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Project Acronym: AUTOIGG – Project Number: 778405
Annex 1 to the Grant Agreement
(Description of the Action)
Part B

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2. Excellence

2.1. *Quality and credibility of the research/innovation project; level of novelty and appropriate consideration of inter/multidisciplinary, intersectoral and gender aspects*

The objectives addressed will be:

- Development of experimental cellular models and procedures with immunoglobulins (IgGs) from patient sera as diagnostic and prognostic technologies related to neurodegenerative diseases, NDs (particularly based on amyotrophic lateral sclerosis - ALS research).
- Defining mark-up characteristics of the standardized in vitro approach for personalized diagnostic protocols for NDs.
- Design of a small-scale platform based on automated fluorescence microscopy.

These objectives are based on previous studies on ALS of the Belgrade group, however the project also proposes to study the applications on other neuroinflammations and neuroinflammatory conditions. This addresses a relevant S&T as well as a socioeconomic medical issue. As can be evidenced by literature search it is the right timing for solving this research challenge towards application by means of networking.

Background studies on ALS

In ALS, production of anti-neuronal IgGs is a significant feature of the immune-inflammatory process. Early studies have shown that human IgGs increase intracellular Ca^{2+} in motor neurons, enhance the glutamate release from synapses residing on lower motor neurons and enhance the release of acetylcholine from the axon terminal at the neuromuscular junction – results being confirmed and further studied also in later years. These crucial events in our own early experiments showed diversity and non-cell autonomous characteristics with effects recorded not only from non-motoneuronal cells (facilitation of synaptic activity and Ca^{2+} transient change) but also on astroglia (Ca^{2+} transients, vesicle trafficking).

Proposed relevance of research and innovation

The idea to use IgGs for *in vitro* diagnostics in NDs is genuinely novel. Based on previous experimental results (see references above) it offers a robust multipurpose processing of a single sample on cell cultures that can give a complex information based on different cellular signalling responses as recorded by electrophysiology/voltage sensitive dyes or fluorescent imaging of Ca^{2+} and ROS generation. This experimental technology will be translated by the SMEs in the project to a clinical platform/devise design based on automated microscopy to be used for point-of-care personalized diagnostics. Standardization of *in vitro* culture media as well as of the multidisciplinary data analysis methodology will improve the performance of diagnosis, prediction, monitoring, intervention or assessment of therapeutic response, with a significant impact on clinical decisions and health outcomes for each ND patient individually. Particular attention will be given to the prediction value of this *in vitro* diagnostics procedure for early diagnostics followed by genetic markers or comorbidities.

Although a vast literature exists on successful preclinical and even on some clinical studies of ALS therapy, this is still a fatal disease without a reliable cure. Often patients being conscious throughout the illness are demanding euthanasia. It is a consensus among researchers and clinicians alike that such a poor prognosis is caused because there is a lack of a robust diagnostic tool that can primarily predict but also follow the therapeutic procedure. **The proposed work is thus highly ambitious and ground breaking in its goal to offer such a tool that can prolong lives and add quality of life and aging to a significant population of patients.** Since ALS is in 90% of cases of sporadic nature the main problem is to recruit patients for screening early enough. This will be achieved first by screening patients for comorbidities (e.g. FTD or muscle cachexia) or recruiting them based on

genetic or proteomic markers (e.g. TDP-43 and FUS/TLS genes) in order to subject them to a **robust point-of-care diagnostics platform** for prediction of disease and/or its stage and later for the follow-up of therapy. Such a multifactorial diagnostic tool is expected to offer a personalized approach to the disease and better patient stratification.

The **diagnostic process of ALS IgG application on stained cell** cultures for an automated recording system is of a **highly innovative potential.** Although experimentally confirmed and used in describing ALS pathogenesis, such phenomena were never used so far in practical terms. Standardizing the procedure for a market pilot will lead to an innovation that could be linked to the *published and patented technology of automated microscopy* (De La Torre-Bueno & McBride, US Patent US 7,272,252, 2007; Gough et al. US Patent US 8,597,899, 2013) for **multidimensional cell profiling for personalized diagnostics**. This approach should lead to the *pilot platform for high-throughput multidimensional cell profile analysis upon ALS IgG challenge*. In such a setup the multivariate single-cell analysis is a starting point for identifying relationships among ALS IgG effects at a systems level and a step toward phenotypic/physiological profiling at the single-cell level.

R & I of this project will outline the relevance of the networking, in terms of increase of interactions among scientists, common utilization of infrastructures, transfer and dissemination of knowledge, and interdisciplinarity. It offers a new approach, based on an innovative combination of state of the art methods, including standardization.

After thorough standardization of in vitro procedures these processes caused by IgGs purified from ALS patient sera will be looked in our animal cell models (cultures of neurons, astrocytes and cell lines) through electrophysiological monitoring (microelectrode array and/or voltage sensitive-dyes), Ca^{2+} and Reactive Oxygen Species (ROS) – imaging (with fluorescent probes). Using this combination of sensitive readouts, ALS IgGs will thus be tested in order to differentiate mark-ups of disease phase and severity for ***personalized*** *in vitro* diagnostics. In order to do this in a *robust point-of-care setup* an ***innovative automate microscopy system*** will be designed.

The project is based on preliminary published results by us and others that have demonstrated easily quantifiable effects of ALS IgGs on cell cultures. This research was of fundamental nature aiming to describe the pathophysiology of the disease. However, in this project we propose to use these findings for establishing **standardized personalized diagnostic technologies**.

The findings obtained with ALS IgGs as milestones for the development of the current project can be divided into the following:

a) **Synaptic activity**.

According to the excitotoxic hypothesis of ALS, overstimulation of glutamate receptors by excessive presynaptic release of glutamate induces a large and sustained increase in intracellular calcium which leads to motoneuronal death. Pathogenic Igs from ALS patients were suggested as the link between the neuroinflammation and the excitotoxic hypothesis. To test if the ALS IgG fraction can indeed affect the presynaptic glutamate release mechanisms we employed an electrophysiological approach based on whole-cell patch clamp recording of spontaneous excitatory postsynaptic currents in rat hippocampal neurons in culture. ALS IgGs (0.1 mg/ml) were focally applied by pressure puffs through a glass pipette placed 100 μm above the patched cell. The main result was **a rise in frequency of post-synaptic currents with ALS IgGs** but not with IgGs from healthy or disease controls. This effect was also proven to be presynaptic. It will be the goal of this project to correlate quantitative parameters of this ALS IgG-induced electrophysiological activity (as the functional biomarker of ALS) with disease specific parameters (ALSFRS, gender, age, duration of the disease and type – spinal/bulbar). The later will be done through long lasting on-going collaboration of neurologists at the Clinical centre of Republic of Serbia in Belgrade recruited through the on-going National project – see below). In addition, more robust technologies of electrophysiological recordings such as the microelectrode array (MEA) or voltage sensitive dyes will be explored.

b) **Ca^{2+} imaging**.

Ca^{2+} imaging with fluorescent dyes gained information on intracellular calcium mobilization in response to ALS IgGs on several cell types (hippocampal neuronal cultures, primary cortical astrocytes, primary microglial cells as well as motoneuronal and microglial BV-2 cell lines). We have previously shown that ALS IgGs induce typical calcium transients in astrocytes where PI3K is activated upstream of PLC. In addition, preliminary (unpublished) experiments on microglial BV-2 cell line (collaboration of Belgrade and Kuopio labs), as well as on primary hippocampal neurons have shown similar effects of ALS IgGs. We intend to explore this ALS IgGs-induced Ca^{2+}-signalling in all *in vitro* models stated above in a systematic manner, with carefully chosen IgGs (from ALS patients with different disease progression, as indicated by ALS functional rating score, ALSFRS, as well as from healthy and disease controls) in order to find the **correlation between the ALS IgGs calcium mobilizing potential (quantified by the amplitude of the Ca^{2+} spike and the integrated calcium elevation over time) and disease specific parameters.** As a corollary this applied research may also give some basic results on the second messengers in this signalling pathway in relevant *in vitro* model system that may facilitate the discovery of cell specific antigens, and consequently significantly simplify the proposed *in vitro* diagnostic technology.

c) **ROS imaging.**

Pilot experiments (collaboration between Belgrade and Kuopio lab in the framework of the recently ended COST Action – see below) on motoneuronal (NSL-34) and microglial (BV-2) cell lines transfected with ROS-sensitive fluorescent constructs HyPer 3 or CyPer have shown that in response to acute exposure to ALS IgGs, ROS signalling is induced in both Vcell lines, with an indicated correlation with disease progression (as measured by ALSFRS). We intend to gather enough data for verification of these results, and test newly available fluorescent constructs designed for site specific ROS signalling, as well as markers of oxidative stress in mitochondria (e.g. Rhodamine 123). Following quantification and estimate of the response in all *in vitro* models results will be correlated with disease parameters which could also lead to **an innovative ROS-based biomarker for the ALS disease that is known to be largely driven by abnormal ROS production.**

d) **Vesicle trafficking.**

We have previously shown that ALS IgGs increase the mobility of acidic vesicles (mostly endosomes and lysosomes) in primary cortical astrocytes, which could suggest long term modulation of endocytosis and/or autophagy. We intend to further **explore this ALS IgGs mobility enhancing effect in all *in vitro* models stated above with the goal to find correlation with disease specific parameters**. In addition, endocytosis and autophagy pathways will be investigated in detail in order to gain close insight into the overall ALS IgGs effect on cellular physiology, with the final goal to simplify the proposed *in vitro* diagnostic technology.

The above approaches evidently need to be considered as *trans-disciplinary* since biologists, physicists, physico-chemists and MDs (neurologists) need to work as a team in order to achieve the proposed methodologies. **Such interdisciplinarity is however already well seeded in the management of the National innovative integrative project** on biomarkers in neurodegeneration (coordinated in Belgrade; see below). The herewith proposed project will also employ software programmers and instrumentation designers.

The project is based on experimental work that is already published as a proof of concept (*Technology Readiness Level* - TRL 3). **The main goal of the project is to bring this concept to a higher level by validating the technology of a small-scale platform in the lab (TRL 4)** by means of standardization and benchmarking (as a necessary phase for the 'lab to market' planning). In order to create sustainability, in the final project phase, with the help of the **SME**, it is planned to propose the technology for an industrially relevant environment (TLR 5).

The work on the project will tightly be linked to the **National Innovative Integrative Research project** "Biomarkers in neurodegenerative and malignant processes" (grant #III41005 to PA) Ministry of Education, Science and Technological Development Republic of Serbia. On the other hand, the collaboration of partners (Belgrade & Kuopio) within the recently ended **COST Action BM1203 EU-ROS** will be continued and strengthened through this project. This proposal is also related and represents a reasonable continuation of a completed (2013) **ROSim Grant No 135179 Photonics Research Program** from the Finnish Academy (with 4 research groups where the Kuopio partner was the coordinator). The ROS imaging techniques optimised through the latter grant will be used within this project.

On the other hand, the participant **YEDITEPE** will bring to the project a clinically relevant immunological research practice.

Two SMEs, **ArGenit** and **ELVESYS**, will bring transectorial knowledge of entrepreneurship and marketing of scientific equipment and software. It is also expected that these participants will share the IPR knowledge with other participants.

In addition, a biophysical approach will be sought through collaboration with the Laboratory of Wim van Drongelen, University of Chicago USA (**UCHIC**) on the application of organotypic brain slices on multielectrode arrays as devices for *in vitro* diagnostics for ALS IgGs as well as with Dr. Antic's lab from University of Connecticut Health, USA (**UCONN**) on voltage-sensitive dyes.

LANOTEC with its state of the art advanced microscopy techniques (AFM, TEM, SEM) adds a complementary biophysical approach to the knowledge sharing of the project.

With one third of female principal investigators, the AUTOIGG consortium compares favourably with most research departments and collaborative networks in the field. Particular attention will be paid to the recruitment of women for secondments as ERs, and ESRs, in order to reach a **minimal 40% participation in the network**. Also, public actions at international level **promoting the participation of women in science and research** will be emphasized and supported during the training events and outreach activities in order to foster scientific vocations among young women and girls. Finally, all research projects on animals and humans delineated within the AUTOIGG program will include equal numbers of male and female subjects and will specifically look for possible sex differences in the biological or medical issues studied by the consortium. In fact, It is shown epidemiologically that there is a gender difference in the infliction of ALS and this was also demonstrated on animal models by the UEF- Kuopio beneficiary on this project. This is going to be included in hope of presenting it as *gender innovation* along with the piloting (WP 2) and sustainability phase.

2.2. *Quality and appropriateness of knowledge sharing among the participating organisations in light of the research and innovation objectives*

The approach for knowledge sharing among participants of AUTOIGG will be presented by the following:

- **A.** Secondments
- **B.** Workshops
- **C.** Training Schools

A. Secondments (see Gantt chart below)

Secondments are the main method for knowledge exchange. The coordinator, FBUB will exchange staff with ELVESYS and ArGenit for intersectorial exchange of expertise on the automated device production. FBUB will also maintain secondments to the TC USA i.e. to UCONN and UCHIC in order to pursue electrophysiology vs fluorescence. FBUB will exchange two staff members with LANOTEC (TC – Costa Rica) in order to synchronize the advanced microscopy protocols with the fluorescence probing in WG1.

Other secondments: UEF – one and YEDITEPE – two staff members will exchange with ELVESYS staff in order to further the expertise in designing the chamber of the automatic system and to validate IgG effects measurement, respectively.

Workshops (Tables 1-3)

Three workshops will be hosted by YEDITEPE in Istanbul. Lecturers will be recruited from Academia and SMEs as well. Two workshops will deal with the fundamentals of the scientific goals of the project - immunology and detection methods for the IgGs and their effects. These workshops will also address the main R & I goal of the project – the automatic system for fluorescent detection of neurodegenerative IgG effects. The third workshop will be organized in the first year of the project and will deal with an important soft skill – Good laboratory practice. In fact, the host, YEDITEPE will be instrumental in preparing the GLP compliant manual for AUTOIGG.

WORKSHOP 1: GOOD LABORATORY PRACTICE (GLP) IN RESEARCH PROJECTS (Month 13)

Organizing Committee: One person from each beneficiary

Venue: Yeditepe University, Rectorate Building, Atasehir, Istanbul, Turkey

PROGRAM		
Time	**Topic**	**Lecturer/Teacher**
Day 1		
09:00-09:20	Welcome and introduction (Pre-workshop questionnaire)	Gulderen Yanikkaya Demirel, Pavle Andjus
09:20-09:50	OECD Principles of GLP	TBA
09:50-10:20	EU regulation on GLP	Authority from EU Commission
10:20-10:40	Coffee break	
10:40-11:10	Resources for GLP (Management/Personnel/Facilities)	TBA (SME representative)
11:10-11:40	Rules for GLP in Research	Gulderen Yanikkaya Demirel
11:40-12:10	How to Handle Results in Research Projects Conforming into GLP	TBA (SME representative)
12:10-13:30	Lunch Break	
13:30-16:15	Small Group Study on Quality Assurance of Research Projects	Pavle Andjus, Gulderen Yanikkaya Demirel
16:15-16:30	Coffee break	
16:30-18:00	Presentations of Small Groups Studies	
18:00-18:15	Concluding Remarks	
Day 2		
09:20-09:50	Notes from the first GLP Workshop	Gulderen Yanikkaya Demirel
09:50-10:20	GLP and Multi-Site Studies	TBA
10.20-10.40	Coffee break	
10.40-11:10	GLP and Computerized Systems	TBA (SME representative)
11:10-11:40	Characterization of Test and Test Systems	TBA (SME representative)

11:40-12:10	GLP and *in vitro* Studies	Gulderen Yanikkaya Demirel
12:10-13:30	Lunch break	
13:30-14:00	How to prepare for an inspection	Pavle Andjus, Gulderen Yanikkaya Demirel
14:00-14:30	Archiving of Research Results	TBA (SME representative)
14:30-15:00	Monitorisation of Research Studies and GLP	TBA
15:00-15.30	Coffee break	
15:30-16:00	Feed-back from the attendees	
16:00-16:30	Post Workshop Questionnaire	
16:30-17:00	Close up and remarks	

WORKSHOP 2: UPDATE on IMMUNOLOGY OF AUTOANTIBODIES (Month 21)

Organizing Committee: One person from each beneficiary

Venue: Yeditepe University, Rectorate Building, Atasehir, Istanbul, Turkey

PROGRAM		
Time	**Topic**	**Lecturer/Teacher**
09:00-09:20	Welcome and introduction	Gulderen Yanikkaya Demirel
09:20-09:50	Autoantibodies and their use in clinical decision making	Sule Yavuz
09:50-10:20	Principles of autoantibody detection	Ishak Tekin
10:20-10:40	Coffee break	
10:40-11:10	Current Biophysical Techniques for Antibody Effects detection	Pavle Andjus
11:10-11:40	Guidelines for Autoantibody Testing	Gulderen Yanikkaya Demirel
11:40-12:10	Future of Autoantibody Detection	TBA
12:10-13:30	Lunch break	
13:30-15:30	Small Group Hands on Training on IFA method*****	Pavle Andjus, Gulderen Yanikkaya Demirel
13:30-15:30	Small Group Hands on Training on Line Blot assays*****	TBA
15:30-16:00	Coffee break	
16:00-17:30	Presentations of Small Group Outcomes	
17:30-18:00	Concluding Remarks	
***** There will be rotations between the groups		

WORKSHOP 3: EVALUATION of AUTOANTIBODY DETECTION and THE NEWLY DEVELOPED AUTOMATIC MEASUREMENT DEVICE (Month 34)

Organizing Committee: One person from each beneficiary

Venue: Yeditepe University, Rectorate Building, Atasehir, Istanbul, Turkey

PROGRAM		
Time	**Topic**	**Lecturer/Teacher**
09:00-09:20	Welcome and introduction	Gulderen Yanikkaya Demirel
09:20-09:50	Recent developments in autoantibody detection	Haner Direskeneli
09:50-10:20	Introduction of the new automated system	Pavle Andjus
10:20-10:40	Coffee break	
10:40-11:10	Advantages of using POC testing for autoantibody detection	TBA
11:10-11:40	TBD	SME representative
11:40-12:10	Future of Autoantibody Detection	TBA
12:10-13:30	Lunch break	
13:30-15:30	Small Group Hands on Training on new system	
15:30-16:00	Coffee break	
16:00-17:30	Feedback from the participants	
17:30-18:00	Concluding Remarks	

C. Training Schools (Tables 4-5)

Two Training Schools will be organized by UEF: one on free radical (ROS) monitoring – essential for one of the tasks in WG1, and one on inducible pluripotent stem cells (iPSCs) and their applications - as an alternative model for the proposed automated recording platform. The Schools host, UEF is in the forefront of stem cell research in Europe as well as in the field of ROS science and detection.

TRAINING SCHOOL 'Mitochondria and ROS signalling' (Month 6)

PROGRAM		
Time	**Topic**	**Lecturer/Teacher**
09:00-09:15	Opening the meeting	
09:15-10:00	Mitochondria as generators of ROS	Andrei Abramov (UCL)
10:00-10:30	ROS induction of ALS IGGs	Pavle Andjus (UB)
10:30-11:00	Redox imaging in neurodegenerative disorders	Pier G Mastroberardino (Rotterdam)
11:00-11:45	Workshop: New applications of the multiphoton microscopy for imaging mitochondria	Leonard Khiroug (U Hki)
11:45-12:15	Mitochondrial production of reactive oxygen species	Gundars Goldsteins (UEF)
12:15-13:00	Lunch break	

13:00-13:30	The role Nrf2 and downstream enzymes in prevention of the oxidative stress primary human retinal pigment epithelium	Anna-Liisa Levonen (UEF)
13:30-14:00	ROS and NOS signalling in modulation of pain stranding ion channels	Rashid Giniatullin (UEF)
14:00-14:30	Diet, antioxidant and ER stress	Mustafa Atalay (UEF)
14:30-15:00	Mechanisms of oxidative stress by microglia	Tarja Malm
15:00-15:30	Sensitivity of mitochondria to neurodegenerative disorders	Eugene Pryagnikov (U Hki)
15:30-15:45	Coffee break	
15:45-17:45	Laboratory demonstrations: Imaging ROS with Flow cytometry	Organized by group of Prof. Jari Koistinaho (UEF)
17:45-19:45	Laboratory demonstrations: Imaging of ROS in neurons and cell lines with pharmacological and genetically encoded ROS sensors	Organized by Prof. Rashid Giniatullin (UEF)
19:45-21:30	Dinner	

TRAINING SCHOOL 'iPSC-based stem cell research' (Month 30)

PROGRAM		
Time	**Topic**	**Lecturer/Teacher**
Day 1 (31.08.)		
09:15-09:30	Opening the meeting	Jari Koistinaho (UEF)
09:30-10:15	Plenary: 3D organoid models from induced pluripotent stem cells	Jürgen Knoblich (U Wien)
10:15-10:45	Derivation of Adult Human Fibroblasts and their Direct Conversion into Expandable Neural Progenitor Cells	Frank Edenhofer (U Innsbruck)
10:45-11:15	Regulation of Human Pluripotent Stem Cell-Derived Hepatic Cell Phenotype by Three-Dimensional Hydrogel Models	Sanna Lehtonen (U Hki)
11:15-12:15	Lunch, at own cost	
12:15-12:45	CRISPR/Cas9-mediate Transcriptional Activation for Maturation of Human Stem Cell derived Beta Cells	Timo Otonkoski (U Hki)
12:45-13:15	Mitochondrial DNA mutations in iPS cells: mtDNA integrity as standard iPSC selection criteria?	Riikka Hämäläinen (UEF)
13:15-13:45	Comparative proteomic analysis of human embryonic stem cell-derived and primary human retinal pigment epithelium	Heli Skottman (U Tre)
13:45-14:00	Coffee break	
14:00-14:30	Modelling Heart: Functional characterization of iPSC/ESC-derived cardiomyocytes	Pasi Tavi (UEF)
14:30-15:00	The Effects of Pharmacological Compounds on Beat Rate Variations in Human Long QT-Syndrome Cardiomyocytes	Katriina Aalto-Setälä (U Tre)

15:00-15:30	Microfluidic 3D cell culture: from tools to tissue models	Paul Vulto (Mimetas, Leiden)
15:30-16:00	Stem cells in as a tool in safety testing	Markku Pasanen (UEF)
18:30-21:00	Posters, Social Networking, Dinner	All
Day 2 (01.09.)		
08:30-09:00	3D modelling of the brain derived from human iPSCs	Katharina Kruszewski (U Innsbruck)
09:00-09:30	iPSC-derived human endothelial cells: Disease mechanisms and biomarkers in Parkinson's Disease and Schizophrenia	Sarka Lehtonen (UEF)
09:30-10:00	iPSC-derived oligodendrocytes in human diseases	Laurent Roybon (U Lund)
10:00-10:15	Coffee break	
10:15-10:45	Role of NeuroD1 in hippocampal neurogenesis	Claire Rampon (U Toulouse)
10:45-11:15	iPSC-derived human astrocytes in neurodegenerative diseases	Jari Koistinaho (UEF)
11:15-11:45	iPSC-derived microglia-like cells in Alzheimer's disease	Tarja Malm (UEF)
11:45-12:45	Lunch, at own cost	
12:45-13:15	Pathology of hiPSC-derived neurons in Alzheimer's disease	Gunnar Gouras (U Lund)
13:15-13:45	Modelling vesicular trafficking by hiPSC-derived brain cell models	Dora Brites (U Lisbon)
13:45-14:15	Neuroinflammation in human neurodegeneration: iPSC-based models	Michael Heneka (U Bonn)
14:15-14:30	Coffee break	
14:30-15:00	Altered differentiation of cells with AMPA receptors from human and mouse fragile X neural progenitors	Maija Castren (U Hki)
15:00-15:30	Development of gonadotropin-releasing hormone-secreting neurons from human pluripotent stem cells	Taneli Raivio (U Hki)
15:30-16:00	Abnormalities in iPSC-derived brain cells of violent substance abusers	Olli Kärkkäinen (UEF)
16:00-16:15	Instructions for Assays based on given articles and lectures: Wrapping up and closing	Tarja Malm (UEF)

2.3. *Quality of the proposed interaction between the participating organisations*

Beneficiaries

1. **Elvflow Microfluidic Innovation Center (**ELVESYS**)** – Paris, France (*SME, MS*) - Device design towards point of care system and diagnostic tool based on microfluidics, will also offer non-scientific soft skills (valorisation & entrepreneurship training) and help in project management and dissemination (website design)

2. **ArGenIt** (linked to the Regenerative and Restorative Medicine Research Center), Technopark of Istanbul Technical University, Turkey (*SME, AC*) – Device design and development of microscopy analysis systems; patient-derived induced pluripotent stem cells, differentiated to motor neurons, oligodendrocytes and astrocytes; stem cell derived cultures for modeling diseases and search for phenotypes.
3. **Yedıtepe University School of Medicine** (YEDITEPE) - Immunology Department, Istanbul, Turkey (*Academic, AC*) – will provide patient samples from different disease groups; patients antibody testing; standardization and method evaluation; Good Laboratory Practice manual for the project; links to SMEs
4. **Dept. Neurobiology, A. I. Virtanen Institute University of Eastern Finland** (UEF) Kuopio (*Academic, AC*) - Fluorescence-probe imaging of ROS signalling and electrophysiology. Expertise in iPSC characterization and applications.
5. **The National Laboratory of Nanotechnology** (LANOTEC) – San Jose, Costa Rica (*TC partner eligible for funding*) – Complementary testing to the fluorescence microscopy analysis and proposing further analysis (AFM, hrTEM, SEM)

Partners

6. **University of Connecticut Health Center** (UCONN), Farmington, USA (*TC*) – Know-how in voltage-sensitive dyes
7. **Dept. Pediatrics, Pritzker School of Medicine, The University of Chicago** (UCHIC), Chicago, USA (*TC*) – Know-how in Microelectrode arrays (MEA) and big data electrophysiology.

All of these participants will interact through the secondments programme (see 2.2A) while participants #3 (YEDITEPE) and #4 (UEF) will be hosting workshops and training schools that rely on their expertise as stated above.

The mobility programme (see 2.2A) involves all participants and is designed around tasks of WG1 and 2. Intersectorial exchange is planned between the academic MS/AC institutions (FBUB, YEDITEPE, UEF) and the two SMEs (ELVESYS and ArGenit). The main goals for these secondments is to exchange experience and know-how on entrepreneurship on one side and translational product development on the other. The staff members of SMEs will also visit Academic participants in order to learn the basic biophysical mechanisms to be exploited in the innovative measuring device. International secondment is also planned with the three TC partners. UCONN will host an ESR followed by an ER from FBUB in order to transfer know-how on its renown expertise in voltage-sensitive dyes as well as the knowledge in optical devise set-ups. Likewise, UCHIC will be the host for know-how on MEA technology. Thus, both of these TC visits are justified for the design of the electrophysiological basis of the proposed measuring device. Regarding LANOTEC since this is a TC legible for funding there will be planned exchanges with FBUB staff in order to exchange expertise in the subcellular markers (also explorable with high resolution advance microscopy techniques at the TC partner) of the observed basic effects of pathological IgGs (to be presented by FBUB as the host).

Two participants #3 (YEDITEPE) and #4 (UEF) will be hosting three workshops and two training schools (see 2.2 B & C, respectively) that are based on their experience as stated above. The staff members of the participant beneficiaries will take part at the workshops and trainings. These workshops as well as trainings will be focused on different aspects of cellular neuroimmunological phenomena to be followed with the proposed device.

The meetings of the Supervisory board organized twice a year will be an opportunity for all beneficiaries to monitor and eventually revise and update the project plan (research, networking and finances). During these meetings, outreach activities will also be planned and evaluated.

Networking with patient organizations through the Supervisory board will bring an added value and a societal dimension to the project.

3. Impact

3.1. *Enhancing the potential and future career perspectives of the staff members*

The project will be open to multidisciplinary staff profiles – biologists, physicists, physical chemists, physicians, technicians, administrators, entrepreneurs. By joining these profiles under the AUTOIGG project we will create conditions for transectorial careers. This will be reinforced through reciprocal secondments between the sectors but also within the Academic sector taking care of different expertise (e.g. neurologist MD vs biologist PhD; biologist PhD vs software designer, academic administrator vs entrepreneur…). It is intended to engage existing staff for secondments but also to recruit new staff in case there will be a lack of some profiles and/or expertise. These new skills will be further strengthened through workshops and training schools. New staff members will thus be offered a career plan (as drafted by the Secondment Committee) for the integration at the host institution after the ending of the grant.

3.2. *Developing new and lasting research collaborations, achieving transfer of knowledge between participating organisations and contribution to improving research and innovation potential at the European and global levels*

This consortium was formed through a NCP-based partner search. A form describing the FBUB Centre in Belgrade and the basics of the project proposal were posted online and the responses collected. A questionnaire was then sent to all relevant organizations. Based on the responses to the questionnaire a selection of organizations was made and these were invited to form the consortium and to start writing their part in the proposal. Thus, except for the previous relationships of FBUB and UEF and TC organizations from USA (UCONN and UCHIC) the partnerships within the consortium are all new. The participants of AUTOIGG are thus carefully selected through a two stage process. The main criteria for the selection of collaborations was based on complementarity of expertise as well as on interdisciplinarity. This selection will be strengthened through the envisaged intersectorial and international secondments reinforced trough a rich programme of workshops and trainings. The main goal of the project – to design a prototype of an automatic diagnostic device for neuroegenerative diseases gathered all participants each demonstrating a particular role and expertise. This engagement of complementary expertise also guarantees a sustained collaboration after the ending of the project period, thus strengthening the European innovation capacity.

The project aims to design a biomedical device for the automatic evaluation of diagnostic potential of ALS IgGs primarily employing primary astrocytes and neurons in culture. There are several lines of this project that will allow for a sustained partnership.

1. This innovative device offers a wider field of applications in respect to possible neurodegenerative / neuroinflammatory phenomena as well as other plated cell populations (stem cells, cell lines etc).
2. The design of the device within the project is planned to reach the prototype level. However, the study of the market and the creation of a business plan for the device production and exploitation needs a sustained collaboration between the academic and SME participants.
3. The project research topics open a new filed of neuroimmunological basic studies to pursue among the academic partners of the AUTOIGG after its completion.

Although the effects of ALS IgGs are well studied and documented in the Belgrade laboratory and elsewhere the process of IgG application for *in vitro* **diagnostics of ALS is completely novel** and **offers a practical multidimensional functional analysis for personalized medicine**. Thus, based on a standard clinical blood sample and upon routine serum separation and IgG purification one can

obtain under an automated microscope actually a pattern of activities in the form of fluorescence intensities in time and space thus establishing a *signature of disease for the individual patient*. The combining of several recording modes raises **the reliability of the obtained diagnostic pattern** still **keeping the cost of the setup effectively low** (main cost is for fluorescent dyes and culture media).

On the other hand, this procedure can give **early diagnosis of ALS** since it has been shown that inflammation markers appear early in the disease model (Beers et al. Proc Natl Acad Sci U S A. 2008 105:15558). The complex multifactorial nature of ALS underlines the need for a personalized treatment and patient stratification. It is strongly believed that the multivariate single-cell analysis offered by the proposed *in vitro* diagnostic technology may present exactly the adequate approach for the **personalized patient care of such a multifocal disease**. At the same time the pattern analysis of the multivariate single-cell response allows for **a robust point-of-care diagnostics necessary for improved and efficient clinical decisions.**

The project will contribute to the *sustainability of the health care* of ALS patients by drafting and planning a large-scale prototype in an operational environment. In addition, the designed personalized diagnostics technology of *in vitro* testing of IgGs from patient sera can be proposed for other motoneuron and neuroinflammatory neurodegenerative processes, thus allowing for **sustainability of the health care system in the particular area of neuroinflammation** as the common mechanism of neurodegenerative diseases.

The proposed project will rise an opportunity not just for the partner **SME** to deliver innovation to the market but also for other **SMEs** that could contribute to the upgrade and strengthening of the designed technology in its many aspects from standardized cell culturing to the hardware and software design for automated microscopy, thus potentially opening new job positions.

3.3. *Quality of the proposed measures to exploit and disseminate the project results*

Publications and scientific meetings. After the IP potential has been evaluated, data and results will be made public through the standard scientific community approaches: scientific and public meetings, posters, high impact publications in peer-reviewed journals. Thesis and dissertation publication of data will be permitted, and specific clause to address this included in the consortium agreement.

Governments and Ministries of Health and Science. These public stakeholders will be addressed by younger researcher with the final goal of reaching the patients. Contacts of developed centres with patient organizations will be particularly emphasized and translated to the emerging centres and their public stakeholders lacking this experience. Thus, a wider transitional societal impact will be attained.

Patients associations. The project participants will take an active part in the dissemination of findings to patients associations. It will be required that they make contact with one patient organizations during the course of the project and that they maintain a regular communication through their representatives. This will be done through the distribution of newsletters and flyers and through the invitation of local patients organizations and patient support groups to the annual meetings of the consortium. The contact with patient groups is of particular interest for emerging countries where this public initiative is just starting.

Press releases. Additionally, publicity to a wider audience will be performed by press release, at the local and national level, interfacing with already contacted publishing desks dedicated to EC programmes (egs. https://danube-inco.net/documents or Projects by British publishers). Existing experience and previous project of partners with TV stations and science programmes will be specifically utilized for this purpose.

Public engagement. Most of the partners belong to institutes that have public engagement plans linked to national (governmental) or international (IBRO, FENS) organizations, such as the

Edu(cational)-Fair, Science fair, NENS Job Fair or Brain Awareness Week. The project will use these links to disseminate its activities to a wider audience. Young researchers funded by AUTOIGG will be required to participate to at least one outreach event during the course of the project. A special initiative named Science and the City will be negotiated with public places of leisure such as town cafes or exhibit halls. It will consist of popular presentations by fellows and experts of science networking for the benefit of society.

***Web site*.** The project will develop a website, based on those the partners have produced for other EU projects and networks. This will be used for internal communication within the network, and will have public area for dissemination of discoveries from the project. The website will be linked to the regional site Danube-inco.net

- A very important dissemination and communication tool will be the web site of AUTOIGG. Besides classical features in line with EC recommendation (i.e., direct links to individual web sites of the partners with information on training activities, recent project developments, and vacancies), the web site shall be central to the dissemination and communication strategy of AUTOIGG. The web site will support an internal interactive communication platform (DokuWiki architecture) as well as an external interactive web tutorial (MediaWiki architecture). In detail this includes:
- Forum for the partners, ER and ESR (membership access only);
- Project-based and scientist-based task pages to facilitate management of closely interlaced multi-host activities (membership access wiki);
- Web tutorial for the underlying experimental protocols, their application and data analysis (this will involve the active participation of many of the partners); AUTOIGG will explore novel collaborative publication techniques via the internet (open access wiki);
- Popular science pages explaining results and problems to the general public and lists of open questions in fields related to the Network.

This area will be the specific responsibility of the Supervisory Board. A critical aspect of the project will be to ensure the protection of IPR without preventing neither creation of new knowledge nor the dissemination of that knowledge to other sectors. IPR activities will comprise: assuring the protection of IPR where appropriate; to promote exploitation via license and patents where appropriate; to maintain an updated list of IPR belonging to each of the partners and generated in the frame of this project; to assist partners in the consortium in any conflict with respect to the IPR with a party outside the consortium.

3.4. *Quality of the proposed measures to communicate the project activities to different target audiences*

Key objectives

- To communicate to key stakeholders the existence of the project and provide regular updates on new data generated
- To ensure that the general population is aware of the impact that the **AUTOIGG** research and innovation has on the community as a whole

Dissemination and promotional activities will include: creating **AUTOIGG** logo, brochure, poster and a web site with web page Forum and a potential Partner list and electronic newsletters, press releases and involving external scientists and SME representatives at project meetings. Considerable efforts will be undertaken regarding interactions with end-users (patients, patient associations, clinicians and other professionals) and the public, addressing new knowledge in neuroscience research and questions arising from its potential application. Senior researchers, in particular, are expected to take a lead in ensuring that research is fruitful and that results are made accessible to the public as well as how they should be exploited commercially. *All this will ensure a*

*continuous process concordant with the European Charter for Researchers, which will enhance visibility and exposure of the **AUTOIGG** initiative, the outcomes and achievements to key end-users at national, regional and international level, and develop general management principles and techniques even beyond the field of neuroscience research.*

Description of work

Every effort will be made to disseminate the existence of the project, creation of an active Academia + SME consortium, its progress and scientific data to as wide an audience as possible.

Publications and scientific meetings. After the IP potential has been evaluated, data and results will be made public through the standard scientific community approaches: scientific and public meetings, posters, high impact publications in peer-reviewed journals. Thesis and dissertation publication of data will be permitted, and specific clause to address this included in the consortium agreement.

Governments and Ministries of Health and Science. The staff and young researchers within the AUTOIGG consortium will take an active part in the dissemination of findings to these public stakeholders with the final goal of reaching the patients. Contacts with patient organizations will be particularly emphasised. Thus a wider transitional societal impact will be attained. This will be done through the distribution of newsletters and flyers and through the invitation of representatives of public stakeholders to the annual meetings of the consortium.

Press releases. Additionally, publicity towards a wider audience will be performed by press release, at the local and national level, interfacing with publishing desks dedicated to EC programmes (egs. see-science.eJournal/wbc-inco.net or Projects by British publishers). Existing experience and previous project of partners with TV stations and science programmes will be specifically utilized for this purpose.

Public engagement. Most of the partners belong to institutes that have public engagement plans linked to national (governmental, National Brain Council) or international (IBRO, FENS, European Brain Council) organizations, such as the *Edu(cational)-Fair*, *Science fair, FENS Job Fair* or *Brain Awareness Week*. The project will use these links to disseminate its activities to a wide audience. This effort will be led by senior researchers but young researchers will also be required to participate to the outreach events during the course of the project. A special initiative named *Science and the City* will be negotiated with public places of leisure such as town cafes or exhibit halls. It will consist of popular presentations by fellows and experts of science networking for the benefit of society.

Website. The project will develop a website, based on those that the partners have produced for other EU projects and networks. This will be used for internal communication within the network, and will have public area for dissemination of discoveries from the project.

AUTOIGG dissemination programme – i.e. **Plan for disseminating and exploiting the project's results -** *intends to continuously and systematically expose knowledge, excellence and project results to the widest possible audience for the **benefit of all end-users at national, regional and international level**.*

- The workshops and congresses planned to be organised will present *the main contribution to the dissemination of knowledge, spreading excellence and exploiting results.*
- The project Web site will be the e-base of the project dissemination strategy (newsletter, Forum, Partner list).
- ***Measures of success*** will be clear, relevant and recognizable communication (*transparency*), delivery of the right message to the targeted group (*focus*), effective delivery mechanism for each message (*effectiveness*) and a feedback system to determine effectiveness (*feedback*).

- **AUTOIGG *target groups (end-users)*** will be young scientists, expert neuroscientists, general physiologists, photonics experts, faculties in the region, research centres, SMEs, neurologists, patients & patient associations and general public.

The strengthening and setting up of a multipurpose biomedical imaging capacity of **FBUB,** within the WB region presents a strategic benefit that opens the research area of the region to the ***development of science and education*** and also brings ***jobs and better career opportunities to EU***. Once set-up, the hi-tech biomedical imaging instrumentation will serve as a necessary *interdisciplinary facility* opened to be used *even beyond neuroscience/neurology* e.g. in field of immunology of inflammation.

- ***FBUB** will* **further develop contacts** *with centres of excellence in the region and EU that were already established through different past and ongoing international biomedical programmes (COST, FP-6, FP7, TEMPUS).*
- *Data management* will be organized by a separate *ICT office* hosted by SME **ArGenit** with access to **AUTOIGG** partners and users. *Feedback on data usage* will also be collected and analysed through the **AUTOIGG** R&I council, and finally also stored on the server.
- Approved data by way of internal and external review – feedback will be put on the *Open access server*
- All research published data coming from **AUTOIGG** will be open access by *gold* or *green* standard (as decided by the R&I council).
- *Ownership and access to new knowledge* will be protected by a *Consortium agreement* that will help to pursue market opportunities by way of dissemination and communication towards SMEs and Industry. Marketing advisor(s) will be sought from **AUTOIGG** SMEs **ELVESYS** and **ArGenit** or will be particularly subcontracted for the latter purpose.
- *Management of IPR data* to be sanctioned by the above agreement(s) will first need a programme of training and education of **AUTOIGG** staff that will be organized by SMEs on the project.

Communication activities

By establishing a multidisciplinary consortium the project aims to bridge the gap between science fields but also *between theoretical and applied science* as well, that can serve the *socio-economic needs* by *supporting medical and pharmaceutical research* through planning interactions with *SMEs and Industry*.

- Complementing the knowledge and technologies within the AUTOIGG consortium *research platforms on neurodegenerative diseases* will serve as a basis for the development and dissemination of programmes within Academia and SMEs. The deliverables of such an action will be reached through inviting representatives of Pharma and Biomed Industry to AUTOIGG meetings and workshops, establishing also the consortium's role as an incubator for entrepreneurship, thus ensuring growth and sustainability of the project outcomes, and *spreading excellence towards SMEs and Industry*.
- Molecular diagnostics and therapy follow up will be promoted by the development of the complex innovative practical technique, i.e. *diagnostic protocol at the cellular imaging level*. It is also envisaged that through this dissemination, Academia + SME research units interact with the clinic (medical doctors and patients) in the process of supportive medicine build-up. It would also set grounds for further development of research and health-care system in the Region and ERA.
- Interaction with *patients associations* will be fostered through special publications about AUTOIGG and scientific achievements in dedicated journals and by participating at respective meetings.
- Special attention will be given to the dialogue with the general public. *Popular lectures and public debates* will be directed in order to raise awareness (*"Science and the City"*

programme in main cafes, open spaces, open universities, and life-long learning centres; Brain Awareness Week; National Brain Councils' events).

Through **reinforcement of Academia + SMEs research potential**, **AUTOIGG** network will ensure the **flow of expertise in the WB region and a trans-European equalization of standards** in the field of molecular physiology of neurodegenerative diseases with neuroinflammation. Once the target groups have successfully integrated within the project framework, it should be possible to envisage *further controlled expansion of the network* and ***further dissemination of knowledge and spreading of excellence*** **within ERA**.

4. Implementation

4.1. *Coherence and effectiveness of the work plan, including appropriateness of the allocation of tasks and resources*

The work will be divided in following workpackages.

WP 1. **Standardization and benchmarking (***research and demonstration***).** The experimental protocols used previously to show proof of concept will be submitted to rigorous standardization in the lab. This will start from the **selection of cell type and standardization of best practice for cell cultures and further elaborate benchmarking of protocols** for detecting (based on electrophysiology and fluorescence microscopy) and analysing the obtained parameters of cell activity (software design and testing). Patterns of correlation of the above parameters to disease stage and severity will be investigated and incorporated in the analysis software.

WP 2. Pilot platform design (*piloting***).** Design and validation of the technology of a small-scale platform in the lab. This pilot will be tested for robustness and technical feasibility in a near to operational environment. The main characteristic will be its *modulability*. The main technological basis will be an **automated microscopic stage and fluorescence detector for dynamical measurements**. This can be combined with a simple electrophysiological unit such as MEA or alternatively, voltage sensitive dyes. By means of digital conversion and analysis of these activities quantitative parameters (e.g. area under transients, halftimes of rise, frequency of events) will be extracted and *matrices of patterns* in pseudocolor related to each parameter value will be designed (see Perlman et al. *Science.* 2004 306:1194).

WP 3. Dissemination, communication and networking *(dissemination)*

Every effort will be made to disseminate the existence of the project, to create a vigorous multidisciplinary/intersectorial/transcultural research and innovation network, and to communicate its progress and scientific data to as wide an audience as possible. This will be achieved through publications and scientific meetings, addressing the stakeholders in ministries of health and science, patients associations, press releases, public engagement, website.

WP 4. Project management and sustainability (*management and prototyping*). In close collaboration with the Coordinator the project will be managed by the **SME** partner through all phases (solution design, prototyping, testing and validation) of the **developing of a small-scale prototype in the laboratory or simulated environment**. By the end of the project its sustainability will be designed based on the obtained results and drafting of a plan for a large-scale prototype in an operational environment. A wider application for related motoneuronal diseases as well as for other neuroinflammatory diseases will be investigated.

WP 5. Ethics requirements. Ethical standards and guidelines will be rigorously applied, including: technical framework and security measures for collection, processing and storage of personal and sensitive information, as well as obtaining relevant authorisations for the animal experiments. Patients sera will only be collected from persons who give informed consent, while the material will be anonymous labelled with the number code in order to protect the patient's identity. All project

participants will comply with these requirements, regardless of the country in which the research is carried out.

Each WP will have a WP leader, These will be elected at the AUTOIGG Kick-off meeting.

4.2. *Appropriateness of the management structures and procedures, including quality management and risk management*

Description of work

The main managing body is the ***Supervisory Board*** which is helped by the ***Secondments Committee*** with its ***R & I Council***.

Supervisory Board

Members

Representatives of all *AUTOIGG beneficiaries* plus one representative of *fellow ESRs* and *external members* - representative from the *INFO Office of the Ministry of Sciences* and/or the *H2020 NCP*. Representative of *patient associations* will also be invited to join the Supervisory Board for the annual conference. The Supervisory Board will also name its Ethics Advisor (see B5).

Responsibilities - General

(a) supporting the co-ordinator in fulfilling all the contractors obligations towards the EC including reporting,

(b) ensuring that all work meets functional requirements

(c) assessing progress of the secondments/training programme

(d) implementing changes in work sharing, budget and participants,

(e) reviewing of the reports regarding workshops and training schools.

(f) decides on general ethical issues and drafts with co-ordinator Ethical guidelines

(g) agreeing on press releases by the contractors with regard to the project

(h) quality control of generated data and milestones prior to reporting.

Responsibilities - Specific

a) Planning and implementation of all workshops

b) Ensuring standards and quality of the secondments/training activities

c) Receiving feedbacks from seconded fellows

d) IPR issues

Decisions

All decisions of the Supervisory Board shall be taken by *simple majority* (if at least three-quarters of the members of the contractors are present or duly represented by proxy).

Meetings

The Supervisory Board shall meet six-monthly and in principle at the request of its co-ordinator. Meetings will take place during workshops and annual meetings between the ***kick-off*** and a ***final meeting***. If necessary due to urgent matters tele- or Skype conferencing will be arranged. Extraordinary meetings may be called at any other time at the request of the co-ordinator or at the request of a quarter of the contractors. Meetings of the Supervisory Board shall constitute a quorum if more than two third of the contractors are present or duly represented by proxy.

Financial and administrative management

With the help of NCP for EC finances the project office will lead the administrative management of AUTOIGG by:

a) Providing and managing a financial plan including timely collection and preparation of the documents for EC

b) Managing resource engagement and level of integration

c) Alerts in case of non-delivery and/or default of partners

d) Coordinating management of intellectual property and dissemination

Secondments Committee

Members

A chairman and 4 other members respecting the gender balance will be appointed by the Supervisory Board within the first month of the project. At least one member will be assigned from the SME sector. One fellow member will be added from the R & I Council (see below).

The formation of this committee and its first meeting will take place at the kick off meeting of the Supervisory Board.

Decisions of the Secondments Committee:

All decisions shall be taken by simple majority. Any decision requiring a vote at a Committee meeting will be identified as such in the pre-meeting agenda, unless there is an unanimous agreement to vote on a decision at that meeting and three quarters of the members of the contractors are present or duly represented by proxy.

Meetings

The Committee shall meet every 6 months or at the request of its chairmen. Meetings will take place principally during workshops and annual meetings and if necessary by tele- or Skype - conferencing.

Responsibilities

The Secondments Committee shall coordinate the staff mobility program. They will assume overall responsibility for liaison between the seconded fellows and the Supervisory Board, and for analysing and approving the progress of each secondment project. The Secondments Committee shall be responsible for:

a) Coordinating and advising the different host institutions

b) Ensuring that all seconded project topics meet the common research goal of the consortium

c) Assessing progress of mobility projects towards a *stabile knowledge gain* for the fellows

d) Solving any problems coming up between the staff visitor and the host representative

Researcher and Innovation Council

Members

All staff from the seconded plan and headed by a fellow representative. Being the managing body of the seconded fellows it aims to provide feedback on the training activities to the senior scientists, and co-organize network-wide events. The Council will also be actively involved in the organization of fellow-days, voting for the rotation of the representative in the Supervisory Board, and construction of the web site.

Meetings

After a ‘Welcome meeting’ (on Month 8), this Council gathers yearly or if summoned by the Secondment Committee.

Recruitment strategy will be based on

- Gender balance and equal opportunities

- "Code of Conduct and the European charter" The principles of *The European Charter for Researchers & The Code of Conduct for the Recruitment of Researchers* (as of March 11, 2005) will be taken as guidelines.

Monitoring Activities *(approx. dates)*

1- at *organisational level*:

a) During **the first month**: a standardized assessment procedure will be provided at the *kick-off meeting* that will be held by the Supervisory Board. The standardized procedure will be agreed considering the following aspects: mutual recognition of secondment topics, lab and office hours, reporting, individual staff fellow differences, skills and know-how acquainted, critical evaluation of research output, fostering interaction; quantification of objectives which have been set, level of progress towards objectives.

b) At **month 15** mid-term meeting will be held. During the meeting, all the partners will review the progress of the project, identify possible problems and define contingency measures

c) At **month 47**: the final meeting will be attended by the Supervisory Board in order to present, discuss and organize the final dissemination of results

2- at *scientific level*:

a) Every **two months**: the Secondment Committee will report to the Supervisory Board on the mobility research/innovation/training progress status. Results will be discussed and eventually adjusted or modified

b) At **months 11/23/35/47**: review meetings, organized by the project co-ordinator with the Supervisory Board in order to verify and eventually adjust the hosting activities and/or approaches

c) **Yearly meetings** will be held by the Supervisory Board in order to monitor the quality of secondments and working conditions and a representative of the EC will be invited to attend these meetings.

During the entire duration of the project a call centre will be active at the project coordinating centre. Regular hosting site visits will be conducted by a member of the Supervisory Board, and a monthly conference call will be held by the Secondments Committee for monitoring progress and troubleshooting. Intranet communications will be used for sharing the Supervisory Board meeting minutes and project information as well as documents.

4.3. *Appropriateness of the institutional environment (hosting arrangements, infrastructure)*

FBUB will run the project through its Centre for laser microscopy were all the basic results have been accumulated for the proposed automatic IgG-based diagnostic system. The FBUB team has 2 expert professors, 3 researchers, 3 ESRs and one technician – veterinarian, and an administrative person.

YEDITEPE will bring necessary expertise in immunology, particularly regarding antibody profiling and analysis, as well as connections with the clinics and the availability of patient siPSC-derived in vitro and in vivo models. Here are some papers and a patent related to ALS and biologicals. YEDITEPE will bring 5 researchers and 2 technicians (biologists and molecular biologists in both profiles) to the project. They will host three workshops.

UEF has expertise in ROS signalling studies with specific fluorescent dyes that is essential for one of the functions of the designed innovative device. In addition, UEF are in the European forefront

of iPSC research. They can differentiate these human cells from ALS, AD and PD to various neurons, astrocytes, microglia, endothelial cells and oligodendrocytes. When transplanted into immunodeficient mice these cells get fully integrated. UEF will host two training schools. The team of this beneficiary will consist, in addition to two expert key persons, of 3 ERs and 4 ESRs.

ArGenit is an SME specialized in developing microscopy analysis systems and thus will be instrumental in the design and production of the optical device for the proposed automated diagnostic system. In addition, they will bring a complementary know-how in iPSC research, namely, stem cell derived cultures are used to model diseases and to search for phenotypes. ArGenit will bring 2 experts in addition to technical staff.

ELVESYS is an SME specialized in developing microfluidics systems. It boasts the world widest brand of microfluidic flow control products. ELVESYS will be instrumental in the design of the microfluidic platform for the proposed automated diagnostic system. In addition, this SME will offer soft skills regarding marketing of scientific instrumentation and entrepreneurship in general. The human potential for the project will be 3 engineers (2 PhDs in Biology and 1 PhD in physics and chemistry).

LANOTEC is a listed TC academic institution that will offer expertise in state of the art biophysical methods for subcellular analysis that will offer closer understanding of the IgG effects to be standardized. The human resources employed consist of 3 researchers, 1 ESR and one administrative person.

UCONN - is a TC organization with world renown expertise (Dr Srdjan Anntic) in voltage-sensitive dyes that will be essential for a functionality of the proposed automatic diagnostic device. The laboratory of Dr Antic will also transfer know-how on optical system for *in vivo* recordings of fluorescence. The scientific environment of this lab consist of experienced electrophysiologists, anatomists, chemists (designing voltage-sensitive dyes) and optical technicians.

UCHIC – is a TC organization with particular expertise in the measurements with multielectrode arrays (MEA). The lab of prof. Wim Van Drongelen will be instrumental in helping define electrophysiologic criteria to be complemented with voltage-sensitive dye measurements.

FBUB has the lab facility (within the Centre for laser microscopy) for cell culturing and video microscopy monitoring of calcium, patch-clamp electrophysiology, and confocal laser scanning microscopy.

YEDITEPE has within the Immunology Department two lab facilities: 1) Autoantibodies Lab, and 2) HLA Typing Lab and a Stem Cell Laboratory within the Hematology Department in the Hospital, all with necessary equipment.

UEF is equipped with a live imaging facility for both *in vitro* and *in vivo* (with multiphoton microscope) experiments. Newly fluorescent constructs are available designed for site specific ROS signalling, as well as for markers of oxidative stress in mitochondria. In addition, this centre will transfer know–how on multiphoton imaging of mitochondria *in vivo*. An advanced facility of stem cell culturing also exists.

ArGenit - fluorescent microscopes that are needed during the project, are available in the company head office in Istanbul.

ELVESYS has more than 300 m² of Lab facilities for microfluidic and flow control instrument development also containing soft lithography facilities for microfluidic device fabrication.

UCONN facility is equipped with 3 advanced electrophysiology setups connected with fluorescence measuring time high-resolution video microscopy. This will be used for the purposes of testing and studying of intracellular voltage-sensitive dyes.

UCHIC Centre for epilepsy research is fully equipped for the investigation of neuronal networks properties from theoretical mathematical and computational approaches modelling, single neuron and local network activity with fully established patch clamp and confocal microscopy setups and expertise. Investigation of network architecture and physiological and pathological behaviours in dissociated neuronal cultures are conducted on Microelectrode Array (MEA) setups that will be essential for collecting data for the electrophysiology signalling for the proposed automatic IgG-based device.

4.4. *Competences, experience and complementarity of the participating organisations and their commitment to the project*

The main aim of **UEF** - Kuopio team is to provide live imaging experiments both *in vitro* and *in vivo* (with multiphoton microscope). Collaboration between **FBUB** and **UEF** was seeded within the framework of the recently completed COST Action EU-ROS. Pilot experiments were thus performed that are the basis of innovative ROS-based biomarker recordings with ALS IgGs. These data will further be confirmed at **UEF** by multiphoton imaging of mitochondria *in vivo*. **UEF** will also transfer knowledge to **ELVESYS** and **ArGenit** regarding the ROS measurement modality of the proposed device.

YEDITEPE with its Immunology department will bring complementary knowledge on autoantibodies and their characterization that is essential to the work on ALSIgGs. This participant will also be a necessary link to the clinic as well as a monitor of good laboratory practice.

UCONN will complement the **FBUB** knowledge on synaptic effects of ALS IgGs with the application of voltage sensitive dyes. **UCONN** has ongoing collaboration with **FBUB**, through Dr Antic who is also an alumni of the University of Belgrade. The collaboration is based on research and education. A member of **FBUB** lab is now a postdoc at **UCONN**.

UCHIC will complement the know-how of **UCONN** on voltage-sensitive dyes with MEA measurements. This will offer a necessary complementary knowledge of network responses to ALS IgGs.

ELVESYS and **ArGenit** as the two SMEs on the project will interact with other participants as well as among themselves. Thus, they will work closely together and within the AUTOIGG consortium towards the production of the prototype of the automated IgGs screening diagnostic device. Thus, **ELVESYS** will be designing the microfluidic chamber and the measuring platform while **ArGenit** will take care of the optical system of the measuring device.

5. Ethics Aspects

Ethics self-assessment:

The ethical issues concern the involvement of human sera and the involvement of animals in the research proposed by the AUTOIGG consortium.

1) Describe how the proposal meets the national legal and ethics requirements of the country or countries where the tasks raising ethical issues are to be carried out.

The Supervisory board of AUTOIGG will monitor and control that all participants involved in AUTOIGG will comply with "The EU Charter of Fundamental Rights" and will perform their research to the relevant EU legislation, in particular:

• Council Directive 83/570/EEC of 26 October 1983 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EEC on the approximation laid down by law, regulation or administrative action relating to proprietary medicinal products

• Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnology inventions

• Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council directive 0/220/EEC

• Council Directive 2010/63/EU corrigendum of 24 Jan 2013 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of Animals used for Scientific purposes

• Directive 2003/65/EC of the European Parliament and of the Council of 22 July 2003 amending Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (Text with EEA relevance)

• Council Decision 1999/575/EC of 23 March 1998 concerning the conclusion by the Community of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes

• Council Decision 2003/584/EC of 22 July 2003 concerning the conclusion of the Protocol of Amendment to the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes

• Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.

Research ethics also concerns research integrity and the AUTOIGG consortium will adopt "the European Code of Conduct for Research integrity" published by the European Science Foundation in 2011.

Ethics issues within the consortium

Objectives:

Dysfunction of the CNS is the main cause of many neurological diseases. A major clinical and societal problem is that there is no cure for the majority of the nervous system diseases, which are often devastating. This affects a huge number of people, as disorders of the nervous system affect 38.2% of the population in Europe, which is a conservative estimate since not all neurological

disorders were taken into account. This underscores that nervous system disorders are currently a core health challenge.

The research proposed by the AUTOIGG consortium will involve human sera and animal experiments.

Methodology:

None of the animal experiments will be initiated before the approval by the appropriate ethical committee has been obtained. The AUTOIGG consortium has ethical approval for most of the proposed experiments and will obtain approvals for the remaining ones.

AUTOIGG participants are fully aware of the ethical issues involved in working with animals.

The Supervisory board of AUTOIGG will monitor and safeguard that all participants involved in AUTOIGG comply with the national and EU legislations.

Every effort will be made to replace, reduce and refine the animal experiments and thus to comply with the 3R's principle.

Replace: whenever possible experiments will be performed on cell culture and human brain tissue;

Reduce: the number of animals in each study will be restricted to the minimum;

Refine: Unnecessary suffering will be prevented at all times. All procedures will be performed by individuals who are adequately trained and competent in the planned procedures. The appropriate anesthesia as approved by the local authorities will be applied and stress will be reduced wherever possible.

Patients sera will be taken from patients based on written informed consent.

Impact:

Insight into nervous system dysfunctions, which is a prerequisite for designing novel therapies that would cure the diseases or improve the neural functioning, requires studies in animal disease models. To reach this goal, basic knowledge about molecular and cellular changes in the nervous system combined with translational and applied research is necessary to connect this knowledge to the patient situation.

Pre –Grant Requirements

HUMANS

2.1 Details on the procedures and criteria that will be used to identify/recruit research participants

Identification and recruitment of human participants will take place through existing co-operation of FBUB and Neurological Clinic, Clinical Center of Serbia. Neurological Clinic is leading neurological center of the region, providing highly specialized diagnostic procedures such as immunological and genetic analyses, neurophysiological and neuroradiological diagnostic procedures.

Patients diagnosed with a neurodgenerative disease with inflammatory symptomatology (e.g. ALS, MS, PD, HD), which have undergone detailed specialist clinical examination and met the entry criteria defined by the research design, will be included in the study.

For the purpose of **AUTOIGG**, during routine blood sampling, additional 10 ml of blood will be collected and only purified IgG fraction from patient sera will further be used in experiments.

Beside neurological patients, samples will be taken from age and sex-matched controls that include healthy individuals and disease-control patients.

Patient specific data that will be collected will include Functional Rating Scales, gender, age, duration of the disease and type. Patients will also be screened for possible genetic mutations linked to inherited form of the disease and potential comorbidities. Additionally, basic demographic and clinical data, relevant to this research, will be gathered. Neurology Clinic has implemented InfoMedis information system for patient data storage.

Patient material will also be provided by the YEDITEPE University (see informed consent form attached).

Sera samples and personal information will only be taken from patients who give informed consent in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. Detailed information on the informed consent procedures that will be implemented for the participation of humans.

Research involving Patients - Informed Consent

Patients sera will be taken from patients based on written informed consent. Informed Consent is the decision, which is written, dated and signed, taken freely after being duly informed of its nature, significance, implications and risks and appropriately documented, by any person capable of giving consent or, where the person is not capable of giving consent e.g. Alzheimer patients, by his or her legal representative; if the person concerned is unable to write, oral consent in the presence of at least one witness may be given in exceptional cases, as national legislation requires. The European Commission - Research Directorate - General provides guidance on informed consent and this will be respected.

The research planned in this proposal only requires persons able to freely understand and question the consent procedure. Vulnerable persons like children, persons with an IQ below 70, severely-injured patients, etc. will be excluded. Information will be given in lay terms and under no circumstances will pressure of any kind be exercised on the individual participant nor her/his family.

The collection of personal data for human patients in **AUTOIGG** will be performed in Serbia and Turkey.

Methods of obtaining Informed Consent: Informed consent documents (ICDs) will explain in simple terms the objectives, scope, and potential risks and benefits of study participation. If the patient decides after an appropriate time for consideration to participate in the experiments, patients will sign the respective standardized ICD document in duplicate in the presence of the investigator (template attached). One copy of the signed and dated ICD is handed to the patient. The other copy of the ICD is filed at the **AUTOIGG** research center. Patients will be informed that participation in the study is voluntary and that consent can be withdrawn at any time without the need to provide reasons, and without disadvantage or prejudice. Information on data protection will be given before the patient is entered into the study and any study-specific procedures are performed.

2.3. Templates of the informed consent forms and information sheet

INFORMED CONSENT

Original in Serbian

Писана сагласност пацијента за учешће у испитивању

Назив истраживачког пројекта: Аутоматизовани функционални скрининг IgG за дијагностику неуродегенеративних обољења

Руководилац пројекта:

Подаци о пацијенту

АЛС/Контролно обољење/Здрава контрола:

Име и презиме:

Датум рођења:

Број картона: Молимо да обележите поља (X)

1.	У оквиру *Информације за пацијенте* описани су циљеви и план истраживања, као и његови могући ризици и користи. Потврђујем да сам пажљиво ишчитао/-ла *Информацију за пацијенте*, да су ми наведена упутства била разумљива и да располажем са довољно података о истраживању у коме бих учествовао/-ла. Имао/-ла сам прилику да поставим питања у вези са овим истраживањем и добио/-ла сам јасне и разумљиве одговоре.	
2.	Схватам да је моје учешће добровољно, да могу да повучем своју сагласност у било ком тренутку без објашњавања разлога и да то ни на који начин неће утицати на стандард мог садашњег или будућег лечења.	
3.	Добровољно пристајем на све поступке описане у плану истраживања *Информације за пацијенте*.	
4.	Схватам да се подаци сакупљени током мог учешћа у наведеном истраживању шифровано уносе у базу података и анализирају, и да ће се користити искључиво у научне сврхе.	
5.	Дајем дозволу да се моји узорци шаљу у друге земље укључене у пројекат.	
6.	Разумем да ми неће бити саопштени резултати истраживања која ће се спроводити са мојим узорцима, сем уколико је то важно за ток моје болести.	

Не одричем се својих законских права потписом овог информисаног пристанка.
Добићу потписани примерак овог информисаног пристанка.

	Име и презиме	Својеручни потпис	Датум
Испитаник			
Особа које је добила пристанак			

Translation in English

Informed consent form

Title of Research Project: Automated Functional Screening of IgGs for Diagnostics of Neurodegenerative Diseases

Principal Investigator:

Patient Details

ALS/Control disease/Healthy control:

Name and Surname:

DOB:

Hospital No: Please initial box (X)

1.	The document *Patient Information Sheet* gives goals of the research, as well as research plan, potential risks and benefits. I confirm that I have carefully read and understood the information given in *Patient Information Sheet.* Provided information was clear to me and I have enough facts about the proposed research to be able to decide whether I would like to participate. I was given the opportunity to ask everything concerning this research, and I got clear answers.	
2.	I understand that my participation is voluntary, that I can withdraw my consent at any time without giving reasons, and that it will not affect in any way the standards of my current or future health care.	
3.	I agree to all the procedures described in the research plan in the document *Patient Information Sheet.*	
4.	I understand that data collected during my participation will be anonymised and imported in database and it will be used only for scientific purposes.	
5.	I give permission for my samples to be sent to other countries included in the project.	
6.	I understand that I will not be told the results of any test which may be carried out with my samples unless it is relevant for my medical condition.	

I do not give up on my legal rights by signing this informed consent.
I will get a signed copy of this informed consent.

	Name and Surname	Signature	Date
Patient			
Person taking consent			

The original consent form from Yeditepe University, Turkey

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	**Poliklinik Genel Onam Formu**

Değerli Hastamız

Sağlık Bakanlığı yasal Hasta Hakları Yönetmelik gereklilikleri, JCI akreditasyon standart gereklilikleri ve ISO 27001 ve 15189 laboratuvar akreditasyon standartlarını uygulayan bir kurum olarak; hastanemizde bulunduğunuz süre içerisinde ilk başvurduğunuzda ve yıllık olarak güncellenmek üzere uygulanacak muayene, kan alma vb işlemler için yazılı onam alınmaktadır.

Hastanın veya yerine onam verecek kişinin okuma, anlama, konuşma, dil sorunu mevcut mu? Evet ☐ Hayır ☐ Cevabınız EVET ise Hasta Hakları Bölümü ile iletişim kurunuz.	**Tercüman gerektiyse;** Tercümanın adı _____ İmza _____ Tarih _____

Yeditepe Üniversitesi Hastaneleri ve Bağlı kurumlarında sorumlu doktorum ve doktorumun talimatıyla ilgili birimler personelinin bakımı altında olduğumun bilincindeyim.

- Doktora / hemşireye / personele mevcut sağlık problemlerimi, alerjilerimi, aldığım ilaç ve tedavileri belirtmem gerektiğini biliyorum. İnceleme amacı ile kullanılan kan, idrar ve diğer vücut sıvılarımın doku ve ürünlerinin alınmasına ve saklama süresi sonunda testlerin tamamlanmış örneklerin imha edilmesine izin veriyorum. Doktorun, sağlık profesyonelinin, çalışanın veya ilk eylemde bulunan yetkilinin perkütan, mukoza zarı, kan veya başka bir vücut sıvısının açık yaradan çıkışı ile HIV (human immunodeficiency virus) ve / veya HBV (hepatit B virus) veya HCV (hepatit C virus) incelemesinin yeni bir onay alınmadan uygulanabileceğini biliyorum
- Bakım sağlayıcılarımın belirttiği rutin işlemler, muayeneler, incelemeler, bağışıklık sağlayıcıları, teşhis amaçlı işlemlerin uygulanmasına onay veriyorum. Tam anlamamam durumunda, uygun sağlık bakımı personeline soru sorma hakkımın olduğunu ve **bunun benim sorumluluğum olduğunun bilincindeyim.**
- Doktorum tarafından gerekli görülen her tür ilave işlem için, ayrıca işleme özel bilgilendirme onay formu imzalamamın istenebileceğini anlıyorum.
- Hastalığım nedeni ile hastanede uygulanacak tüm tanı ve tedavi yöntemlerinin olası maliyeti konusunda işlem öncesinde bilgilendirileceğim bana söylendi.

- Bağlı olduğum özel ve resmi kurumların talep ettiği bilgileri vermekle yükümlü olduğunuzu biliyor ve kabul ediyorum. Kullandığım, Özel sağlık sigortam nedeniyle, kimlik ve sağlık bilgilerimin, sigorta şirketimle ve onun yetkilendirdiği ağ içinde bulunan diğer şirketlerle paylaşılmasına muvaffakat ediyorum.
- Tesis içerisinde bulunuşum sırasında personele teslim ettiğim kişisel eşyalarımın dışında, kayıp veya zarar görmüş kişisel eşyadan Yeditepe Üniversitesi Hastanesi'ni sorumlu tutamayacağımı anlıyor ve kabul ediyorum.
- Benden alınan kan, idrar vb örneklerimin gerekli tetkiklerim çalışıldıktan sonra kalanlarından (Genetik çalışmalar hariç olmak üzere) ismim kullanılmadan deney / araştırma örneği olarak kullanılabileceğini biliyor ve izin veriyorum.
- Hastanemiz bir üniversite, eğitim ve araştırma hastanesi olup, gerek tıp eğitimi, gerekse hemşirelik, eczacılık, fizyoterapi gibi diğer sağlık alanlarında eğitim verilmektedir. Bu alanlarda eğitim alan stajyer, intörn, tıpta uzmanlık öğrencisi ve diğer öğrenciler, öğretim üyelerinin gözetiminde zaman zaman tedavinizde yer alabilirler.
  Belirtilen ifadelerin çerçevesinde YUKARIDA BAHSEDİLEN İŞLEMİN YAPILMASINI TALEP EDİYORUM.

AŞAĞIDAKİ İMZAM KABUL ETTİĞİMİ GÖSTERİR:

1) Bu form üzerindeki tüm bilgileri okudum veya okuttum ve anladım
2) Soru sorma şansı tanındı ve bu form ile ilgili tüm sorularıma tatmin edici cevaplar verildi.

HASTANIN BİLİNCİ AÇIK ve 15 YAŞ ÜZERİNDE İSE;	**HASTANIN BİLİNCİ KAPALI VEYA 18 YAŞ ALTINDA İSE VE YANINDA YASAL TEMSİLCİSİ VAR İSE;**
Hastanın	**Yasal Temsilci*(Vasi) veya Veli**
Adı Soyadı :	Adı Soyadı :
Adresi :	Adresi :
Telefon Numarası :	Telefon Numarası:
İmza:	**İmza:**
Şahit:**	**Şahit**:**

Adı Soyadı: **İmza:**	**Adı Soyadı:** **İmza:**

Poliklinik Onam Hasta dışındaki Yasal Temsilcisi ve ya Velisi(Vasi) tarafından verildi ise lütfen yakınlık derecesini ve hastanın kendisinin imzalamama gerekçesini belirtiniz.

……

……

Informed consent form from Yeditepe University, Turkey translated into English

YEDİTEPE ÜNİVERSİTESİ HASTANESİ	**General Polyclinic Approval Form**

Dear Patient,

As required by the Ministry of Health in accordance with Patient Rights Regulations, and as an health organization complying to JCI accreditation, ISO 27001 and ISO 15189 standards; we take your written approval for blood collection, therapeutic applications and such; when you first apply and update this approval on yearly basis.

Do patient or patient's custodian have any problem with reading, comprehension, speaking, language problem? Yes ☐ No ☐ If your answer is YES please contact Patient Rights Office	**If a translator was necessary;** Name and surname of the translator _____ Signature _____ Date _____

I am aware that I am under the care of my doctor and personnel from related departments with the instructions of my doctor in Yeditepe University Hospitals and associated organizations.

I know that I need to declare my present health problems, allergies, medical and other therapies to doctors/nurses/hospital personnel. I give permission to collection and storage of samples of my blood, urine and other body fluids, tissues; and their annihilation after expiry date. I know that in case of percutaneus, through mucosa membrane, blood or other body fluid's leakage to the doctor, health professionals or the initial care persons HIV (Human Immunodeficiency Virus) and/or HBV (Hepatitis B Virus), and/or HCV (Hepatit C virus) will be tested without additional approval.

I give consent to application of routine procedures, examinations, analysis, vaccines, procedures for diagnosis given by the healthcare providers. I realize that, for any issue that I have not understood clearly, I have the right to ask questions to related health care person and that is in my own responsibility.

I comprehend that for any additional procedure that is accepted as necessary by my doctor/s, I may be asked to sign a procedure specific approval.

- I have been informed that I will be given information about the possible cost of all diagnostic and therapeutic applications for my illness before these procedures.
- I know and accept that you are required to provide information to my affliated private or state organizations when requested. I give consent to share of my identity and health information with my insurance company, and their approved companies due to my private health insurance.
- I know that, while I am in the hospital, I cannot hold the Yeditepe University Hospital responsible for lost or damaged belongings except the personal belongings I have declared to personnel at first.

- I have been informed and consent that my left over blood, urine etc. samples can be used for experiments/researches after routine testing except genetic testing without use of my name.
- I have been informed that this hospital is a university's training and research hospital, that gives education to medicine, nursing, pharmacy and physiotherapy. The students in training for these professions, interns, residents and other students may take role in your care from time to time under the supervision of their professors.

  I REQUEST THAT PROCEDURES TO BE COMPLETED in framework of the above mentioned.

MY SIGNATURE BELOW INDICATES THAT I APPROVE:

1) I have read/instructed and understood all of the information provided on this form
2) I was given the chance to ask questions and have received satisfying answers to all my questions

IF THE PATIENT IS CONCIOUS AND OVER 18 YEARS OLD;	**PATIENTS IS UNCONCIOUS AND LESS THAN 18 YEARS OLD AND A LEGAL CUSTODIAN;**
Patient's Name Surname : Address : Phone number : **Signature:**	**Legal Representative*(Custodian) or Parent** Name Surname : Address : Phone Number: **Signature:**
Observant:** **Name/Surname:** **Signature:**	**Observant**:** **Name/Surname:** **Signature:**

If the Polyclinic Approval is given by the legal custodian or parents, please indicate the relation and explain why the patient have signed himself/herself.

……

……

Original in Serbian

Информација за пацијенте

Назив истраживачког пројекта: **Аутоматизована функционална анализа IgG за дијагностику неуродегенеративних обољења**

Овај документ садржи податке које је потребно да имате у виду пре него што пристанете да учествујете у овом истраживању. Врло нам је важно да разумете ове информације и да питате све што желите у вези са овим истраживањем. Пројекат „ Аутоматизовани функционални скрининг IgG за дијагностику неуродегенеративних обољења“ је настао са циљем да се омогући што раније успостављање дијагнозе и прецизније предвиђање тока неуродегенеративних обољења као и персонализован приступ лечењу пацијената. На Вама је да одлучите да ли желите да учествујете у истраживању. Објаснићемо Вам студију и детаљно проћи са Вама кроз овај документ. Уколико се одлучите да дате свој пристанак, замолићемо Вас да дате писану сагласност.

У било ком тренутку студије можете да повучете своју сагласност, без објашњавања својих разлога. Такав поступак неће утицати на стандард лечења који добијате.

Поштовани,

Позивамо Вас да учествујете у научном истраживању. Пре него што одлучите да ли ћете дати свој добровољни пристанак, неопходно је да добро разумете разлоге због којих се изводи ово истраживање, циљеве којима тежи и на који начин Ви доприносите њиховом остварењу. Због тога Вас молимо да одвојите довољно времена да пажљиво ишчитате податке о истраживању, да нас питате све што Вас занима и да потом одлучите да ли желите да учествујете.

Уколико одлучите да не желите да учествујете, то неће ни на који начин утицати на стандард лечења и терапије коју добијате. У било ком тренутку студије можете да повучете своју сагласност, без објашњавања својих разлога. Такав поступак неће утицати на стандард лечења који добијате.

Циљ истраживања

Упркос бројим истраживањима међу којима су и врло успешне преклиничке, па чак и клиничке студије о потенцијалним терапијама за амиотрофичну латералну склерозу (АЛС), поуздана и делотворна терапија још увек не постоји. Истраживачи и лекари се слажу да је разлог томе недовољно усавршен процес успостављања дијагнозе што је могуће раније и прецизније, као и система за праћење тока болести на ћелијском и молекуларном нивоу.

Установљено је да се код пацијената оболелих од АЛС стварају анти-неурални имуноглобулини Г (IgG), као важна одлика запаљенског процеса, који доводе до патофизиолошких промена основих параметара ћелија нервног система. Ово истраживање ће омогућити стандардизацију процеса којима се ове промене прате као и повезивање конкретних промена са специфностима развојних фаза болести . На основу прикупљених података, на крају ће се дизајнирати дијагностичка апаратура која ће помоћи предвиђање, праћење болести, процену адекватне терапије и одговора на исту, за сваког пацијента појединачно.

План истраживања

Фокус истраживања је испитивање могућности употребе имуноглобулина Г изолованих из серума пацијената као дијагностичких и прогностичких параметара неуродегенеративних обољења. Пацијенти оболели од амиотрофичне латералне склерозе, који су прошли детаљан клинички преглед и који задовољавају улазни критеријум дефинисан дизајном истраживања, биће укључени у истраживање. Посебно ће се узимати у обзир да ли је код пацијента установљена спорадична форма болести или су идентификоване најчешће узрочне мутације које доводе до развоја наследне форме АЛС, као и да ли постоје и друга пропратна обољења, како би се узели у обзир сви фактори и унапредила дијагностика амиотрофичне латералне склерозе. У истраживање ће бити укључени и пацијенти оболели од других обољења, као и здрави појединци као контролне групе. За потребе овог истраживања, учесницима који дају добровољни пристанак, ће приликом редовног вађења крви бити узет додатни узорак крви (до 10мл), а потом ће се из тог узорка пречистити имуноглобулини Г који ће се користити даље за испитивање патофизиолошких процеса АЛС или као контролини IgG. Од учесника ће се прикупити и основни демографски и клинички подаци од значаја за ово истраживање и унети у базу података ради детаљне анализе. Сакупљени биолошки материјал и резултати овог истраживања биће коришћени само, једино и искључиво у научне сврхе, а у циљу бољег разумевања и прогнозе болести.

Ризик

У стручној литератури нису описани ризици у вези са поступцима који ће бити примењени у овом истраживању.

Тајност података

Узорци Ваше крви, пречишћени имуноглобулини Г и Ваши подаци неће бити означени Вашим личним именом, већ лабораторијском ознаком (бројем) ради заштите Вашег идентитета. Резултати добијени у овом истраживању биће коришћени у оквиру научног пројекта „Аутоматизовани функционални скрининг IgG за дијагностику неуродегенеративних обољења“ , у стручним радовима објављеним у научним часописима и

саопштењима на научним скуповима. Истраживање мора бити одобрено од стране Етичког одбора, што представља потврду о заштити и поштовању права пацијента.

Новчани трошкови испитаника

Испитаници укључени у истраживање неће сносити никакве новчане трошкове за поступке који ће бити примењени у овом истраживању.

Translation in English

Patient Information Sheet

Title of Research Project: Automated Functional Screening of IgGs for Diagnostics of Neurodegenerative Diseases

This document provides information that you need to take into consideration before you agree to take part in this research project. It is very important to us that you completely understand this information and ask us anything concerning this research. Research project “Automated Functional Screening of IgGs for Diagnostics of Neurodegenerative Diseases” includes activities aimed at enabling early diagnostics, precise monitoring of the course of neurodegenerative diseases as well as point-of care personalized diagnostics. You are free to decide whether you want to participate in this research. We will explain the study and go through this information sheet. If you decide to participate, we will then ask you to sign a consent form.

At any point during the study you are free to withdraw, without giving a reason. This will not affect in any way the current or future standard of care and treatment you receive.

Respected Sir/Madame,

We would like to invite you to take part in a research project. Before you decide whether you wish to participate or not, it is necessary for you to understand the reasons for this research, its goals and how you can contribute. Therefore, we kindly ask you to take time and read carefully this Information Sheet, ask us anything you would like to know and then decide whether you would like to participate.

If you decide not to take part, your standard of care will not be affected by your decision. At any point during the study you are free to withdraw, without giving a reason. This will not affect in any way the current or future standard of care and treatment you receive.

The purpose of the study

Although there are many successful preclinical, and even some clinical studies of potential therapy for amyotrophic lateral sclerosis (ALS), there is still no reliable and effective therapy. Researchers and practitioners agree that the reason for this is insufficiently perfected process of establishing diagnosis early enough, and monitoring of the course of the disease on cellular and molecular level. In ALS, production of anti-neuronal immunoglobulin G (IgGs) is a significant feature of the immune-inflammatory process. IgGs further provoke pathophysiological changes of basic parameters in the cells of the central nervous system. This research will enable standardization of processes through which these changes are being monitored as well as linking them with the specificities of the developmental stages of the disease. Based on collected data, fine diagnostic tool will be designed to help prediction and monitoring of the disease, assessment of adequate therapy and responsiveness for each patient.

Research plan

The aim of this research is to examine whether IgGs isolated form sera of patients diagnosed with ALS can be used as diagnostic and prognostic parameters of neurodegenerative disease. ALS patients who have undergone detailed clinical examination and met the entry criteria defined by the experimental design, will be included in the study. In order to include all the potential factors and improve diagnostics, it will be taken into account whether patients are diagnosed with sporadic form of the disease or the presence of some of the mutations related to the inherited form is detected. Patients will be also screened for comorbidities. Patients diagnosed with other disease, as well as healthy individuals will be included in the study as control groups. For the purposes of this research, participants who give informed consent, will be asked to give additional 10 ml of blood during their routine blood sampling. Thereafter, IgG will be purified from sera and used to examine pathophysiological processes in ALS or as control IgGs. Basic demographic and clinical data relevant to the study will also be collected and imported into database for further correlation analysis. Gathered data, biological material and research results will only be used for scientific purposes in order to gain better understanding of disease pathophysiology.

Risks

There are no risks associated with the procedures that will be used in this research.

Confidentiality and Privacy of Personal Data

Your blood samples, purified IgGs and personal data will not be labeled with your name but with laboratory index (number) for protection of your identity. Results obtained throughout this research will be used for the purposes of scientific project “Automated Functional Screening of IgGs for Diagnostics of Neurodegenerative Diseases”, in scientific articles and in announcements on scientific meetings. Research must be approved by Ethic Committee, which represents confirmation of respect and protection of patients` rights.

Patients` expenses

Participants included in this research will not bare any costs for the procedures that will be applied in this research.

PROTECTION OF PERSONAL DATA

4.4. Detailed information must be provided on the procedures that will be implemented for data collection, storage, protection, retention and destruction and confirmation that they comply with national and EU legislation.

AUTOIGG project require the processing and/or storage of personal and sensitive information. Personal and sensitive information that is collected from research will be kept confidential. Such information will be anonymised and kept safe. **AUTOIGG** research complies with relevant legislation and processes/stores all personal information in accordance with it. Personal data will be handled according to the national regulations in vigor regarding research involving humans and personal data protection.

Each patient after signing informed consent will enter the study and study-specific procedures will be performed. Patients blood samples, purified IgGs and personal data will not be labeled with the patients name but with the medical laboratory index (the number code) for protection of the patients identity. Each patient will be assigned a number code. This code is the only fact that will be known about the patient throughout the project. The consortium will exchange the results obtained from the IgG with the code. Only the code will be available, but not patient personal data.

4.5. Detailed information on the informed consent procedures that will be implemented in regard to the collection, storage and protection of personal data must be submitted.

All handling of personal data within this project will be conducted in accordance with legal requirements and only after voluntary consent of the participating patients.

Archiving of Data / Access to Records: Originals of all report forms, administrative documents, patient information, logs, consent forms and documentations will be stored at the **AUTOIGG** research facility for at least 10 years. A list allowing patient identification will be kept for 15 years.

Neurology Clinic, Clinical Center of Serbia has implemented InfoMedis information system for patient data storage. Keeping all information for 10 years is standard procedure. This information will at no time be kept with the participants of the **AUTOIGG** project.

Partner laboratories in the **AUTOIGG** consortium are located in Turkey, Costa Rica, and USA. Patient data will be exchanged between these partners and the rest of the consortium located in the European Union. These will include data of patients IgGs effects on synaptic activity, vesicle trafficking, as well as $Ca^{2+}$and ROS imaging and cellular and subcellular morphology. However, all these data will be rendered anonymous labelled with the number code, before any analysis or exchange is considered. This procedure ensures that no personal data will be exchanged between the EU and the rest of the consortium, and eliminates possible ethical risks related to the exchange of data.

THIRD COUNTRIES

6.1. The applicant must confirm that the ethical standards and guidelines of Horizon2020 will be rigorously applied, regardless of the country in which the research is carried out.

We confirm that the ethical standards and guidelines of Horizon2020 will be rigorously applied, regardless of the country in which the research is carried out.

All experiments performed in the research labs of the **AUTOIGG** partners have received permission by the respective national legal authorities as required by the European Commission guidelines (86/609/CEE), and comply with the national and EU legislations in particular: • Directive

98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnology inventions • Council Directive 2010/63/EU corrigendum of 24 Jan 2013 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of Animals used for Scientific purposes • Directive 2003/65/EC of the European Parliament and of the Council of 22 July 2003 amending Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (Text with EEA relevance) • Council Decision 1999/575/EC of 23 March 1998 concerning the conclusion by the Community of the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes • Council Decision 2003/584/EC of 22 July 2003 concerning the conclusion of the Protocol of Amendment to the European Convention for the Protection of vertebrate animals used for experimental and other scientific purposes • Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells.

Research ethics also concerns research integrity and the **AUTOIGG** consortium will adopt "the European Code of Conduct for Research integrity" published by the European Science Foundation in 2011.

The **AUTOIGG** projects will fulfill the national criteria for laboratory and safety and the conduction of experimental procedures will be regularly inspected. Disposal of hazardous material will be performed according to the national rules. Protective material and easily readable documents on their use are available as prescribed by the current laws.

6.2. Detailed information that fair benefit-sharing arrangements with stakeholders from low and/or lower-middle income countries are ensured during the project.

Two points have to be taken care off to confirm that fair benefit-sharing arrangements have been assured with stakeholders from low or lower-middle income countries.

A. Sharing the benefits and allocation of the intellectual property rights.

B. Contribution to the capacity building in the developing countries.

AdA:

*We confirm that fair benefit-sharing arrangements with stakeholders from Third Countries are ensured throughout the project. The sharing of the benefits and allocation of the intellectual property rights with the Third countries will follow the Consortium agreement. In general, the benefits will depend on contributions related to design of experiments, software, and the small-scale platform that will be used to accomplish the building of the new diagnostic and prognostic technology.

AdB:

Intellectual capacity: the researchers from the Third country will participate as involved observers in the different stages and development processes of the project. This will allow them to learn the methodologies and protocols related to research and development used by companies and institutes for future developments; respecting the copywriter and agreements.

Infrastructure: The LANOTEC- Costa Rica will be allowed to acquire a prototype of the new technology to be installed in the institute to be payed regarding materials and manufacturing; for future diagnostics in Costa Rica while allowing other scientist from the region to learn about the technique and protocols.

6.3. The applicant must provide details on the material which will be imported to/exported from EU.

The only materials which will be imported to/exported from EU are patients IgGs, and the adequate authorizations will be obtained. This exchange will be kept to minimum between YEDITEPE and/or FBUB on one side and UEF on the other.

6. Letters of Commitment of partner organisations

CeNAT
Centro Nacional de Alta Tecnología

FunCeNAT

CONARE
CONSEJO NACIONAL DE RECTORES
Centro Nacional de Alta Tecnología

2017
Las Universidades Públicas por la Vida, el Diálogo y la Paz

Letter of Commitment

With this letter we would like to express our real and active participation if the project proposal AUTOMATED FUNCTIONAL SCREENING OF IgGs FOR DIAGNOSTICS OF NEURODEGENERATIVE DISEASES (**AUTOIGG**), (coordinator Prof. Pavle R. Andjus, Faculty of Biology University of Belgrade) will be funded.

The person responsible for this collaboration will be Dr.José Vega Baudrit and Dra. Yendry Corrales Ureña.

Signature of legal representative
MBA. Cynthia Cordero Solis
Administrative Director FUNCENAT

Date: 27/3/2017.

FUNCENAT

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UCONN HEALTH

Center for Molecular Medicine
263 Farmington Avenue
Farmington, CT 06030-3101

31 March 2017

To Whom It May Concern:

This letter confirms that Srdjan D. Antic, M.D., in the Department of Neuroscience at UConn Health is able to host visiting postdoctoral fellows in his laboratory in order to fulfill his obligation to the proposed project entitled:

"AUTOMATED FUNCTIONAL SCREENING OF IgGs FOR DIAGNOSTIC OF NEURODEGENERATIVE DISEASS (AUTOIGG)"

(Coordinator: Prof. Pavle R. Andjus, Faculty of Biology, University of Belgrade)

Visiting scientists may perform their training at UConn Health for up to one year contingent upon obtaining valid immigration status and availability of funds.

Sincerely yours,

Christopher D. Heinen, Ph.D.
Director of Postdoctoral Affairs
Associate Professor of Medicine
UConn Health
Tel: 1-860-679-8859
cheinen@uchc.edu

The University of Chicago
Pritzker School of Medicine
Department of Pediatrics

5841 S Maryland Avenue
Chicago, Illinois 60637-1470

March 16, 2017

Letter of Commitment

With this letter we would like to express our real and active participation if the project proposal AUTOMATED FUNCTIONAL SCREENING OF IgGs FOR DIAGNOSTICS OF NEURODEGENERATIVE DISEASES (**AUTOIGG**),
(coordinator Prof. Pavle R. Andjus, Faculty of Biology University of Belrgade)
will be funded.
The person responsible for this collaboration will be ***Dr Wim van Drongelen***.

Sincerely,

Wim van Drongelen, PhD
Professor of Pediatrics, Neurology, Computational Neuroscience
Technical Director of the Pediatric Epilepsy Center
Senior Fellow Computation Institute
KCBD, Room 4124
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Marie Skłodowska-Curie Actions (MSCA)
Research and Innovation Staff Exchange (RISE)
H2020-MSCA-RISE-2017

Project Acronym: AUTOIGG – Project Number: 778405
Annex 1 to the Grant Agreement
(Description of the Action)
Part B