H2020 Topic: New concepts in patient stratification
Topic identifier: SC1-PM-02-2017

Title of Proposal

Post Stroke Improvement and Outcome Monitoring (PSIOM)

List of participants:

Participant No	Participant organization name	Country
1.	Fundatia Bio-Forum	Romania
2.	Electronic Record Services B.V.	Netherlands
3.	Biovista	Greece
4.	Servicio Andaluz de Salud - SAS	Spain

1. Excellence

1.1 Objectives

Specific objectives: clear, measurable, realistic and achievable within the duration of the project

- 1) *Identify functional and molecular stroke-related markers*: new electrophysiological function markers and a new molecular composite panel in stroke patients related to neurogenesis and improving neurological function; at least one new electrophysiological marker (giant depolarizing potentials with preceding potentials) and one new composite molecular panel (with 4-5 components: neuronal structural integrity, neurotrophin, oxidative stress, inflammation, immunovascular/neuroendocrine) is to be defined;
- 2) *Monitor post-stroke neurogenesis*: to evidentiate neuronal electrical activities during post-stroke neurogenesis and correlate them with neurological improvements; an EEG monitoring device will record continuously for the first 3 weeks post-stroke, and a bioimpedance spectroscopy device will be used at specific times in order to give more information on cellular and molecular activities which take place during post-stroke recovery;
- 3) *Evaluate new combination therapy:* obtain a significant neurological recovery in patients with severe stroke by using a new combination therapy; specifically more than 50% of stroke patients with an initial National Institutes of Health Stroke Scale (NIHSS) score above 14 (severe neurological deficit) are expected to have at the completion of the 3-week treatment an improvement in NIHSS score by 4 or more points, and further improvement at 3 and 6 months;
- 4) *Set new criteria for patient stratification:* utilize the data from this trial in order to stratify stroke patients according to composite panel of blood markers, brain electrical activity, and clinical response to specific treatment; this will provide the clinicians additional tools for administering optimal therapy and assessing recurrence risk.

1.2 Relation to the work programme

This proposal relates the following work programme topic:

TOPIC: New concepts in patient stratification

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Stroke is a complex, multifaceted disease which has been amply studied because of its prevalence and impact on patients' life and healthcare resources; the current patient stratification is based on clinical evaluation, imaging macroscopic brain structures (CT, MRI) and infrequently the levels of biomarkers (inflammatory, oxidative stress, thrombotic/fibrinolytic, neuroendocrine, etc.) which are reflecting various degrees of neuronal structural damage taking place in stroke; even so it is not considering a most important aspect of recovery from stroke, namely neurogenesis and functional integration of newly formed neurons.

PSIOM project is approaching stroke patients evaluation, stratification and treatment by using a completely new concept: the direct monitoring of neurogenesis, the formation and functional integration of newly formed neurons, and the main process leading to neurological recovery after stroke via continuous recording of EEG signals and other electrical activity during the acute phase of stroke recovery.

This approach emphasizes not on the modifications of anatomical structures nor the molecular environment associated with the loss and recovery of neuronal networks functionality which is difficult to standardize because of differences in infarct sizes and subtype as well as patient co-existent conditions and functional reserve status (eg. differences in levels of inflammatory cytokines in lacunar vs. large cerebral artery stroke), but focuses on neurogenesis and finds common ground in all types of stroke the direct monitoring of neurogenesis and the recovery of neuronal network function.

This is achieved by recording the discrete electrical events preceding and related to the integration of newly formed neurons in the damaged neural networks, and it will allow for better individualization of treatment with specific substances which can be individually tailored.

Scope: Novel concepts for patient stratification for stratified or personalised therapeutic interventions.

PSIOM project is addressing both the need for improved patient stratification and for more efficient treatment in stroke patients. It plans to achieve this by studying an essential aspect of stroke recovery through direct monitoring of neurogenesis with electrophysiologic markers and individually tailoring treatments according to both patient's characteristics and their response to treatment. Compared to known biomarkers (inflammatory, neuroendocrine, thromboembolic, oxidative, etc.) which are associated closer to neuronal death and neurological deficit (mostly a *reactive* association), the recording of electrical activity surrounding new neuron maturation and functional integration for neurological recovery is a *proactive* approach and will make possible prompt clinical decision-making for optimal course of treatment.

Integrates multidimensional and longitudinal data, -omics, including pharmacogenomics, systems biomedicine approaches, network analysis and of computational modelling.

The new combination treatment used in the PSIOM clinical study addresses simultaneously many of the pathological aspects known to take place in stroke patients (inflammation, oxidative stress, metabolic and mitochondrial modifications, neuroendocrine, immune and vascular modifications) Monitoring electrical activity related to neurogenesis and the complex molecular modifications taking place simultaneously, will make possible a new approach on patient stratification.

Systems biomedicine – COSS™ from BioVista - will be used to:

- identify and validate novel therapeutic options (and combinations) for control group of stroke patients
- identify and validate molecular and functional markers for stroke patients
- predict adverse events (AEs) related to a particular therapy in the proposed stroke population, and
- make use of potential AEs or possible comorbidities identified to propose the most appropriate patient sub-population(s) that could benefit from this treatment

Validation in clinical study taking into account sex and gender differences.

PSIOM project is organized as a randomized controlled multicentric clinical study which will evaluate neurogenesis directly as well a new combination treatment for stroke with the aim of their validation for clinical use; sex differences are being accounted for in the statistical analysis and gender is considered by selecting a treatment which is widely accepted on cultural and religious grounds.

Actively involve patient associations.

Even though the study applies to acute stroke patients, associations in the countries participating in the project will be contacted to increase patient awareness and possible applicability of study results. Partner Biovista has a long expertise in collaborating with patient advocacy groups (PAGs), notable examples being the Chronic Fatigue and Immune Deficiency Syndrome Association of America, the Thalassemia International Federation, and the Wylder Nation Foundation.

The proposals observes regulatory aspects of clinical practice and commercialisation opportunities.

The clinical trial proposed by the PSIOM project will be using medications already approved for human use, and for ethical purposes it will not use a placebo comparator (the control group will also receive active substances which are known to be beneficial to stroke patients) so that the regulatory aspects of clinical trials should be observed with ease. PSIOM will be registered with each national authority and with the respective Institutional Review Board and Ethics Committee.

PSIOM project will also comply with Horizon Open Access and Data Management Policies.

Proposals should focus on complex diseases having high prevalence and high economic impact.

Stroke is a multifactorial disease which was linked to high blood pressure, atherosclerosis, atrial fibrillation, vasculitis and other blood vessel disease, hematologic disorders, autoimmune diseases, metabolic syndrome, type 2 diabetes, central obesity, respiratory tract infections, paraneoplastic syndromes, and other less frequent pathologies. According to World Health Organization data for

Europe in 2014 (latest available), cerebrovascular disease (ischemic and hemorrhagic stroke) accounted for 1.056.983 or 12% of total deaths in that year alone. Stroke is estimated by the European Stroke Organisation to be the most important cause of morbidity and long-term disability and such it imposes an enormous economic burden and those aspects can be much improved with the PSIOM project.

1.3 Concept and methodology

(a) Concept Overall concept underpinning the project.

Stroke is associated with multiple pathophysiologic modifications at cellular and molecular level, and although many clinical studies were performed, so far there are no widely accepted biomarkers to help clinicians with patient stratification and treatment options. This may happen because most biomarkers studied so far are mainly reactive, reflecting the pathological changes surrounding neuronal destruction with the ensuing loss of neurological function. Improvement in their levels is mostly related to reduction of neuronal damage (mitigation of the negative factors affecting the neurons) but are not indicative of neurogenesis, the process in which newly formed neurons replace damaged neurons and make possible the recovery of lost function. For neurogenesis different, proactive parameters need to be defined and studied.

EEG can directly monitor neuronal function, it shows modifications of electrical activity in different brain areas and was used in stroke patients recovering to show improving neurological function with modifying patterns of electrical waves of different frequencies (alpha, beta, delta, theta). Serial EEGs can show an increase in alpha waves during useful stroke treatments, however two important improvements can be added: first is continuous (vs. serial) monitoring of brain waves, which so far was not performed especially due to technical limitations. The second and more important is the possibility to directly evidentiate neurogenesis and the electrical events associated with the 4 main stages of neural network function recovery: 1.generation of new neurons, 2.differentiation, 3.migration, and 4.network integration with neurological recovery. The latter is preceded by specific electrical activity – giant depolarizing potentials (GDPs) – which are electrical spikes of huge amplitude (more than 100 times normal activity potentials) and very short duration, thought to be caused by unison uninhibited firing of new neurons and their connections while integrating in an existing network, these GDPs so far were evidentiated only in the laboratory. As expected, the 4 stages have specific modulators (cytokines, etc) and it makes sense to adjust the clinical intervention accordingly for obtaining optimal recovery. While the first three stages are less active from an electrical standpoint, we can still look for their specific markers on EEG recordings starting backwards from GDPs and having an approximate duration for each stage from laboratory data; a data-mining software with specific characteristics will be used to look for these type of bio-electric markers.

Another tool used in this project in a novel way to record electrical activity at organ and cell level is bioimpedance spectroscopy, which has proved valuable in studying the extracellular as well as intracellular medium. It is using multiple frequencies in the kHz range (the EEG records discrete neuronal activities in the Hz range) and measures modifications of the properties of the electrical signal and its propagation medium (body tissues) to give additional information on the cell function and the molecules influencing their activities. It is currently used and validated for lymphedema patients, and it will be evaluated for its use in estimating brain edema and/or hemorrhage for point-of-care use.

Besides making patient prognosis more directly and proactively linked to neurogenesis and functional recovery and allowing for an improved stratification, this approach has the advantage of showing real-time modifications induced in neuronal recovery by different treatments, and ultimately selecting the optimal course for clinical intervention.

The treatment aspect is also very important in this context, and having a new combination treatment which was shown to offer prompt improvement in stroke (Stancioiu 2016) is paramount for recording neuronal functional improvement.

Main ideas, models and assumptions involved. Inter-disciplinary aspects, use of stakeholder knowledge

There are different components of the ischemic cascade, including **microglial activation, inflammation, oxidative stress, neuronal injury, hemostasis**, and **endothelial dysfunction** (Kernagis 2012); the respective aspects are tied to the levels of certain molecules:

Inflammation molecules (interleukins *IL-1β*, *IL-6*, tumor necrosis factor *TNF-α*, *inflammasome* activation, *C-reactive protein*, *fibrinogen*, *interferons*, etc.). TNF-α, IL-6 and IL-1β levels tend to be higher in cardioembolic stroke but lower in the ischemic lacunar stroke, are probably proportional to the infarct size; their levels peak around 72 hours post-infarction, and there is an important reactive component to inflammation. However, a 2014 metaanalysis done on 24 clinical studies (4112 stroke patients) by Bustamante et al (Dr. Bustamante is Co-Principal Investigator for the PSIOM study) showed that despite a high association of IL-6 with stroke outcome, the additional predictive value of IL-6 was moderate and unlikely to be used in clinical practice.

Microglial activation and cerebrovascular **hemostasis** were analyzed in the 2012 article by Tuttolomondo et al. through multiple clinical studies; the molecular (thrombotic/hemostatic selectins and adhesion molecules) and cellular (leukocytes, microglia) components of post-stroke recovery were shown to be important, but no significative difference was observed between each TOAST subtype with regard of *ICAM-1*, *VCAM-1*, *E-selectin*, *P-selectin*, *VWF*, *PAI-1* and *TPA* plasma levels. *CD40L* (a pro-thrombotic platelet factor) was also elevated in stroke patients compared to controls.

Oxidative stress is a major contributing factor to ischemic cerebral injury as it activates matrix metalloproteinases (MMPs, specifically *MMP-9*) and blood-brain barrier injury (Kelly 2008). Among other molecules, F2-isoprostanes (*F2IPs*), free-radical induced products of neuronal arachadonic acid peroxidation, and *laminin* are increased in acute ischemic stroke (BEAT-Stroke study).

Neuronal injury can be estimated with levels of *neuron-specific enolase*, *S100*, F2IP, etc.

The **neuro-endocrine** system, especially the activity of hipotalamus-pituitary-adrenal axis, was shown to be closely correlated with post-stroke recovery, as reflected by the levels of *copeptin* (metaanalysis by Bustamante, 2015); also *B-type natriuretic peptide (BNP)* and N-terminal fragment of BNP (*NT-proBNP*) (Bustamante 2013).

Other biomarkers reflecting mitochondrial/metabolic function and coagulation in stroke severity and prognosis are: *visfatin*, *insulin-like growth factor*, *lymphocyte succinate dehydrogenase*, *thioredoxin*, *hemopexin*, *d-dimer*, *proenkephalin A (PENK-A) and protachykinin (PTA)*, *sphingolipids*, *glial fibrillary acid protein*, *soluble tumor necrosis factor α receptor-1*, *von Willebrand factor*, *heart-type fatty-acid-binding protein*, etc.

Additional important factors shown in clinical studies to influence outcome in stroke are:

- **hyperglycemia,** closely associated with an impaired recovery of neurological function post-stroke, seems to be an independent predictor of poor outcome for stroke, even though inflammation (elevated cytokines, inflammasome activation, etc.) is present in both hyperglycemic states and ischemic injury.
- **homocysteine** levels are correlated with cardiovascular risk, stroke incidence and recurrence. The HOPE 2 trial (Saposnik 2009) with 5522 patients for 5 years showed that in adults with cardiovascular disease, daily administration of folic acid, B6 and B12 decreased risk of stroke (but not stroke severity or disability) simultaneously with lowering of homocysteine levels. However in the VISP trial homocysteine lowering was not statistically significant for stroke secondary prevention, but baseline homocysteine levels were linked to vascular events morbidity and mortality.

On the treatment side, in line with the association between stroke and inflammatory activation of platelets, aspirin was shown to confer protection against stroke occurrence in some ischemic stroke subtypes (except lacunar and TIA), and it is very likely that anti-inflammatory and anti-oxidant molecules with pleiotropic actions are beneficial in stroke prevention and treatment. This was confirmed in a placebo-controlled study by Ullegaddi (2006) on 96 stroke patients given antioxidants (vit E and C) and/or vit B2, B6, B12 showed a decrease in inflammatory marker CRP, increase in antioxidant status (measured by total plasma antioxidant capacity, malondialdehyde) and decrease in homocysteine, all of which are known to be beneficial in stroke. Interestingly, antioxidants did not

reduce homocysteine levels and B vitamins did not improve antioxidant status, so both of them are required for overall improvement. This study provides support for the simultaneous utilization of those vitamins in the new combination treatment.

The new treatment combination proposed for stroke has multiple actions on the pathological pathways known to be activated in stroke (mentioned above): inflammation, oxidative stress, neuronal structural damage and apoptosis, vascular, neuroendocrine and immune responses to ischemic stress. Actovegin was shown in multiple clinical trials to be efficacious in improving status of stroke patients; it consists of deproteinated veal serum with more than 200 substances of less than 5000 Daltons with pleiotropic effects. Actovegin was shown to improve cell utilization of glucose through insulin-like growth factor, thus improving *mitochondrial function*, which in turn is involved in neuronal survival and new neuron formation. Dimethylsulfoxide was shown to inhibit the action of many interleukins and *inflammatory molecules*, and is FDA-approved for treatment of chronic interstitial cystitis, a difficult to treat inflammatory condition. Dimethylsulfoxide is also widely utilized for protecting and maintaining vitality of stem cell during cryopreservation and in organ transplant; its pleiotropic actions - anti-inflammatory, *reducing edema*, strong *antioxidant -* are beneficial for the protection of newly formed and developing neurons in conditions of relative ischemia. Together with vitamin C and glutathion, DMSO will counteract and protect against the oxidative damage known to take place during stroke.
The vitamins of the B group (B1, B6, B12, etc) are known to improve neuronal function and importantly to *lower homocysteine* levels, a molecule associated with poor vascular status and poor outcome in stroke patients. As shown by Ullegadi, their actions are independent and additive to that of the anti-oxidants.
Finally, oxytocin is known to have stimulatory effects on dopaminergic pathways and help modulate the hipotalamic-pituitary-adrenal axis response to stress - *neuroendocrine modulation*.
This new combination treatment was already administered successfully in a few patients (Stancioiu, 2016).

On the system medicine side PSIOM will employ the COSS platform from BioVista (Clinical Outcome Search Space) to perform a risk assessment analysis of each drug, integrate the new clinical and molecular data, assess the benefit/risk profile of therapeutic interventions and identify subpopulations for stratifying patients and personalizing therapies. Prior to the study, the literature mining platform will be used to assess therapy in terms of its repositioning to stroke patients, identify suitable patient subpopulations that could mostly benefit from this drug combination, and identify suitable drugs (or combinations of drugs) to be used for the control arm patients.

Positioning of the project: from ‘idea to application’

Many aspects of the projects are ready to be implemented; on both the treatment side and on the testing side, the substances and devices are already on the market. The innovation consists of using those combination of substances and devices for stroke treatment, and the same novel utilization is on the testing devices, which will be used to monitor brain activity during stroke recovery.
It is expected that data from the PSIOM project will generate new directions for research and new application for devices, ex:
- the EEG monitoring device will be used to track changes in brain activity specific to neurogenesis and adjust stroke treatment optimally according to this data;
- bioimpedance spectroscopy will be used to investigate brain edema and hemorrhage as well as neurogenesis activity and have the potential to become regular tools in assessment of stroke patients, especially in ambulatory and emergency situations, due to their low cost, ease of use and noninvasive character.

National or international research and innovation activities linked with the project:

The consortium will actively disseminate the results of this study and will pursue new research directions and opportunities which will arise from this project.

(b) Methodology

To achieve the objectives proposed (establish neurogenesis markers, define a composite panel of markers for patient stratification, obtain significant neurological improvement in more than 50% of severe stroke patients, etc), a randomized, controlled clinical study is planned to take place at 3 clinical sites (hospitals), with a total of up to 200 inpatients enrolled, and 150 stroke patients are expected to complete the trial (anything over 130 patients ensures a good statistical power for the study). There is a 36 month time frame for the whole project:

- 6 months clinical sites setup, medical devices and medication acquisition and installation, training;
- 12 months patient enrollment, treatment and data collection;
- 12 months patient follow-up;
- 3 months for covering eventual delays in patient enrollment, treatment and follow-up;
- 3 months for data centralization, analysis, publication.

Each of the 3 sites will have 4-6 simultaneous patients a month for 12 months (total about 65-70 patients at each clinical site), and 4-5 providers at each site will treat and monitor each inpatient continuously for 3 weeks; afterward each patient will be evaluated at 1 month, 3, 6 and 12 months. Neurological evaluation will include NIH Stroke Scale, Barthel Index, NEUROTEST, Rankin/EUQOL.
Inclusion criteria:- adults (>18 years old); - recent stroke (acute, 1-7 days old), ischemic or hemorrhagic; - severe neurological dysfunction: NIHSS score > 14
Exclusion criteria:- administration of tPA; - mild severity stroke (NIHSS $\leq$ 14); - current viral/bacterial/fungal infection, with fever, leukocytosis >10.000/dL; - acute myocardial infarction; - venous trombembolism (deep vein thrombosis or pulmonary embolism); - severe liver or renal failure; - at least 7 days medication-free with steroids, non-steroidal antiinflammatory drugs, chemotherapy, immunosupressive medication)

At admission or during the first 72 hours is recorded information on presence of diabetes, hypertension, atrial fibrillation, cardiovascular disease, other systemic disease, current medication. eCRF for each patient will be completed at days 1, 4, 7, 10, 14, 17, 20, 30, 90, 180, 360 with assessment of the neurological function using standardized tests (NIH Stroke Scale, Barthel Index, etc).

Blood samples will be drawn also at these time points, some of which will be sent to an outside lab for processing. The modifications in the neurological status and functions are to be documented shortly but promptly (time included) in the eCRF in writing or by recording a short video of the patient as soon as it is observed. Blood also drawn at these time-points will be tested for various markers related to stroke (such as neuron-specific enolase, interleukins, etc) and also microRNAs and some -omics (aspects of metabolome, proteome, etc.).

EEG data will be recorded and uploaded from each patient from the SD card of the EEG device to the tablet/netbook manually every 12 hrs for 21 days; it may be possible though to have wireless data sent to the tablet directly from the EEG monitor (the device has the capability) and monitored remotely; continuous EEG monitoring (the respective cap with 8/20 dry electrodes) will be performed for 3 weeks. Recording of electrical activity using a bioimpedance device will be done at the same time with collecting the EEG recordings and upon investigators' requests.

The new combination treatment (Actovegin, dimethylsulfoxide, oxytocin, vit C, B1, B6, B12, glutathion, calcium) will be one of the 2 treatments given, and will be started within 24 hours of patient admission, at which time patients will be randomized to receive either this combination or a combination of piracetam plus essential phospholipids or another medication which is also known and approved substances; no new medications or substances will be administered.

Clinical data recorded in eCRFs will be centralized and statistical analysis will be performed with specific software (SPSS) and input from biostatistics specialists.

The COSS™ platform for Systematic Drug Repositioning (SDR) is a discovery suite of applications which creates multi-dimensional profiles of all biologically relevant concepts (every specific gene, disease, pathway, drug, PTM, and more than 30 additional classes of concepts) to find non-obvious connections between them, e.g. the pathophysiology of diseases and mechanisms of action

(MoA) of drugs. The COSS™ platform allows Biovista to perform drug repurposing, and off-target toxicity prediction offering these as services to the pharmaceutical and biotechnology community. The analytical COSS-based methodology is described here (Lekka et al., 2011; Persidis et al., 2004) as well as its use in expanding the understanding of important biological pathways (Mastellos et al., 2005).
Budget estimate: The cost for each patient completing the study (150) is estimated to be 35.000-40.000 euro, and the total cost for the project (up to 200 enrolled patients) is estimated to be 6 million euros; 25% of total budget is for patient care in the 3 clinical sites, 25% for laboratory testing and imaging, 25% for IT (software, hardware by ERS, Biovista, others), and 25% clinical study coordination, medication, biostatistics, administrative, travel, accommodations (Bio-Forum, others).
Sex and gender analysis: There are differences in stroke incidence linked to estrogen status (estrogen confers some degree of protection in cardiovascular disease) and possibly other factors (stress, testosterone-linked behavior, etc.) important for assessing stroke incidence. Certain subtypes of ischemic stroke were shown to occur more frequently in men than in women and this may reflect in study enrollment, however this project addresses post-stroke neurogenesis and recovery of neurological function, and based on prior experience in the field, sex and gender is not considered to play a statistically significant role in patients' improvement during post-stroke treatment.
Nevertheless, a specific statistical analysis which will consider the possible influence of patients' sex on recovery will be done, analysis which will stratify patients according to estrogen status (monthly period, pre-menopausal vs menopausal vs men) and will adjust accordingly the statistical analysis.

1.4 Ambition

Advance beyond the state-of-the-art, and the extent the proposed work is ambitious.

An existing limitation of patient monitoring and stratification after stroke is that it is mostly focusing on the structural aspects of the brain and minimally and indirectly on the functional aspect of neurons; and such an essential aspect of post-stroke recovery is not considered.
It is known that recovery from stroke is achieved by neurogenesis (differentiation of new neurons from their progenitors), their migration to the site of injury and subsequent integration in the neural network which was previously damaged.

EEG was previously used to monitor brain wave activity (alpha, beta, delta, theta) and the modification of their patterns of occurrence in different brain areas with serial fragmented recordings. Electrical activity related to newly formed neurons and especially their functional integration in the neural networks was not studied so far in man, although in the laboratory it is known that such developmental milestones are accompanied by distinct, specific electrical signals of high amplitude (Giant Depolarizing Potentials - GDPs). These electrical signals are relatively easy to record because their amplitude is much higher than regular neuronal activity (GDP is measured in mV, while regular waves recorded by EEG are measured in μV). This will be a first recording in vivo of neurogenesis and it may be possible therefore to see other biological processes taking place before this neuronal network integration (neuronal maturation and migration), and to characterize more precisely the stage of neurogenesis in stroke patients during recovery (neuronal differentiation vs. migration vs. functional maturation vs. neuronal integration) and with this information it is possible to better adjust the therapeutic interventions in an optimal manner for each patient.

Bioimpedance spectroscopy has so far been used and validated to quantify intra- and extra-cellular water levels, and its main use is for estimating lymphedema (mostly upper limb) in cancer patients after surgery, chemo- and radio-therapy. In our study will be used to assess brain edema/hemorrhage as a simple patient bedside procedure, and also to study brain electrical activity linked to neurogenesis, both being completely new research directions.

Finally and at least as important, the new stratification concept is integrated with the therapeutic concept. The new combination treatment is the first complex treatment for stroke patients which directly addresses most of the pathophysiological modifications known to take place during stroke

(inflammation, oxidative stress, neuronal death and regeneration, neuroendocrine, vascular modifications, etc.), and its components will be adjusted according to individual characteristics and responses in a manner which cannot be achieved by mono-therapy (individualizing components and reducing side effects). With the integration of literature mining and related technologies, we aim to provide new criteria for the better stratification of stroke patients in future clinical trials, use knowledge gained as to re-design and optimize future clinical trial protocols, altogether optimizing personalized therapeutics.

Innovation potential (ground-breaking objectives, novel concepts and approaches, new products)

A new, specific EEG monitoring device can be developed specially designed for bedridden stroke patients (better autonomy for the battery if a charging device is used while patient is bedridden, software added to monitoring device to indicate detection of neurogenesis modifications/stages; Bioimpedance spectroscopy can also be specifically adjusted to neurologic recording by modifying frequency spectrum, sensitivity, etc.

The new combination treatment is also expected to give clinicians a new therapeutic tool which can be individualized for optimal benefits of the patient according to the electrophysiological data and the composite panel.

Where relevant, refer to products and services already available on the market.

All the devices and medications used for the PSIOM project are off-the-shelf, purchased on the open market from specialized manufacturers and pharmaceutical companies.

2. Impact

2.1 Expected impacts

Quantified indicators and targets.

The PSIOM project will provide important advances in stroke patient stratification and treatment:

I. it will develop a new concept for stroke patients stratification by including and evaluating neurogenesis stages as the fundament for neurological recovery. EEG markers for the 4 neurogenesis stages will be inferred. Bioimpedance spectroscopy will be evaluated for assessing brain edema and hemorrhage). A new composite panel with electrophysiological and molecular markers will be evaluated for clinical use.

II. evaluate the efficacy of a new therapeutic approach for stroke recovery which addresses adverse pathological processes (inflammation, oxidative stress, neuroendocrine, immune and vascular reactions) individually and simultaneously and adjusts treatment according to neuronal functionality.

By focusing on neurogenesis and recovery, the new stratification and treatment individualization can be applied to all stroke patients, ischemic and hemorrhagic, including those who cannot benefit from thrombolytic therapy (tPA), and those who have hemorrhagic stroke. A NHSS score improvement of 4 or more is expected after 3 weeks of treatment in more than 50% of patients.

The two components (diagnostic stratification and treatment individualization), together with the -in silico approach, will allow for greater efficiency which will directly translate into better health outcomes, less disability and costs, which will be quantified by using the study results.

Expected Impacts mentioned in the work programme and addressed by the project:

New models for patient stratification to inform clinical decision making.

The PSIOM project will allow direct observation of neurogenesis after stroke and most importantly monitoring of neuronal function and new neuron integration in functional networks; as such will be an exceptional tool for directly assessing recovery stage and status of stroke patients.

Integration of COSS™ and related in silico technologies will help identify molecular markers that are of clinical importance in stroke (e.g associated to stroke recovery) and predict treatment-associated AEs, aiming to identify new criteria that could be used by clinicians to stratify stroke patients into meaningful patient sub-populations.

Accelerate the translation of biomedical and clinical research results to medical use.

The results of PSIOM project can be translated immediately into clinical practice. The monitoring devices used (EEG monitor and bioimpedance spectroscope) are already available on the market, and the blood markers are performed with kits and apparatus produced by companies already established in the field; testing with these devices can be done in an ambulatory setting (outside of hospitals and clinics, point-of-care settings) and the testing itself is non-invasive, economical, and can be performed at patient's bedside, allowing use by emergency crews, and in medically under-served areas.

Increased cost-effectiveness of the novel concepts in comparison to already established practices.

Cost-effectiveness is an important aspect of this project; the EEG monitoring and bioimpedance devices cost between 3.000-5.000 euro each, and the disposables used for each patient are less than 30 euro each. These prices compare favorably with the acquisition and running costs of any imaging device (computed tomography or magnetic resonance machines cost more than 100 times) or laboratory equipment (more than 10 times the cost, and added time for processing samples), and it is possible that widespread use will decrease costs further.

The most important effect on costs will be given by the new possibility of adjusting the treatment in real time and clinicians being more efficacious in their ability to treat patients. Monitoring in real time the effect of treatment on neurogenesis and neuronal function integration as predictor of neurological and psychological recovery will allow for prompt and precise adjustments. It is known that different subtypes of strokes have different molecular characteristics and adjusting the treatment according to this but especially to individual patients will be most efficacious from a clinical standpoint. By consequence, more efficacious treatments translate in faster and better patient recovery, with lower immediate and long-term healthcare costs.

Increased research and innovation opportunities, particularly for small or medium-sized enterprises (SMEs).

The PSIOM project uses devices which are already on the market and will be used for new applications; this opens possibilities for completely new directions for research, improvements and refinements. Some of the foreseen areas for research and innovation are:

- validation of new electrophysiological markers for neurogenesis and stroke types, patient evolution under treatment, development of new software for EEG recording directly related to neurogenesis, etc.
-use of bioimpedance spectroscopy to quantify brain hemorrhage/edema; test a new spectrum range for evaluation of cellular and molecular status of neurons
- development of stroke-specific kits (ELISA, RT-PCR, NASBA, Lab-on-chip, etc) for markers in blood or cerebrospinal fluid for a new composite panel of stroke biomarkers.

For partner Biovista (and indeed other SMEs in this space) this work will open up significant opportunities in the prediction of ADRs (Adverse Drug Reactions) and the optimization of therapies for specific sub-populations. This is an emerging field demanding innovation both at the scientific level (a scientific method for predicting ADRs that is robust enough to support business decision-making) and at the business level. At the business level this will create a need for new business models that capture the value created and allow its sharing among the involved stakeholders (pharmaceutical companies, medical insurance companies and national social security agencies

Cross-cutting Priorities: Gender

Gender refers to cultural attitudes and behaviors that shape "feminine" and "masculine" behaviors, products, technologies, environments, and knowledge (accepted definition of gender in EU).

Patients' sex was shown to play a role in stroke incidence (men are at higher risk before age of 75 than women), and post-stroke disability seems to be higher in women; however an influence by gender on the therapeutic outcome or the efficacy of the stroke treatment is not shown by current scientific literature. Nevertheless a specific statistical analysis will be done for those aspects of stroke treatment efficacy and age and sex-adjusted stratification will be specifically performed.