CYTOMETRY
Journal of Quantitative Cell Science PART A

Life-Saving Microscopy Method for Amyotrophic Lateral Sclerosis Patients

Walter Schubert*

• **Abstract**

The submission describes in the form of a communication recent experiences with a promising new therapeutic approach for amyotrophic lateral sclerosis (ALS). This approach is based on imaging cycler microscopy that led to the discovery of ALS specific cells in the blood, which invade the pyramidal system (lateral corticospinal tract) of ALS patients, where they compress motor axons. The depletion of these cells leads to remission of clinical symptoms and demonstrates the important role of these cells in ALS. The therapy will be offered to ALS patients in licensed and certified centers (in progress).

• **Key terms**

ICM robotics; amyotrophic lateral sclerosis; toponome; therapy

IMAGING cycler microscopy (ICM) is a device that can resolve randomly large toponomes in intact cells and tissues (1–3). Toponomes are defined as the spatial network code of proteins and other biomolecules (3). ICM has led to new insights into amyotrophic lateral sclerosis (ALS) (4). Toponomes are achieved when ICM overcomes the spectral resolution limit between 300 and 400 nm (visible light) by any number of incubation imaging bleaching cycles with scattered light similar to sunlight (1, 2). This leads to "molecular landscapes" in one and the same cell or tissue, which were previously unknown (2, 4, 5).

The application of imaging cycler microscopy (ICM) led to the description of the molecular geometry of ALS (4). The topological combinatorial molecular resolution, which fulfills the criteria of a highly organized molecular system (Zipf's law) (2, 6), revealed that in ALS high-dimensional molecular patterns occur in individual mononuclear blood cells (4, 7). These cells proved to be highly pathogenic since they penetrate the postcapillary venules of the tractus corticospinalis lateralis of the pyramidal tract (in ALS postmortem tissue Fig. 1a) (4). Inside the aforementioned tract they compress motor axons (Fig. 1d) (4). This mechanism is carried out by means of two cell extensions, which express CD8 and CD3 at a distance of 855 nm (Fig. 1d, image on the right). This distance does not allow a cytotoxic action against the motor axon, but is in keeping with an axon-compression-associated accumulation of transactive response DNA-binding protein 43 kDa (TDP-43) in ALS (4). These pathogenetic mononuclear cells are detectable in peripheral blood only of ALS patients (4, 7, 8) (Fig. 1b). As already discussed motor axon compression by axotomy-competent cells (ACC) might be a proximal cause of TDP-43 accumulation (4). Together these data suggest that ACC appear to be involved in the pathogenesis of ALS. Depletion of ACC by extracorporal photopheresis (ECP) showed that ACC were sensitive to ultraviolet A (UVA) irradiation (Fig. 1c). A 59-year-old male patient with suspected ALS showed ACC in his blood (Fig. 1b) and was therefore treated with ECP series over seven cycles at 14-day intervals (8). Figure 1c,e (gallery image) shows that the pathogenetic mononuclear cells (ACC) react very sensitively to UVA irradiation: while the molecular ICM-phenotype is retained (topological profiles below the gallery in Fig. 1e), the morphology of the cell is strongly altered, so that the cell polarization required for invasion of the pyramidal tract

Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany

Received 27 March 2020; Accepted 27 April 2020

*Correspondence to: Walter Schubert, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany Email: walter.schubert@med.ovgu.de

Published online in Wiley Online Library (wileyonlinelibrary.com)
DOI: 10.1002/cyto.a.24039

(as in Fig. 1a) (4) and axon compression (Fig. 1d, on the right) can no longer be functionally achieved. Therefore, seven ECP were performed to determine whether this finding can be observed constantly over a period of about 7 months. The latter turned out to be the case (Fig. 1e). After this time, ACC were no longer detectable in the blood, while the initial symptoms of ALS (as described in the figure legend) disappeared and were permanently undetectable over a period of

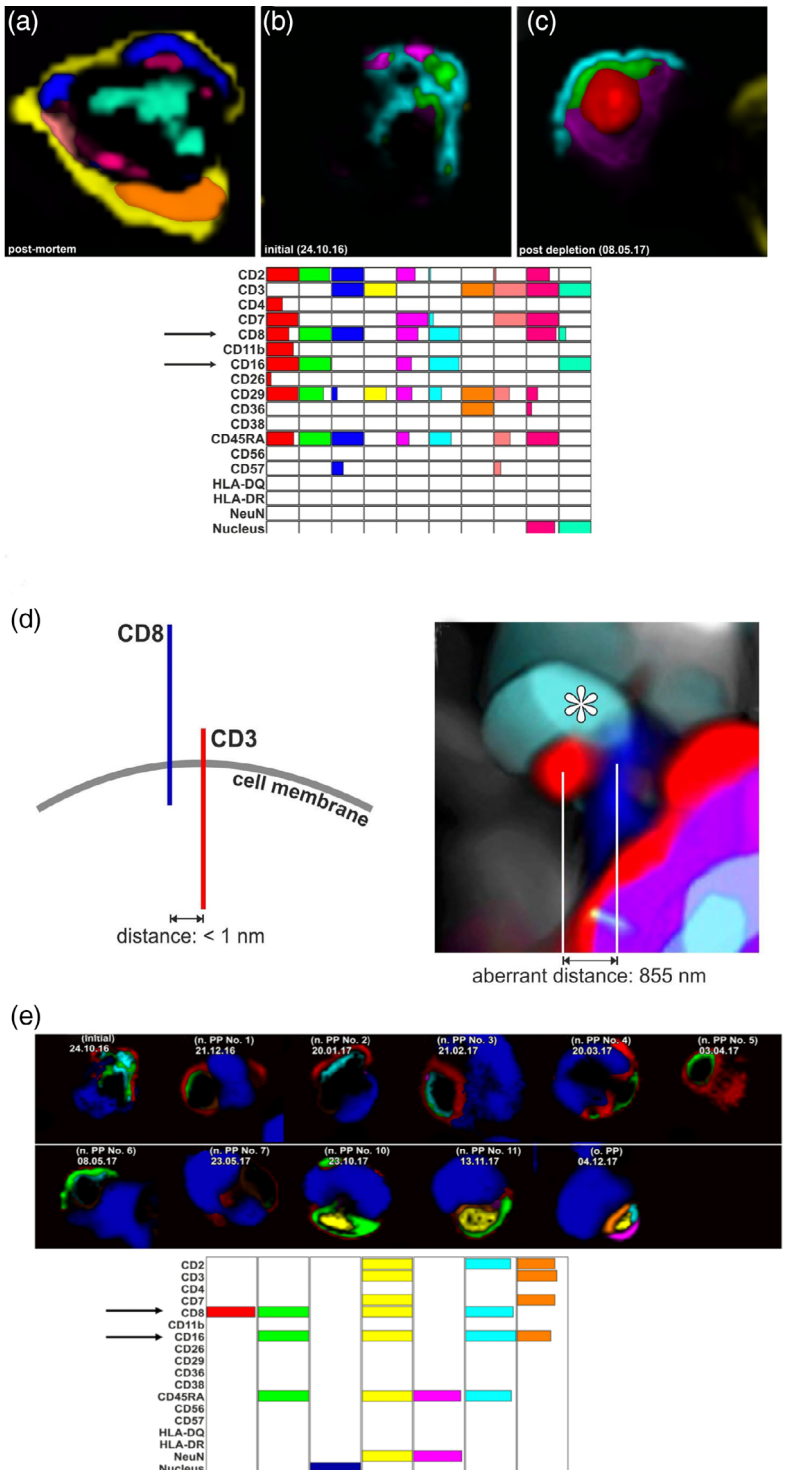

FIGURE 1. Legend on next page.

1 year. Since ALS does not remit spontaneously, the effect described is likely due to the therapeutic depletion of the ACC cell type. This can be explained by the above-mentioned pathophysiology of the sequence of events (ACC invasion, ACC axon compression). All patients diagnosed with ALS examined to date showed ACC in the blood, with the number per liter of blood corresponding to the rate of progression: ALS with 140 million ACC per liter of blood progressed three times as fast as ALS with 50 million ACC per liter of blood (8). This finding also supports the pathogenetic role of ACC in ALS and since therapeutic depletion is associated with clinical remission of symptoms, the relationships described above demonstrate a central role of ACC in ALS.

In conclusion, the facts briefly described here show that the systematic mapping of very high-dimensional, combinatorial functions of protein networks, also called the biological code (9) or the toponome (3), can lead to significant insights into disease mechanisms and new efficient therapeutic approaches. Similar topological approaches to the problem of spatial networks in cancer (10, 11) are also expected to lead to new forms of therapy, as they are based on topological disease mechanisms that cannot be identified by biochemical proteomics approaches (12).

Note that certified/licensed centers offering the described therapy are in progress.

Remark: The ICM-control of the ECP procedure is mandatory in order to readily detect unwanted sideeffects and determine the endpoint of the ECP treatment.

REFERENCES

1. Schubert W. Multiple antigen—mapping microscopy of human tissue. In: Burger G, Oberholzer M, Vooijs GP, editors. Advances in Analytical Cellular Pathology. Amsterdam: Elsevier, 1990; p. 97–98.
2. Schubert W, Bonnekoh B, Pommer AJ, Philipsen L, Boeckelmann R, Maliykh J, Gollnick H, Friedenberger M, Bode M, Dress AW. Analyzing proteome topology and function by automated multidimensional fluorescence microscopy. Nat Biotechnol 2006;24:1270–1278.
3. Schubert W. Topological proteomics, toponomics, MELK-technology. Adv Biochem Eng Biotechnol 2003;83:189–209.
4. Schubert W. A platform for parameter unlimited molecular geometry imaging obviously enabling life saving measures in ALS. Adv Pure Math 2018;8:321–334.
5. Abbott A. Mapping togetherness (research highlight). Nature 2006;443:609.
6. Friedenberger M, Bode M, Krusche A, Schubert W. Fluorescence detection of protein clusters in individual cells and tissue sections by using toponome imaging system: Sample preparation and measuring procedures. Nat Protocol 2007;2:2285–2294.
7. Schubert W. Advances in toponomics drug discovery: Imaging cycler microscopy correctly predicts a therapy method of amyotrophic lateral sclerosis. Cytometry A 2015;87(8):696–703. https://doi.org/10.1002/cyto.a.22671.
8. Schubert W. Therapeutic depletion of axotomy competent cells in amyotrophic lateral sclerosis (ALS). Adv Neur Neur Sci 2019;2(1). https://irp-cdn.multiscreensite.com/d33e6613/files/uploaded/therapeutic-depletion-of-axotomy-competent-cells-in-amyotrophic-lateral-sclerosis-als-anns-19.pdf.
9. Schubert W. Breaking the biological code. Cytometry A 2007;71:771–772.
10. Sage L. The molecular face of prostate cancer. J Proteome Res 2009;8:2616.
11. Cottingham K. Human toponome project—Human proteinpedia is open for (free) business. J Proteome Res 2008;7:1806.
12. Di Meo A, Diamandis EP, Rodriguez H, Hoofnagle AN, Ioannidis J, Lopez M. What is wrong with clinical proteomics? Clin Chem 2014 Oct;60(10):1258–1266. https://doi.org/10.1373/clinchem.2014.225185.

FIGURE 1 (a) Mononuclear cell in the process of transmigration through the postcapillary endothelium (yellow) in the tractus corticospinalis lateralis (postmortem study (4)). (b) The same cell type as seen in (a) in the blood circulation of a patient with initial ALS symptoms (same profile). (c) The cell type under (b) was irradiated with UVA light (extracorporal photopheresis); it can be seen that the molecular phenotype (high-dimensional protein profile below (a–c)) in (b) is preserved, while the cell morphology is strongly altered. The protein profile shown in (a–c) is topologically resolved and contains the so-called lead proteins CD8/CD16 (arrows), while the morphology of the cell (c) is severely altered, so that pathological homing to the tractus corticospinalis lateralis is most likely not possible anymore. (d) The micrograph on the right taken by an ICM robot (in the postmortem study (4)) shows neural cell adhesion molecule (NCAM)-expresssing axon (asterisk), which is compressed by the two cell-extensions expressing CD3 (red) and CD8 (blue) with an aberrant distance of 855 nm. This distance does not allow for cytotoxic actions against the motor axon (blue). For an active cytotoxic action against the axon a distance of CD3 and CD8 receptors as schematically shown in (d, on the left) would be required to generate cytotoxic cascades. The cell extensions belong to the invasive mononuclear cell in ALS, which is described in the text. (e) ICM robotics was used to analyze mononuclear cells of the above-mentioned patient with early stage suspected ALS. Image gallery cell top left (date October 24, 2016): the so-called axotomy competent cell (ACC) as above under (b) before photopheresis treatment (note: blue color = cell nucleus). All images of the gallery in (e) were taken by an ICM robot for precise subcellular localization of 18 cell surface proteins (see profile below the images; note: proteins indicated by horizontal arrows are the so-called lead proteins (4)). The corresponding cell form was treated with an extracorporal photopheresis series from October 24, 2016 to December 4, 2017 and subjected to the Imaging Cycler control in the respective treatment sessions (from December 21, 2016 to December 4, 2017). A clear morphological UVA-induced change can be seen, which no longer permits physiological cell functions such as cell polarization. During the whole session of ECP treatment the molecular phenotype is preserved (protein profiles below the image gallery in (e)). After this phase (see image gallery) the cell type was no longer detectable in the patient's blood circulation. Results of photopheresis treatment (e) were accompanied by neurological controls with the results as follows (neurological findings sorted by date of clinical examination): December 1, 2016: suspected motoneuron disease; EMG: chronic neurogenic remodeling of Musculus biceps brachii on the right and left hand side, in Musculus interossius dorsalis on the right-hand side; December 12, 2016: same findings as on December 1, 2016; 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; Since December 4, 2017 recurrences of clinical signs were not reported. These clinical results were documented by neurological facilities of the Ludwig-Maximilians-University, Munich. (a–d) Cell diameter approximately 10 μm; (e) cell diameter approximately 7 μm. ALS, amyotrophic lateral sclerosis; ECP, extracorporal photopheresis; EMG, electromyography