Figure S1. Flow cytometry gating strategy for analysis of T cell subsets. A representative example of the gating strategy used for the phenotypic characterization is shown. Cellular debris and non-leucocytes were excluded as CD45RA⁻CD45RO⁻ (**A**); among leukocytes (CD45RA⁺ and/or CD45RO⁺), lymphocytes were identified based on FSC/SSC profile (**B**). T cells (CD3⁺) were selected from lymphocytes (**C**) and divided in CD4⁺ and CD8⁺ cells (**D**). Naïve and memory T cells were defined as CD45RA⁺CD45RO⁻ and CD45RA⁻CD45RO⁺, respectively, among CD8⁺ T cells (**E**). Expression of the activation markers CD69 and HLA-DR was analyzed among total CD8⁺ cells (**H** and **I**). Gates from naïve, memory, HLA-DR⁺ and CD69⁺ cells were transposed to CD4⁺ T cells (not shown).
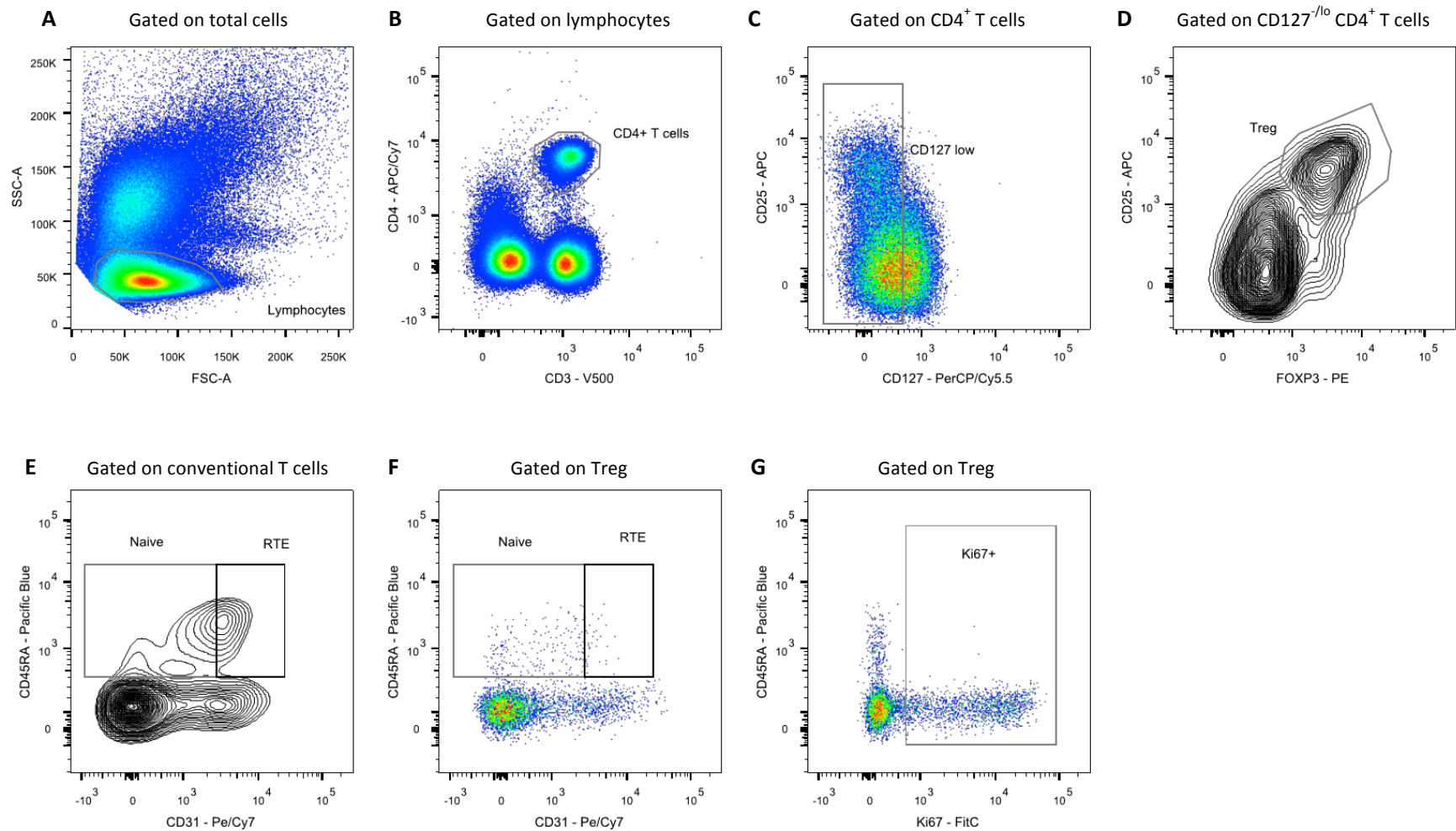
Figure S2. Flow cytometry gating strategy for analysis of regulatory T cells (Treg). A representative example of the gating strategy used for the phenotypic characterization of Treg is shown. Lymphocytes were identified based on FSC/SSC profile (**A**) and CD4$^+$ T cells (CD4$^+$CD3$^+$) were selected within these (**B**). Treg were defined as CD127$^{-/low}$ CD4$^+$ T cells (**C**) that were CD25$^+$FOXP3$^+$ (**D**). Conventional T cells (Tconv) were defined using a "no gate" from Treg (not shown). Naïve (CD45RA$^+$) and recent thymic emigrant cells (CD45RA$^+$ CD31^{Hi}) were selected within conventional T cells (**E**) and gates were transposed to Treg (**F**). Ki67 expression was analyzed within Treg (**G**).
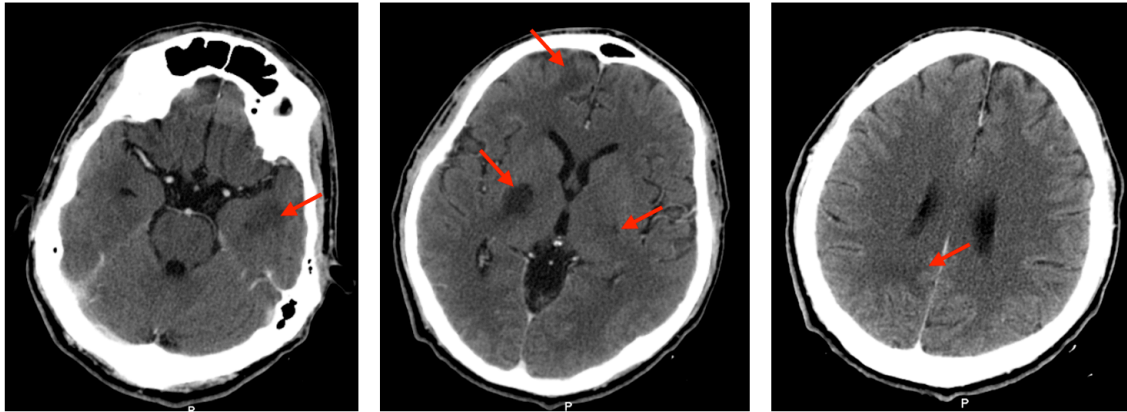
Figure S3. Brain CT performed after 14 days under specific anti-toxoplasma therapy, after contrast injection. Improvement of previous lesions is seen; mass effect and ring contrast enhancement are no longer evident.
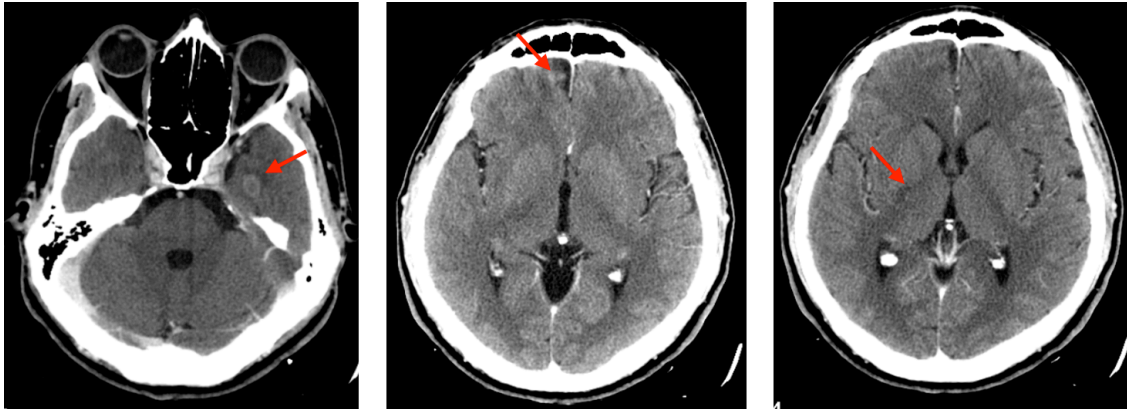

Figure S4. Brain CT performed after hospital admission, after contrast injection.
Several hypo-density lesions are present, some of them with slight perilesional edema and ring enhancement.

Table S1: Comparison between the T cell subsets dynamics of control group and the patient with toxoplasmosis associated CNS-IRIS.

		Baseline			1 Month			2 Months			3 Months			6 Months		
		Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]
Absolute count (cells/µL) — CD4+	Total CD4+	20.0	38.3 ± 30.7	p=0.111	67.0	109.0 ± 65.2	p=0.223	143.0	137.2 ± 58.9	p=0.776	128.0	107.4 ± 57.9	p=0.471	165.0	136.1 ± 44.6	p=0.088
	Naïve	3.2	3.4 ± 3.3	p=0.885	0.4	9.6 ± 10.6	p=0.122	1.0	7.1 ± 7.8	**p=0.048**	3.2	8.2 ± 6.8	p=0.172	26.2	15.3 ± 11.7	**p=0.024**
	Memory	14.0	32.5 ± 26.6	p=0.071	65.1	92.7 ± 53.9	p=0.316	137.6	124.4 ± 54.1	p=0.484	122.2	89.0 ± 47.9	p=0.196	129.4	112.9 ± 37.8	p=0.226
	CD69+	7.1	7.4 ± 9.2	p=0.933	15.4	19.8 ± 22.2	p=0.679	5.0	12.6 ± 6.6	**p=0.009**	23.2	15.5 ± 8.9	p=0.126	31.7	15.4 ± 8.1	**p<0.001**
	HLA-DR+	13.8	15.5 ± 15.5	p=0.748	38.3	45.9 ± 40.4	p=0.697	51.5	60.2 ± 29.9	p=0.408	79.5	43.4 ± 25.8	**p=0.035**	53.1	40.8 ± 13.3	**p=0.025**
CD8+	Total CD8+	1186.0	746.6 ± 415.0	**p=0.013**	1078.0	1288.8 ± 537.6	p=0.430	1308.0	1087.0 ± 448.7	p=0.178	852.0	920.4 ± 384.8	p=0.711	1132.0	957.6 ± 590.9	p=0.402
	Naïve	498.0	207.1 ± 155.2	**p<0.001**	538.0	377.8 ± 203.8	p=0.154	695.0	329.8 ± 226.5	**p=0.001**	344.0	297.6 ± 219.0	p=0.660	503.0	326.4 ± 256.1	p=0.104
	Memory	442.0	391.0 [191.0; 1446.0]	p=0.314	314.0	780.0 ± 646.7	p=0.182	419.0	458.0 [155.0; 1447.0]	p=0.214	244.0	313.0 [216.0 ; 977.0]	p=0.080	237.0	462.3 ± 296.3	p=0.052
	CD69+	248.0	173.8 ± 103.4	p=0.063	232.0	266.2 ± 154.0	p=0.645	84.0	154.8 ± 64.0	**p=0.011**	159.0	184.6 ± 118.0	p=0.653	191.0	138.1 ± 83.1	p=0.093
	HLA-DR+	306.0	170.0 [28.9; 966.0]	p=0.110	344.0	210.0 [181.0; 837.0]	p=0.686	229.0	333.9 ± 220.5	p=0.191	264.0	195.0 [140.0; 666.0]	p=0.500	307.0	235.7 ± 193.3	p=0.300
Percentage (%) — CD4+	Naïve (% among CD4+)	15.9	9.9 ± 8.7	p=0.073	0.6	5.1 ± 5.4	**p=0.050**	0.7	4.6 ± 4.8	**p=0.041**	2.5	5.2 ± 4.1	p=0.083	15.9	10.5 ± 7.2	p=0.053
	Memory (% among CD4+)	69.9	81.9 ± 10.9	**p=0.011**	97.1	89.5 ± 7.8	**p=0.029**	96.2	93.3 [75.5; 97.5]	**p=0.015**	95.5	92.2 [71.3; 95.0]	**p=0.008**	78.4	83.6 ± 10.0	p=0.155
	CD69+ (% among CD4+)	35.7	16.0 ± 7.9	**p<0.001**	23.0	10.0 [6.6; 38.3]	p=0.069	3.5	9.9 ± 5.2	**p=0.006**	18.1	11.8 ± 5.1	**p=0.006**	19.2	9.3 [7.2; 21.5]	**p=0.021**
	HLA-DR+ (% among CD4+)	69.0	36.8 ± 15.1	**p<0.001**	57.2	42.2 ± 19.1	p=0.061	36.0	43.9 ± 10.1	**p=0.046**	62.1	39.2 ± 14.5	**p=0.001**	32.2	32.1 ± 10.9	p=0.986
CD8+	Naïve (% among CD8+)	42.0	31.0 ± 18.3	p=0.107	49.9	32.5 ± 21.01	p=0.052	53.1	31.4 ± 19.9	**p=0.011**	40.4	32.9 ± 20.3	p=0.300	44.4	34.1 ± 19.7	p=0.155
	Memory (% among CD8+)	37.3	48.4 [39.2;85.3]	**p=0.008**	29.1	57.4 ± 24.4	**p=0.014**	32.0	55.2 ± 18.8	**p=0.006**	28.6	50.5 ± 15.4	**p=0.003**	20.9	50.0 ± 17.8	**p=0.001**
	CD69+ (% among CD8+)	20.9	23.7 ± 7.0	p=0.262	21.5	17.7 [11.5; 37.2]	p=0.208	6.4	15.0 ± 5.6	**p=0.002**	18.7	15.8 ± 5.5	p=0.151	16.9	11.9 [10.9; 24.0]	p=0.173
	HLA-DR+ (% among CD8+)	25.8	27.3 ± 14.1	p=0.752	31.9	30.6 ± 19.0	p=0.856	17.5	29.8 ± 10.7	**p=0.009**	31.0	25.8 ± 8.8	p=0.116	27.1	22.0 [9.7; 50.3]	p=0.110

[1] Mean ± SD are represented for variables normally distributed; median and [min; max] are represented for the variables not normally distributed.

[2] For normally distributed variables, comparison of the control group with the clinical case was performed by one-sample T-test; for the other variables a one-sample Wilcoxon signed rank test was applied. Cells in bold show significant differences (p<0.05) between the value of the clinical case and the mean/median of the control group.

Table S2: Comparison between regulatory T cell (Treg) subsets dynamics of control group and the patient with toxoplasmosis associated CNS-IRIS.

		Baseline			1 Month			2 Months			3 Months			6 Months		
		Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]	Case	Controls[1]	p value[2]
Absolute count (cells/µL)	**Treg**	3.2	6.1 ± 5.1	p=0.155	4.9	12.4 ± 5.8	**p=0.044**	17.4	23.7 ± 18.0	p=0.325	27.6	12.3 ± 6.8	**p=0.007**	22.1	15.8 ± 7.5	**p=0.036**
	Naïve Treg	1.1	0.1 ± 0.1	**p<0.001**	0.2	0.5 ± 0.4	p=0.154	0.6	1.3 ± 1.0	p=0.086	3.8	0.9 ± 1.1	**p=0.005**	3.23	1.5 ± 1.6	**p=0.015**
	RTE Treg	0.8	0.0 ± 0.1	**p<0.001**	0.2	0.2 ± 0.1	p=0.955	0.4	0.4 ± 0.3	p=0.824	1.0	0.3 ± 0.3	**p=0.012**	0.8	0.4 ± 0.6	p=0.117
	Ki67+ Treg	0.9	1.3 ± 1.2	p=0.514	2.2	3.6 ± 2.2	p=0.230	5.51	4.8 ± 3.3	p=0.622	3.2	2.9 ± 1.9	p=0.781	5.66	2.8 ± 1.3	**p<0.001**
Percentage (%)	**Treg (% among CD4+)**	16.2	19.4 ± 9.4	p=0.362	7.4	18.9 ± 10.5	**p=0.024**	12.2	16.4 [3.6; 38.7]	p=0.374	21.6	12.7 [8.9; 48.6]	p=0.110	13.4	12.3 ± 5.7	p=0.569
	Naïve (% among Treg)	33.6	1.7 ± 1.3	**p<0.001**	4.3	3.8 ± 2.4	p=0.634	3.5	6.3 ± 5.8	p=0.214	13.6	2.5 [0.2; 15.8]	**p=0.017**	14.6	5.4 [3.2; 25.0]	p=0.098
	RTE (% among Treg)	23.1	0.6 ± 0.6	**p<0.001**	2.9	1.4 ± 0.8	**p=0.001**	2.3	1.1 [0.6; 6.2]	p=0.161	3.5	1.1 [0.6; 5.2]	**p=0.025**	3.5	1.7 [0.6; 11.0]	p=0.161
	Ki67+ (% among Treg)	28.4	30.5 ± 19.4	p=0.820	44.8	28.3 ± 9.1	**p=0.007**	31.6	25.2 ± 6.1	**p=0.049**	11.6	20.0 ± 9.0	**p=0.049**	25.6	15.3 [13.1; 35.9]	p=0.069

[1] Mean ± SD are represented for variables normally distributed; median and [min; max] are represented for the variables not normally distributed.

[2] For normally distributed variables, comparison of the control group with the clinical case was performed by one-sample T-test; for the other variables a one-sample Wilcoxon signed rank test was applied. Cells in bold show significant differences (p<0.05) between the value of the clinical case and the mean/median of the control group.