss of FRS-R points per month: #1 from 2.6 loss to 0.4 loss; #6 from 0.7 loss to 1 point gain; #11 from 3.5 loss to 0.5 loss (Table 1). The patients #1, #6, #11 have been also receiving nutritional supplementation with Smartfish<sup>R</sup> drink.

### Discussion

In our previous study, ALS patients were distinguished into Group 1 with strong inflammation

and Group 2 with weak inflammation, and in vitro tocilizumab appropriately attenuated (strongly down in Group 1 and weakly down in Group 2) inflammatory activation in ALS PBMCs in comparison to control PBMCs [4]. In the current study, we have tested 10 sporadic ALS patients and found that one half belonged to Group 1 and the other half to Group 2. Two patients in each group were treated with Actemra<sup>R</sup>. In Group 1 patients, Actemra<sup>R</sup> infusions were associated with dramatic down regulation of inflammatory cytokines (in particular IL1 and IL6) and chemokine mRNAs and proteins or only IL1 and IL6 proteins. In Group 2, IL1 and IL6 mRNAs were generally up regulated and IL6 protein was strongly up regulated. The results support a hypothesis that Actemra<sup>R</sup> infusions may benefit sALS patients by normalizing IL1 and IL6 expression, but the effects are individual and time- and dose-dependent. The in vivo activity is supported by the observation that the concentrations of tocilizumab in the serum of patient #1 were at least 100-fold higher than the concentration required for inhibiting activation of caspase-1 in vitro, and the concentration was just adequate in the spinal fluid for this purpose (**Table 2**).

Patient #1 (Group 1) has been observed for over 2 years and showed decreased rate of FRS decline after start of Actemra<sup>R</sup>. His C-reactive protein level acutely decreased and remained low (**Figure 6**). His PBMCs displayed up regulation of inflammatory cytokines and chemokines prior to Actemra<sup>R</sup> infusions but, after the start of Actemra<sup>R</sup>, showed strong down regulation of the cytokine and chemokine mRNAs and proteins for the first 6 to 12 weeks, followed by a spike of inflammation at 19 and 23 weeks and a return of the down regulation of inflammatory genes at week 27 (**Figure 2**). In the first four months of Actemra<sup>R</sup> therapy, mRNAs and cytokines were down regulated after each infusion, but the acute effects of Actemra<sup>R</sup> diminished later. Thus, the recurrence of inflammation in this patient could be related to (a) development of resistance to the acute effect of tocilizumab, (b) blockade of IL6 clearance by tocilizumab according to the “bath tub theory” [17], (c) sub therapeutic dose of tocilizumab. Patient #1 showed attenuation of ALS FRS-R decline while his inflammation was down regulated but, following the spike of inflammation, his ALS FRS-R decline pro-

gressed. We speculate that the recurrence of inflammation at weeks 19 and 23 caused irreversible damage in the ALS spinal cord by inflammatory macrophages [3].

In conclusion, at baseline ALS patients' PBMCs show heterogeneous inflammatory responses, up regulated in Group 1 and down regulated in Group 2. Actemra<sup>R</sup> infusion attenuated strong inflammation in Group 1 patients but actually increased weak inflammation in Group 2 patients. IL1 $\beta$  was a crucial cytokine affected by Actemra<sup>R</sup>: it was down regulated by Actemra<sup>R</sup> therapy when its transcription was up regulated, and it was up regulated when its transcription was down regulated. In comparison to the rate of neurological decline before Actemra<sup>R</sup> therapy, following Actemra<sup>R</sup> therapy the progression was attenuated in three patients. Because this study was not a controlled double-blind trial, clinical efficacy could not be ascertained and awaits a future clinical trial of tocilizumab, which is now warranted.

### Acknowledgements

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### References

- [1] Henkel JS, Engelhardt JI, Siklos L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR and Appel SH. Presence of dendritic cells, MCP-1, and activated microglia/macrophages

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- in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol* 2004; 55: 221-235.
- [2] Fiala M, Chattopadhyay M, La Cava A, Tse E, Liu G, Lourenco E, Eskin A, Liu PT, Magpantay L, Tse S, Mahanian M, Weitzman R, Tong J, Nguyen C, Cho T, Koo P, Sayre J, Martinez-Maza O, Rosenthal MJ and Wiedau-Pazos M. IL-17A is increased in the serum and in spinal cord CD8 and mast cells of ALS patients. *J Neuroinflammation* 2010; 7: 76-90.
- [3] Liu G, Fiala M, Mizwicki MT, Sayre J, Magpantay L, Siani A, Mahanian M, Chattopadhyay M, La Cava A and Wiedau-Pazos M. Neuronal phagocytosis by inflammatory macrophages in ALS spinal cord: inhibition of inflammation by resolvin D1. *Am J Neurodegener Dis* 2012; 1: 60-74.
- [4] Mizwicki MT, Fiala M, Magpantay L, Aziz N, Sayre J, Liu G, Siani A, Chan D, Martinez-Maza O, Chattopadhyay M, La Cava A. Tocilizumab attenuates inflammation in ALS patients through inhibition of IL6 receptor signaling. *Am J Neurodegener Dis* 2012; 1: 305-15.
- [5] Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, Liao B and Appel SH. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. *Brain* 2011; 134: 1293-1314.
- [6] Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann Neurol* 2009; 65 Suppl 1: S3-9.
- [7] Audet JN, Gowing G, Paradis R, Soucy G and Julien JP. Ablation of proliferating cells in the CNS exacerbates motor neuron disease caused by mutant superoxide dismutase. *PLoS One* 2012; 7: e34932.
- [8] Cudkovicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD and Drachman DB. Trial of celecoxib in amyotrophic lateral sclerosis. *Ann Neurol* 2006; 60: 22-31.
- [9] Serhan CN and Petasis NA. Resolvins and protectins in inflammation resolution. *Chem Rev* 2011; 111: 5922-5943.
- [10] Levy BD, Clish CB, Schmidt B, Gronert K and Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001; 2: 612-619.
- [11] Nishimoto N, Ito K and Takagi N. Safety and efficacy profiles of tocilizumab monotherapy in Japanese patients with rheumatoid arthritis: meta-analysis of six initial trials and five long-term extensions. *Mod Rheumatol* 2010; 20: 222-232.
- [12] Jones SA, Scheller J and Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J Clin Invest* 2011; 121: 3375-3383.
- [13] Kieseier BC, Stüve O, Dehmel T, Goebels N, Leussink VI, Mausberg AK, Ringelstein M, Turowski B, Aktas O, Antoch G and Hartung HP. Disease Amelioration With Tocilizumab in a Treatment-Resistant Patient With Neuromyelitis Optica: Implication for Cellular Immune Responses. *Arch Neurol* 2012; 1-4.
- [14] Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000; 1: 293-299.
- [15] Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B and Nakanishi A. The ALS-FRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci* 1999; 169: 13-21.
- [16] Nishimoto N, Yoshizaki K, Maeda K, Kuritani T, Deguchi H, Sato B, Imai N, Suemura M, Kakehi T, Takagi N and Kishimoto T. Toxicity, pharmacokinetics, and dose-finding study of repetitive treatment with the humanized anti-interleukin 6 receptor antibody MRA in rheumatoid arthritis. Phase I/II clinical study. *J Rheumatol* 2003; 30: 1426-1435.
- [17] Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N and Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood* 2008; 112: 3959-3964.