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1 **Online only methods**

2 Immunohistochemistry of human biopsy samples

3 Formalin-fixed, paraffin-embedded tissue sections (3-4 $\mu$ m thick) from right frontal biopsy  
4 material of a patient with anti-NMDAR encephalitis (case #5<sup>1</sup>) were deparaffinized and the  
5 antigen retrieved as reported. Sections were then serially incubated with 0.3% hydrogen  
6 peroxide for 15 minutes at room temperature, 10% horse serum in PBS for 1h, and the  
7 primary antibody overnight at 4°C. The following primary antibodies were used: polyclonal  
8 (goat) antibody CXCL13 (1:15; R&D System, Minneapolis, USA), CD68 (1:5000; activated  
9 microglia/macrophages, DAKO, Glostrup, Denmark), CD138 (1:50; plasma cells,  
10 plasmablasts, DAKO, Glostrup, Denmark), CD3 (1:100; Leica, Bannockburn, IL), CD8 (1:20;  
11 DAKO), CD4 (1:20; Biocare, Concord, CA), and CD20 (1:250; DAKO). The next day,  
12 sections were incubated with the appropriate secondary antibody (1:1000) for 1h at room  
13 temperature (Vector lab, Burlingame, CA, USA), avidin-biotin-peroxidase for 40 minutes,  
14 and visualized with diaminobenzidine (DAB) (Vector lab). Sections were then counterstained  
15 with Mayer's hematoxylin.

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17 **Online-only references:**

18 1. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M,  
19 Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-  
20 NMDAR encephalitis. *Neurology*. 2011;77(6):589–593.

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1 eTable 1: Statistical analysis of CSF CXCL13 in initial and follow-up samples of patients  
 2 with anti-NMDAR encephalitis

All patients n=167 first available sample					
General linear model			Post-hoc testing		
	P-value	Effect size <sup>o</sup>	Group	Mean <sup>&amp;</sup> (95% CI), n	P-Value
Corrected model	<b>0.001</b>	0.241			
Age	<b>0.005</b>	0.062			
Gender	0.914	0.000			
Maximum mRS 5	0.811	0.000			
Onset-to-sample time	<b>0.004</b>	0.065	months 1-2 <sup>¥</sup>	14.9pg/ml (10.6-21.0) n=113	
			months 3-4 <sup>¥</sup>	4.4pg/ml (2.0-9.7) n=21	<b>p=0.017</b>
			months 5-12 <sup>¥</sup>	3.5pg/ml (1.9-6.7) n=33	<b>p=0.0003</b>
Prodromal symptoms	<b>0.011</b>	0.051	prodrome <sup>#</sup>	20.3pg/ml (12.5-33.0) n=67	n/a
			no prodrome <sup>#</sup>	10.0pg/ml (6.2-16.0) n=44	
MRI abnormal	0.448	0.005			
CSF abnormal	0.117	0.020			
Stay in ICU	0.398	0.006			
Tumor found	0.104	0.021			
Prior immunosuppression <sup>§</sup>	0.302	0.009			
Interaction Prodrome * Tumor	<b>0.039</b>	0.034	no tumor, prodrome <sup>#</sup>	16.9pg/ml (9.9-28.9) n=56	p=1.0
			no tumor, no prodrome <sup>#</sup>	11.0pg/ml (6.6-18.4) n=36	
			tumor, prodrome <sup>#</sup>	26.2pg/ml (9.7-70.5) n=20	
			tumor, no prodrome <sup>#</sup>	6.4pg/ml (1.7-24.4) n=8	p=0.45

  

Patients treated within 90 days of onset n=137 first available sample					
General linear model			Group means		
	P-value	Effect size <sup>o</sup>	Group	Mean (95% CI), n	P-Value
Limited response at 8 months	<b>0.003</b>	0.078	Limited response at 8 months	16.4 pg/ml (10.0-26.8), n=57	n/a
			Favorable response at 8 months	8.6 pg/ml (CI 5.6-13.1), n=80	

  

Monophasic patients treated within 90 days from onset and follow-up samples available n=35						
Two-way ANOVA			Post-hoc testing <sup>@</sup>			
Variable	Source of variation	P-value	Months after treatment	Limited response, mean [95% CI],n	Favorable response, mean [95% CI],n	Multiplicity-adjusted p-value <sup>@</sup>
CSF CXCL13 in pg/ml in initial and follow-up samples n=35	Months after treatment initiation	<b>&lt;0.001</b>	1-2 months	24.7 [5.98-101.8],13	9.00 [2.37-34.1],13	0.23
	Response to therapy	<b>0.003</b>	3-6 months	12.7 [4.43-36.11],15	1.69 [0.99-2.88],13	<b>0.019</b>
	Interaction	0.36	> 6 months	1.96 [1.09-3.53],18	1 [1-1],9	0.32

  

Relapsing patients n=13				
Kruskal-Wallis-Test		Dunn's post-hoc testing		
Variable	P-value	Variable	Mean rank diff.	Multiplicity-adjusted P-value
CSF CXCL13 in pg/ml in initial and follow-up samples n=13	<b>0.004</b>	Initial vs. last sample	11.75	<b>0.044</b>
		Remission versus last sample	5.61	0.78
		Relapse versus last sample	11.5	<b>0.027</b>

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 4 <sup>&</sup>Logarithmical mean, recalculated after creation of the model leading to skewed 95% CI of  
 5 mean intervals. <sup>¥</sup>post hoc testing done using one-way ANOVA with Sidak-Holm post-hoc test  
 6 and multiplicity-adjusted p-values of difference to samples from months 1-2. <sup>#</sup>Post-hoc  
 7 testing done using unpaired t-tests and data from samples from months 1 and 2. <sup>§</sup>To correct  
 8 for effects of samples acquired before and after initiation of immunosuppression and allow for  
 9 the immunosuppressive effect on cytokine levels, any immunosuppressive treatment >14 days

1 before sample was included as a factor. °partial eta squared. \* Interaction analysis in  
2 univariate general linear model. @Post-hoc testing using Sidak-Holm procedure with  
3 multiplicity-adjusted p-values. ICU intensive care unit, n/a not applicable.

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### Supplemental figure legends:

8 eFigure 1: Serum concentration of CXCL13 is not different between anti-NMDAR

9 encephalitis and controls

10 Serum CXCL13 levels of patients with anti-NMDAR encephalitis (NMDAR) are not  
11 significantly different from those of controls with non-inflammatory conditions (NID).

12 Data is presented as logarithmic mean and 95% CI. CXCL13 concentrations below 1pg/ml are  
13 depicted as negative (neg).

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15 eFigure 2: CSF CXCL13 correlates with age but not with CSF titer of NMDAR antibodies.

16 **A)** CSF CXCL13 measured in samples from patients with anti-NMDAR encephalitis,  
17 showing patients' age in years at time of diagnosis. Samples obtained during months 1-2.

18 **B)** CSF CXCL13 concentration plotted against the CSF NMDAR antibody titer in the first  
19 available CSF sample of patients with monophasic anti-NMDAR encephalitis (n=30) shows  
20 lack of significant correlation.

21 Pearson  $R^2$  and significance p indicated. CSF CXCL13 concentrations below 1pg/ml are  
22 depicted as negative (neg).

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24 eFigure 3: Perivascular macrophages and activated microglia express CXCL13

25 **A-B)** Brain biopsy of a patient with anti-NMDAR encephalitis showing perivascular  
26 infiltrates expressing CXCL13 (**A**, arrow heads); these infiltrates were mainly composed of  
27 monocytes and macrophages (**B**, arrow heads indicate CD68 expressing monocytes and

- 1 macrophages).
- 2 **C-D**) CXCL13 was also expressed by activated microglia in the brain parenchyma (**C**,
- 3 CXCL13, arrow head shows a microglial cell; **D**, CD68, arrow heads show microglial cells).
- 4 No complement deposition was observed (Case #5<sup>1</sup>). Scale bar A-B 14  $\mu\text{m}$ , C-D 10  $\mu\text{m}$ .
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