Short Communication

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Therapeutic Depletion of Axotomy Competent Cells in Amyotrophic Lateral Sclerosis (ALS)

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The present paper addresses anatomically resolved protein networks by using the Imaging Cycler Microscopy (ICM/TIS) [1,2]. ICM is capable of resolving protein networks in intact anatomical structures at a power of combinatorial molecular resolution of $65{,}553^{k}$, where k is the number of co-mapped proteins, e.g. 100 proteins [1-4]. This method provides insight into the laws of the spatial communication of large protein networks in health and disease, which is essential for new therapy options in diseases.

As the ICM has shown, protein networks in intact anatomical structures tend to form several molecular emergent networks. For example, it has been shown that up to six different emergent protein networks can be detected within the Lamina lucida of the skin [1].

These networks have a diameter of 15 nm, and obviously serve as an assembly of biological quantum network structures that serve the real-time adaptation to shear forces in these skin structures (details to be published elsewhere). A partial network of this protein complement was found in mononuclear blood cells of Amyotrophic Lateral Sclerosis (ALS) patients (Figure 1b) [5]. These cells cross the post-capillary venules of the blood brain barrier (Figure 1a) and compress motor axons in ALS (Figure 2) in the first motoneuron. These invasive axotomy competent cells (ACC) were tested for their resistance/sensitivity related to UVA irradiation (Figure 1c).

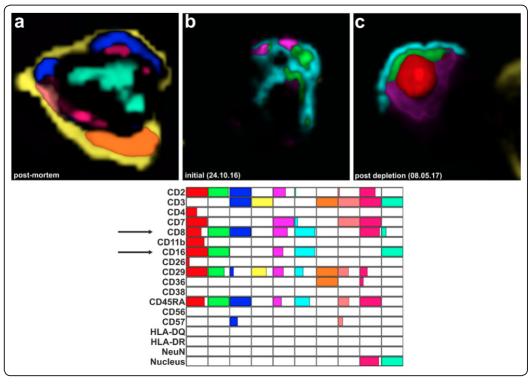

Figure 1: (a) Snapshot of an invasive ACC in postmortem tissue of ALS showing initial stage of invasion across the blood brain barrier (yellow), corresponding cells having been mapped by ICM profiles with lead proteins CD8/CD16 (arrows). Note that the ACC in the blood (b) of an ALS patient displays the identical ICM fingerprint as shown in the invasive postmortem ACC in Figure 1a. (c) Vital ACC (as in b) was tested for sensitivity of UVA irradiation as methodological principle in extracorporal photopheresis (ECP). The subcellular expression of the ICM profile is preserved, but the physiological cell structure is severely damaged. This indicates that ICM-controlled ECP is likely to be a therapeutical means for destruction of ACC's pathophysiological mechanism.

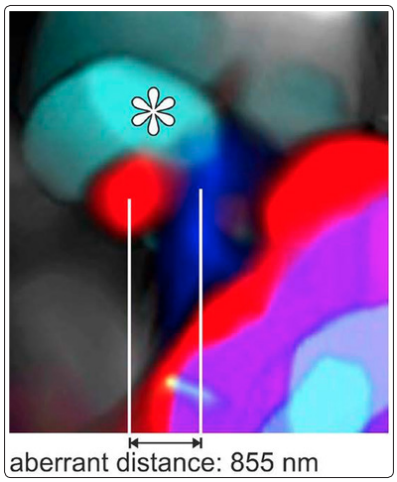

Figure 2: Detail showing NCAM-positive (light blue) axon of the first motoneuron (asterisk) being compressed by cell extension formed by CD3/CD8-positive invasive mononuclear cell. Note: The CD3 receptor (red) and the CD8 receptor (blue) are expressed in an extreme distance that does not allow for efficient cytotoxic action against the motoraxon (asterisk) [1], not preventing compression.

This connection implies a pathogenically important role of these cells. Here it is shown that these cells can be significantly damaged in the peripheral blood circulation by UVA light irradiation by using extracorporal photopheresis (ECP). In this damaged state (Figure 1c) these cells can no longer perform cell biological processes for the post-capillary pathological invasion into the pyramidal tract. As Figure 3 shows, the clinical findings of a male patient correlate with the progressive morphological sign of ECP-induced damage of the ACC. This fact suggests that the damage to the cells prevents

the progression of ALS symptoms and even causes a regression of the initial symptoms (Figure 3). As analyses in several advanced ALS cases indicate, the ACC are present in all patients, and additional clinical information indicates that the number of ACC per litre blood correlates with the progression rates of ALS: 140 mio ACC per litre blood are correlated with the disease progression that is 3 times faster than an ALS case with 50 mio ACC per litre blood. Corresponding blind samples were correctly assigned (Table 1 probe 1 C1).

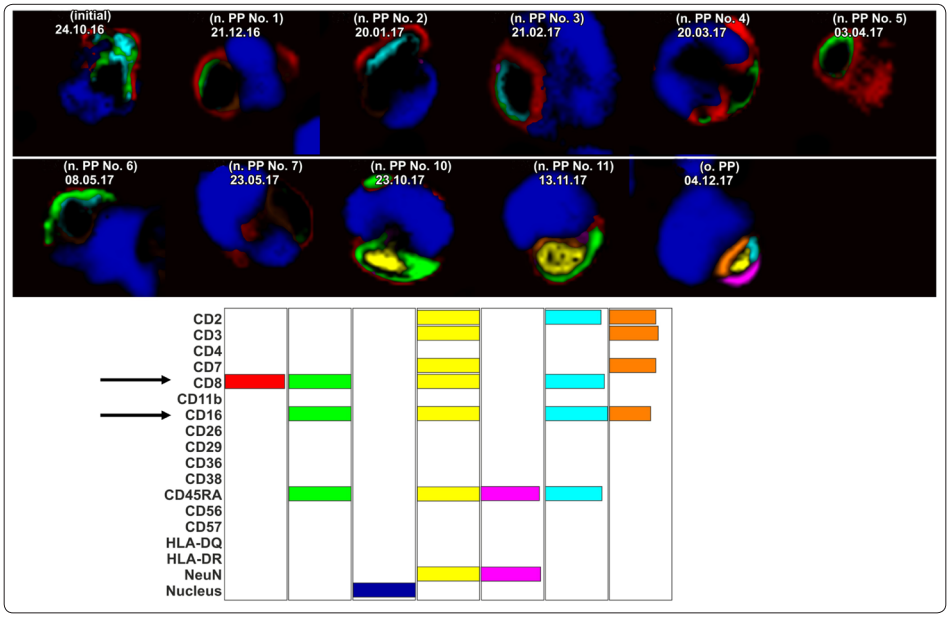

Figure 3: Synopsis of clinical examination/results and ICM-ECP controlled photopheresis of the initial ACC (cell panel, upper image on the left hand side); all remaining images from top left to bottom right show ICM-controlled ECP damaging process over a period of several months. It is seen that ACC undergo severe morphological alteration.

Neurological findings sorted by date of clinical examination:

December 1, 2016: suspected motoneuron disease (G12.2)

EMG: Chronic neurogenic remodeling of M. biceps brachii on the right and left hand side, in M. interossius dorsalis on the right hand side.

December 12, 2016: same findings as on December 1

February 27, 2017: No generalized fasciculations. Little fluidity in the disease process.

Condition stabilized. Subjective findings significantly improved. Good prognosis

May 12, 2017: Pain completely gone. Condition stabilized

August 24, 2017: No pathological spontaneous activity in the EMG. No evidence of disease susceptibility

The conclusion of these analyses is that the presence of pathogenic ALS cells in the blood circulation indicates to the clinician to immediately start with ECP induced ACC destruction accompanied by clinical reexaminations until the ACC cannot be verified anymore in the blood circulation. Present observations indicate that control of ACC in the blood of the patient, whose clinical remission has been documented in Table 1, have led to good clinical prognosis. Since spontaneous remission of ALS is usually not observed, the ICM-controlled destruction of ACC is the therapy of choice as a life-saving measure for ALS.

Table 1: More ALS patients, age 25 – 60 years:
Patients P1-P5 were tested for presence of ACC in the blood. All patients having been diagnosed for progressive ALS displayed ACC. Blind probes were correctly assigned to ALS. Healthy human blood did not display ACC (probe 1, C1).

probe	date	further details	CD2	CD3	CD4	CD7	CD8	CD11B	CD16	CD26	CD29	CD36	CD38	CD45RA	CD56	CD62L	CD71	HLA-DQ	HLA-DR	(approx. cells per liter)
probe 1	19.11.15	C1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	2.000M
probe 2	01.12.15	P1	*	*	*	*	1	*	1	*	*	*	0	*	0	0	0	0	0	84M
probe 3	02.12.15	P2	*	0	0	*	1	0	1	0	0	0	0	*	0	0	0	0	0	46M
probe 4	12.01.16	B1	*	*	*	*	*	*	*	*	*	*	0	*	0	0	0	*	*	0
probe 5	13.01.16	B2	*	*	*	0	1	0	1	0	*	*	0	0	0	0	0	0	0	50M
probe 6	14.01.16	B3	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	8M
-	09.01.16	P3	0	0	0	*	1	0	1	0	0	0	0	*	0	0	0	0	0	12M
-	09.01.16	P3	*	*	0	1	*	*	1	0	*	*	0	1	0	0	0	0	0	12M
probe 16	12.14.16	P2 (prior to leukapheresis)	0	*	0	*	1	*	1	0	*	0	0	*	0	0	*	0	0	58M
probe 17	14.04.16	P2 (after leukapheresis)	*	0	0	*	1	0	1	0	*	0	0	*	0	0	0	0	0	30M
probe 18	15.04.16	P2 (after photopheresis)	*	0	0	*	1	*	1	0	0	0	0	1	0	0	*	0	0	24M
probe 20	25.04.16	P2 (after photopheresis No. 2)	*	*	0	*	1	*	1	0	0	0	0	*	0	0	0	0	0	38M
probe 21	29.04.16	P4	*	*	*	*	1	*	1	*	*	0	0	*	0	0	0	0	0	140M
probe 22	02.05.16	P2 (after photopheresis No. 3)	*	0	0	*	1	0	1	0	0	0	0	1	0	0	0	0	0	8M
probe 23	04.05.16	P2 (after leukapheresis 2)	no ALS toponome present																	0
probe 24	18.05.16	P2	*	*	*	*	1	0	1	0	0	0	0	1	0	0	0	0	0	16M
probe 25	01.06.16	P2	*	0	0	1	1	*	1	0	0	0	0	1	0	0	0	0	0	16M
-	08.06.16	P3 (prior to photopheresis)	no ALS toponome present																	0
-	10.06.16	P3 (after photopheresis)	no ALS toponome present																	0
probe 26	13.06.16	P2	no ALS toponome present																	0
probe 27	24.10.16	P5	*	0	0	0	1	0	1	0	0	*	0	1	0	n/g	0	0	0	30M
DD 01	14.12.16	P5 (prior to photopheresis No. 1)	*	*	n/a	*	1	*	1	0	0	0	0	*	0	n/g	0	0	0	10M
DD 02	15.12.16	P5 (after photopheresis No. 1)	*	*	*	*	1	*	1	*	*	*	0	*	0	n/g	0	0	0	52M
probe 28	21.12.16	P5 (after photopheresis No. 1)	0	0	0	1	1	1	1	0	0	0	0	1	0	n/g	0	0	0	2M
probe 29	23.01.17	P5 (after photopheresis No. 2)	0	*	0	*	1	*	1	0	0	0	0	*	0	n/g	0	0	0	12M
DD 03	19.01.17	P5 (prior to photopheresis No. 2)	no ALS toponome present																	0
DD 04	20.01.17	P5 (after photopheresis No. 2)	no ALS toponome present																	0
probe 30	21.02.17	P5 (after photopheresis No. 3)	*	0	0	*	1	*	1	0	0	0	0	*	0	n/g	0	0	0	8M
DD 05	14.03.17	P5 (prior to photopheresis No. 4)	*	0	0	*	1	*	1	0	*	*	*	*	0	n/g	0	0	0	30M
Control	27.02.14	C2	no ALS toponome present																	0
probe 27	24.10.16	P5 (initial)	*	*	0	0	1	0	1	0	0	0	0	1	0	n/g	0	0	0	18M
probe 31	20.03.17	P5 (after photopheresis No.4)	*	0	0	*	1	*	1	0	*	*	0	*	*	n/g	0	0	0	28M
DD 06	29.03.17	P5 (prior to photopheresis No.5)	0	0	0	*	1	*	1	0	0	*	*	*	0	n/g	0	0	0	24M
probe 32	03.04.17	P5 (after photopheresis No.5)	*	0	0	*	1	*	1	0	0	0	0	*	0	n/g	0	0	0	14M
probe 32	03.04.17	P5 (after photopheresis No.5)	no ALS toponome present																	0
probe 32	03.04.17	P5 (after photopheresis No.5)	*	0	0	*	1	0	1	0	0	0	0	1	0	n/g	0	0	0	6M
probe 33	08.05.17	P5 (after photopheresis No.6)	*	0	*	*	1	*	1	0	*	*	0	*	0	n/g	0	0	0	22M
DD 07	05.05.17	P5 (prior to Photpherese No.6)	*	*	*	*	1	*	1	*	*	*	*	*	0	n/g	0	*	0	242M
probe 35	23.05.17	P5 (5d after photopheresis No.7)	*	*	0	*	1	*	1	0	0	0	0	1	0	n/g	0	0	0	4M
DD 10	19.06.17	P5 (prior to photopheresis No. 8)	no ALS toponome present																	0
probe 36	22.06.17	P5 (3d after photopheresis No.8)	no ALS toponome present																	0
DD 11	13.07.17	P5 (prior to photopheresis No.9)	no ALS toponome present																	0
probe 37	17.07.17	P5 (4d after photopheresis No.9)	no ALS toponome present																	0
DD 12	10.08.17	P5 (without photopheresis)	no ALS toponome present																	0
DD 12	10.08.17	P5 (without photopheresis)	no ALS toponome present																	0
probe 38	24.08.17	P5 (without photopheresis)	no ALS toponome present																	0
probe 39	26.09.17	P5 (without photopheresis)	*	*	0	*	1	0	1	0	*	0	0	*	0	n/g	0	*	0	30M
probe 40	23.10.17	P5 (14d after photopheresis No. 10)	*	0	0	*	1	0	1	0	0	0	0	1	0	n/g	0	0	0	34M
DD 14	08.11.17	P5 (prior to photopheresis No. 11)	no ALS toponome present																	0
probe 41	13.11.17	P5 (5d after photopheresis No. 11)	*	*	*	*	1	0	1	0	*	0	0	1	0	n/g	0	0	0	16M
probe 42	04.12.17	P5 (without photopheresis)	1	0	0	1	1	0	1	0	0	0	0	1	0	n/g	0	0	0	10M
probe 43	17.01.18	P5 (without photopheresis)	no ALS toponome present																	0
probe 44	11.04.18	P5 (without photopheresis)	no ALS toponome present																	0

These findings show, that

i. Axotomy competent cells (ACC) can readily be identified in ALS blood samples by ICM technology [1-5];
ii. ACC can be efficiently depleted by extracorporal photopheresis (ECP);
iii. successful depletion of ACC correlates with clinically documented regression of symptoms of initial stage of ALS.

These findings are supported by analyses of blood mononuclear cells in ALS patients with progressed stages of the disease (Table 1). Since ACC have been shown to invade the pyramidal tract, where they compress motor axons explaining the progressive clinical signs of ALS, these findings indicate that the ECP treatment of ACC can lead to severely functionally damage of ACC, so that these cells are incapacitated to invade the pyramidal tract at the post-capillary venules [1]. The data also suggest that the protein combinatorics at the cell surface of these cells represent a disease specific invasion address for the post-capillary venules of the first motoneuron. Hence, the decline of the clinical symptoms are explained by the ECP-induced damage of the specific combinatorial protein address code at the cell surface of the ACC. This therapy is well-tolerated and is likely to be a life-saving measure for ALS patients: If ACC are detected in the blood of any patient with suspected ALS, ACC destruction by ICM-controlled ECP is a new option in ALS clinical management.

These ACC must be immediately depleted in the blood circulation of these patients and must be controlled in the blood circulation over a time period of approximately 1 year. Together the present observations and conclusion are the result of hypothesis-free anatomical resolution of an ALS specific protein network in morphologically intact patient's cells and postmortem studies [1-5].

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