Project Final Report

Grant Agreement number: 201024
Project acronym: ARISE
Project title: Affording recovery in stroke
Funding Scheme: Collaborative project
Date of latest version of Annex I against which the assessment will be made: 16.07.2012
Final report
Period covered: from 2008-03-01 to 2013-08-31

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ARISE

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1.1 Executive summary

Stroke presents a major global burden to patients, their relatives, and whole economies. Worldwide intense efforts are being undertaken to understand the pathobiology of stroke and to develop novel, effective treatments. This has led to the discovery of a plethora of pre-clinically promising treatment strategies, but intravenous thrombolysis remains the only specific pharmacological treatment with proven efficacy in acute ischemic stroke. Apparently, of several hundred controlled clinical trials (The Internet Stroke Center) aiming to protect the brain against ischemia ('neuroprotection') with compounds previously found effective in animal experiments, not a single one has led to the regulatory approval of a drug for this indication. Many pharmaceutical companies have therefore stopped developing treatments for this devastating disease, and nihilism is spreading in the field.

In ARISE, leading European stroke researchers and clinicians with a track record of established cooperation were sharing their complementary expertise, and teamed up with industrial partners with high profile R&D and ongoing, promising clinical trials. Importantly, the ARISE consortium combined expertise in clinical as well as preclinical stroke research, expertise which was further expanded by a strong collaboration with the EU-funded consortium EUSTROKE (grant agreement n° 202213). ARISE and EUSTROKE have joined to form the European Stroke Network (ESN). Within the ESN, ARISE and EUSTROKE gathered for consortia meetings, shared their results, planned joint preclinical and clinical trials, disseminated their results, and were advised by a joint scientific advisory board. At the outset, our consortium had formulated the following, highly interconnected objectives:

- To develop innovative strategies to prevent the delayed neurotoxic effects of tissue plasminogen activator (tPA)
- To understand and therapeutically target inflammation in stroke
- To understand and modulate brain-immune interactions after stroke
- To induce endogenous neuroprotection in stroke
- To improve recovery by inducing repair
- To improve targeting of therapeutics to the brain after stroke

These were ambitious and wide ranging aims, reaching from the development of novel preclinical models to the discovery of new diagnostic and therapeutic targets, and finally obtaining proof of concept for the most promising approaches identified by the group. Indeed, the interaction of leading preclinical and clinical stroke researchers in Europe helped to make a number of highly relevant contributions to the understanding of stroke pathophysiology, and had impact on the clinical treatment of stroke. For a number of approaches which were discovered and validated by the consortium during the funding period, clinical testing has only recently begun, or is in planning. Therefore, further impact of ARISE research will materialize in the future. Major contributions with impact on reducing the burden of stroke were already made through ARISE research by:

- Discovering and developing novel strategies to improve the efficacy and safety of thrombolysis, the currently only pharmacological therapy of acute ischemic stroke of proven efficacy
- Discovering mechanisms of brain immune system interactions after stroke and the development of therapeutic strategies (preventive antibiotics: phase IIb RCT positive; anti-inflammation: IL1RA trial in planning)
- Discovering novel pharmacological strategies to boost endogenous brain protective and regenerative strategies. One was already tested in an international phase II RCT (unfortunately with neutral results), and initiation of planning of clinical testing of other candidates.
- Development and Phase I testing of a novel device for brain cooling, and initiation of clinical testing in large RCT (EuroHyp-1).

ARISE (and EUSTROKE) were instrumental in creating a novel atmosphere of collaboration and sharing of results among European stroke researchers, which has led to a number of follow up activities (ERA-Net projects, MULTIPART, EuroHYP-1, etc.) as well as transatlantic collaborations (ESN-Canadian Stroke Network, etc.). This has created a spirit of collaboration previously non-existent, which will surely have major impact on future discoveries in the stroke field which help to reduce the burden of this disease on patients, relatives, and societies. Internationally, ARISE research had impact on the Stroke Research Program Group (SPRG) process of the NINDS/NIH. Improving the quality of preclinical stroke research, and multicenter, international phase III Type preclinical stroke trials were selected by the SPRG group as the top priority across all areas (prevention-neuroprotection-neurorehabiliation/repair).

1.2 Summary description of project context and objectives

Background and Aims

Stroke accounts for a substantial and growing share of overall mortality and disease burden worldwide. Annually, approximately 15 million people worldwide suffer a stroke, of whom 5 million die and 5 million are left severely impaired. Stroke burden is projected to rise from 38 million disability-adjusted life years (DALYs) globally in 1990 to 61 million in 2020. Comprehensive specialized stroke care (including stroke units) that incorporates rehabilitation has demonstrated clear benefit for patients, and treatment of acute ischemic stroke with thrombolysis with intravenous rtPA within 3 to 4.5 hours can be highly effective. However, due to contraindications or lack of access only a minor portion of stroke victims worldwide benefit from thrombolysis. Emergency medical systems and stroke unit care are available only to a minority of stroke victims worldwide. Currently, no neuroprotective or neurorestorative therapy has demonstrated efficacy in large, randomized controlled clinical trials (RCTs). Pharmacological protection for the brain against focal cerebral ischemia or hemorrhage, and fostering functional recovery through induced plasticity and repair remain the leitmotifs of stroke research.

Against this background, the objective of ARISE was to provide the basis for improving the outcome of patients after stroke. We aimed at developing novel approaches to minimise the propagation of brain damage after stroke, and to repair such damage if it cannot be prevented. Our collaborative approach brought together preclinical and clinical research, as well as pharmaceutical industry in a joint effort to overcoming the translational roadblock in this field.

This inadequacy of current stroke treatments is in treatment is in spite of intensive research efforts and numerous failed clinical trials. The latest of these (ICTUS, testing the putative neuroprotectant Citicolin in acute stroke) showed no efficacy in spite of extensive and convincing pre-clinical data, though limited experimental studies in man. These failures demand a re-examination of the pathophysiology of ischemic brain injury and subsequent repair processes. At a workshop hosted by the European Commission in Oct. 2005 (Meairs et al. Cerebrovasc Dis. 2006;22:75-82), and in greater detail in a review (Endres et al. Cerebrovasc Dis. 2008;25:268-78), the members of this consortium had identified critical areas which in our view hold the key to improving the neurological outcome of stroke patients by innovative therapies in the future. These were the focus of our research:

1) *Ischemic stroke is the result of vascular occlusion:* Reperfusion is the only current specific therapy of proven efficacy. However, recanalisation strategies are of limited effectiveness, and may even contribute to the propagation of the lesion.
2) *Complexity of stroke pathobiology:* Brain ischaemia initiates a cascade of events which evolve in time and space and include deleterious as well as protective mechanisms. So far, neuroprotective therapy has focused on single, putatively destructive mechanisms. However, most processes which contribute to tissue damage have also been implicated in protection or repair. Thus, therapy must acknowledge the Janus-faced nature of many stroke targets, needs to be instructed by the timing and context of individual pathobiology, and must identify/take advantage of endogenous neuroprotective and repair mechanisms. Other highly relevant aspects of complexity include the large variety of stroke types characterized as permanent vs. transient (reperfusion) occlusion, small vs. large vessel disease, affected brain region, and hemorrhage vs. ischaemia.
3) *Inflammation and brain-immune interaction:* Inflammation contributes to brain injury but is also instrumental in lesion containment and repair. Stroke outcome is modulated by interaction between the injured brain and the immune system. This has important consequences for outcome. For example, the number one cause of death in stroke victims is infection. Not only does stroke induce immunodepression, and therefore increase susceptibility to infection. Infection (and thus systemic inflammation) also alters the secondary progression of the brain lesion, as well as regeneration.
4) *Regeneration:* Recently it has been realized that the potential of the brain for reorganization, plasticity and even repair after injury is much greater than previously thought. Nevertheless, endogenous mechanisms as well as therapeutic strategies of brain repair after stroke remain largely unexplored.
5) *Confounding factors, long-term outcome:* Stroke pathobiology is critically affected by comorbidity, age, and gender. Stroke patients often are of advanced age, atherosclerotic and hypertensive and/or diabetic. In contrast, to date, stroke has been almost exclusively modelled in healthy, young animals, and outcome is studied at very early time points, which are of little relevance to the patient. Integrating confounding factors, long-term outcome and biomarkers as well as brain imaging will result in improved prediction of toxicity and efficacy.
6) *Novel strategies* are required to target therapeutic agents to the affected brain regions.

Work strategy and general description

These six focus areas are linked on all levels, and therefore were tackled by an integrative approach. Stroke pathobiology, as well as brain reorganisation and repair, are highly complex. Developing successful strategies for brain protection and repair therefore required a joint effort of experts on basic neuroscience, vascular biology, neuroimmunology, neuroprotection, neuroregeneration, drug delivery, and clinical stroke neurology. Leading European stroke researchers and clinicians with a track record of established cooperation shared their complementary expertise, and teamed up with industrial partners with high profile R&D and ongoing, promising clinical trials. We worked on a common model and methods platforms, where a particular emphasis was on relevant comorbidities, gender, age, and long-term outcomes. The training of young researchers led to a standardisation and harmonisation of laboratory practices within the consortium, and implementation of standard operating procedures (SOPs) generated by the consortium. Importantly, the ARISE consortium combined expertise in clinical as well as preclinical stroke research. ARISE researchers were and are coordinating a number of investigator initiated Phase II and Phase III trials, or are presently preparing for them. Our small and medium enterprise (SME) partners from biotech and pharmaceutical industries SYGNIS; QUICK COOL; PAION, NSGENE) were conducting Phase I, II and III stroke trials. Our initiative served as hub for the recruitment of additional European centres within and outside the consortium, assisted in obtaining funding at the national and European level (ERANET, MULTIPART, WAKEUP, EUROPHYP-1), and improve overall organization of European stroke research and its ties to the US and Asian stroke research centres in a structured way (e.g. participation in NINDS/NIH panels), and enhanced trans-European flow of information on stroke research within the whole European society, from stroke patients to governmental agencies. In addition, a clinical platform, consisting of outstanding European stroke clinicians, provided advice to the basic researchers of the consortium on clinically relevant questions and modelling, and periodically reviewed the ongoing development of innovative therapies.

Management structure and procedures

The Project Coordinator ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. He submitted all required progress reports, deliverables, financial statements to the European Commission, and, with the assistance of GABO:mi he was responsible for the proper use of funds and their transfers to participants. The ARISE office was established by and based at the coordinator in Berlin and at GABO:mi in Munich. The Project Office at the Coordinator was concerned with the scientific management and the co-ordination of all research activities. The Project Office at GABO:mi was responsible for administrative, financial and contractual management and the organisational co-ordination of the project activities.

The Project Governing Board was in charge of the political and strategic orientation of the project and acted as the arbitration body. It met once a year unless the interest of the project required intermediate meetings. The Project Coordination Committee consisted of all work package leaders and the Coordinator and was in charge of monitoring all activities towards the objective of the project in order to deliver as promised, in due time and in the budget. The Project Coordination Committee met every six months during the funding period. Furthermore, a scientific advisory board was implemented to ensure a high standard of research and monitor the progress of the project by taking part in the annual Governing Board Meetings.

Objectives of ARISE:

Objective 1: To develop innovative strategies to prevent the delayed neurotoxic effects of tissue plasminogen activator (tPA)

There is a clear need to improve thrombolytic therapy of ischaemic stroke. Based on the ground-breaking research from academic and industrial partners of this consortium, studies of in-vitro and in-vivo models of stroke in WP01 increased the therapeutic time-window for thrombolysis; identified and tested non-neurotoxic (and/or non-haemorrhagic) thrombolytics; developed adjunctive therapies for reperfusion and neuroprotection; and developed clinical markers to identify individual variability in response to thromboembolic arterial occlusion. Innovations were the engineering and the characterization of rtPA muteins devoid of neurotoxic effects, and the development of immunotherapy to target the neurotoxic effect of tPA. We obtained proof of concept for immunization to protect the brain against reperfusion damage by tPA, identified tPA-variants with reduced neurotoxicity, and validated microparticles as a potential stroke biomarker in humans.

Objective 2: To understand and therapeutically target inflammation in stroke

In stroke, inflammation modulates infarct size, but also brain oedema, immune depression, and repair processes. While potentially damaging the brain and worsening outcome, it is also a major component of a physiological response which contains injury, and facilitates repair and regeneration. In addition, systemic inflammation driven by underlying atherosclerosis, and/or infection in the course of stroke, has a tremendous impact on the propagation of brain damage and hence neurological outcome. We aimed to disentangle the specific effects on the brain of immunodepression (in concert with objective 3, below), infection, and brain parenchymal inflammation. WP02 has developed novel therapeutic strategies which allow inhibition of the negative consequences of inflammation while preserving its regenerative aspects, and thereby deepened our understanding of the pathophysiological mechanisms and the neurological consequences of ischaemic stroke.

Objective 3: To understand and modulate brain-immune interactions after stroke

Stroke affects the normally well balanced interplay of two supersystems - the nervous and the immune system. Stroke induces immunodepression and increases the susceptibility to infection, the most relevant complication in stroke patients. However, immunodepression after stroke may also have beneficial effects, for example by suppressing autoaggressive responses during lesion-induced exposure of CNS specific antigens to the immune system. WP03 developed innovative therapeutic strategies to modulate the immune response after stroke and instruct it towards protection and repair, and to prevent infectious complications after stroke. We established predictive and sensitive clinical markers for the effect a stroke had on the immune system in a given patient, and in particular for susceptibility to infection. This enables selective and innovative treatments, such as preventive therapy with antibiotics. To this end, we evaluated neuroprotective and immunomodulatory effects of antibiotics suitable for the treatment of stroke patients.

Objective 4: To induce endogenous neuroprotection in stroke

Tissue damage and functional impairment after cerebral ischaemia is the result of the interaction of endogenous protective mechanisms and those events that ultimately lead to cell death. Partners of this consortium have been able to identify a number of such protective mechanisms and molecules (e.g. erythropoeitin and granulocyte colony stimulating factor), some of which are currently evaluated in clinical phase II and III trials. WP04, in preparation for clinical trials planned by our industrial partners, aimed to investigate the interaction of tPA with therapeutically applied endogenous neuroprotectants. Furthermore, we identified novel endogenous neuroprotectants potentially suitable for stroke therapy. Finally, hypothermia, the most effective natural mechanism of endogenous neuroprotection, was used to guide us to novel pharmacological targets for stroke therapy, and the safety and feasibility of a novel therapeutic device for rapid and selective brain cooling in acute stroke patients was established.

Objective 5: To improve recovery by inducing repair

After stroke, function is lost due to neuronal cell loss and disrupted connectivity. Recent research, including work by members of our consortium, has revealed that recovery after stroke can be stimulated successfully and neurological function improved beyond spontaneous recovery. By elucidating the mechanisms of post-stroke brain plasticity WP05 we established novel therapies which will extend the therapeutic window for improving the neurological outcome of stroke patients to days and weeks after the event. We developed new therapies to foster neuro- and angiogenesis, encapsulated cells to attract new neurons to the stroke-damaged area, and developed compounds that modulate lipid trafficking and enhance functional recovery after stroke.

Objective 6: To improve targeting of therapeutics to the brain after stroke

Targeting drugs to the brain after stroke is a formidable challenge: there is an urgent need to establish effective strategies to target therapeutics (small molecule drugs, peptides, proteins, vectors, therapeutic cells) towards and into the part of the brain where their actions are required. Based on groundbreaking work by members of our consortium, WP06 aimed to develop innovative lipoplex-loaded microbubbles that utilize targeted drug release with ultrasound. This might result in novel, non-invasive stroke therapies with substances such as immunoglobulins, viral vectors, plasmid DNA, siRNA, mRNA and high molecular weight drugs.

1.3 Description of the main S&T results/foregrounds of ARISE

WP01 – Overcoming the neurotoxic effects of thrombolysis

Fibrinolysis and extracellular proteolysis are key mechanisms in vascular remodelling that also play a critical role in neuronal plasticity and survival. Two major proteolytic systems induce fibrinolysis, extracellular matrix (ECM) remodelling and cell migration in the vasculature and in the brain: the serine proteinases of the plasminogen activation system and the matrix metalloproteinases (MMPs). Plasminogen (Plg) is converted into plasmin (Pln) at the surface of cells, fibrin or matrix surfaces by either the tissue- or the urokinase-type plasminogen activator (tPA or uPA). In vessels, tPA generates Pln on clots leading to fibrinolysis, whereas uPA is mainly involved in vascular remodelling and cell migration. The activities of tPA and uPA are regulated by serine protease inhibitors such as plasminogen activator inhibitor-1 (PAI-1), the protease nexin-1 (PN-1), whereas tPA is also selectively inhibited by neuroserpin (NS). The latter is primarily localized in neurons in regions of the brain where tPA is also found.

Like other serine proteases, human tPA is synthesized as a single chain zymogen (sc-tPA), composed of five domains, which from the N-terminal to the C-terminal positions are: fibronectin type I finger domain (F), epidermal growth factor-like domain (GF), kringles 1 (K1) and 2 (K2), and a trypsin-like proteolytic region. The sc-tPA is transformed by Pln into a two-chain enzyme (tc-tPA) with similar proteolytic activity. In addition to its ability to promote thrombolysis through Plg activation in the vascular compartment, tPA controls critical functions in the extravascular space (such as cell migration, tissue remodelling, neuronal plasticity and neurodegeneration), by acting either as an enzyme, a cytokine-like molecule or a neuromodulator. Several substrates (both direct and indirect), binding proteins or receptors have been identified to mediate the pleiotropic cerebral functions of tPA, including laminin, the N-methyl-D-aspartate receptor (NMDAR), the low density lipoprotein receptor-related protein (LRP), annexin-II, matrix metalloproteases, pro-Brain-Derived Neurotrophic Factor or pro-Platelet-Derived Growth Factor-C. All these observations illustrate how tPA has a multifaceted role in the ischemic brain: a Plg-dependent, beneficial effect in the intravascular space where it acts as a thrombolytic enzyme, and both Plg-dependent and -independent deleterious roles on and above the vascular wall, including the development of cerebral edema and hemorrhage, as well as damages to brain cells.

A number of stressors and inflammatory mediators can activate blood and neurovascular cells. One of the early manifestations of cell activation is plasma membrane blebbing and shedding of membrane fragments known as microparticles (MPs) or microvesicles. MPs originating from platelets, leukocytes, endothelial cells, and erythrocytes are found in circulating blood at relative concentrations determined by the pathophysiological context. The procoagulant activity of MPs is their most characterized property as a determinant of thrombosis in various vascular and systemic diseases, including myocardial infarction, ischemic cerebrovascular accidents, transient ischemic attacks, multiple sclerosis, and diabetes. Recent data indicate that besides their procoagulant components and identity antigens, MPs bear a number of bioactive effectors that can be disseminated, exchanged, and transferred via their interactions with cells. Interestingly, MPs may be a major source of plasminogen activators and efficient Pln generation in vivo. Furthermore, leukocyte or endothelial cell-derived MPs bearing uPA are able to transform fibrin- or platelet-bound Plg into Pln. Thus, MPs are now emerging as new messengers/biomarkers from a specific tissue undergoing activation or damage as they are cell-specific and are released early in the pathophysiological cascade of a disease.

Main objectives

After quite a long period of pessimism due to the lack of translation from the bench to the beside, the area of cerebral ischemia has now regained much interest thanks to the development of new tools and technologies bringing molecular/cellular/animal biology closer to the clinical situations.

During these last 5 years, our ARISE collaborative program has fully participated to this exciting period of progress, thanks to our constant effort to develop a real translational approach, going from the molecule to the integrated pathophysiology, offering multiple possibilities to identify new ways to understand and treat stroke.

We focused our investigations on the influence of the serine proteases, the tissue type plasminogen activator (tPA).

In order to reach an internationally competitive level in the field, our original objectives were divided into the three following complementary tasks. These tasks include both fundamental and translational approaches, first to further understand the functions of tPA in the central nervous system (molecular and cellular mechanisms), and second to possibly develop original tools for diagnosis and therapeutic.

- To develop tPA-related muteins for optimization of thrombolysis

- To develop a method of immunotherapy targeting the neurtoxicity of tPA
- To investigate wether circulating microparticles could be used for diagnosis

Main achievements

Providing a 3D model of how tPA interacts with NR1 as a molecular basis to design an optimized tPA: We generated an array of tPA-related muteins and tested their ability to influence the interaction of tPA with the amino-terminal domain of NR1 (ATD-NR1), to promote NMDAR signalling and/or to compete with tPA for some of its other effects by using a set of approaches for protein-protein interactions (IP/IB, SPR, computational modelling), ability to cross the blood-brain barrier, neurotoxicity and calcium video-imaging. The combined data led us to propose a 3D model of the tPA-NR1 interaction, which occurs through a two-sites system (KD = 24 nM): the first critical step is the interaction of tPA with NR1 through its kringle 2 (K2) domain, followed by the binding of its catalytic domain, which in turn, cleaves the ATD-NR1, leading to a subsequent potentiation of NMDA-induced calcium influx and neurotoxicity. We have also shown that in contrast to tPA, DSPA (desmoteplase, a vampire bat-derived tPA-related molecule, but lacking the K2 domain) does not promote excitotoxicity. More recently, we have evidenced for the first time a differential function of the two forms of tPA, single chain tPA (sc-tPA) and two chain tPA (tc-tPA), with sc-tPA the only one capable to interact and control NMDA receptor signaling. Based on point mutations targeting both the cleavage site of the sc-tPA necessary for its convertion into tc-tPA and the Lysine Binding Site contained in its kringle 2 domain, we have generated an optimized tPA which is still fibrinolytic but devoided of deleterious effects such as neurotoxicity and risk of hemorhagic transformations. This new tPA is now patented (Inserm-trasnfert), the subject of a start-up project, called “Theraprot”.

Preventing the neurotoxic effect of tPA in stroke treatment: We have developed a protocol of active immunization in mice by using the ATD-NR1 as an antigen, enabling to transiently prevent the interaction of tPA with NR1 in vivo. We demonstrated that binding and cleavage of the NR1 subunit of the NMDAR by tPA control tPA-promoted NMDA neurotoxicity and tPA-dependent encoding for novel spatial experiences. Such a strategy could be of cardinal importance to improve existing therapeutic strategies for stroke. More recently, we have evaluated the efficiency of passive immunization using purified polyclonal antibodies against the cleavage site of NR1. These antibodies were found to prevent the worsening effect of tPA on NMDAR-mediated neuronal death in vitro. Importantly, in a clinically relevant model of in situ thromboembolic stroke in mice (Orset et al., 2007), we found that a single early or late intravenous administration of the antibody can strongly and durably (at least 1 month) improve brain outcome after stroke, by limiting BBB alterations (PDGF-cc formation, PDGF-Ralpha activation, MMP- 3 and 9 activation and Evan’s Blue extravasation) and brain damages (lesion volumes - thionine staining and MRI analysis), neurological scores/functional recovery). Interestingly, we also evidenced that such a strategy can increase the therapeutic window of tPA-driven thrombolysis. We thus provide strong evidence for the possibility of a larger proportion of treatable stroke patients and for an alternative treatment for stroke patients who are not eligible for thrombolysis thanks to an original antibody-based immunotherapy for stroke. Based on the work described above, we have now generated the corresponding monoclonal antibodies, its humanized form is in process. This antibody is now patented and under licence with a pharmaceutical group for clinical development.

Microparticles for diagnosis of stroke: We have demonstrated that tPA constitutively expressed by adherent vascular cells can generate plasmin at their surface and induce thereby the proteolysis of extracellular matrix proteins (e.g. fibronectin and laminin), cell retraction and structural changes including the release of membrane microparticles (MPs) and finally cell detachment and apoptosis. It is well known that practically any cell type has the ability to release MPs upon proper stimulation. Here, we demonstrated that neurons can also release MPs. In parallel experiments we have found that neuronal MPs are similar in size and in their ability to generate plasmin as those produced by TNFα-activated human microvascular endothelial cells, which express the uPA/uPAR system instead of tPA. Our recent studies on MPs isolated from blood of patients with autoimmune, cardiovascular and neurovascular diseases, including stroke, provide the proof-of-concept that in vivo, circulating MPs derived from endothelial cells and leukocytes but not from red cell or platelets are present and able to generate plasmin at their surface. Whether neuron-derived MPs are also present remains to be elucidated. In stroke, released MP could be actors linking the inflammatory status, hemorrhagic risk and severity of ischemic lesion to brain damage and the repair processes. Accordingly, we have shown that cell-derived MPs bearing uPA but not tPA are able to approach and contact fibrin and platelets bearing plasminogen that is transformed into plasmin. This cross-talk mechanism bypasses the requirement for molecular co-assembly of plasminogen and its activator on the same surface thus increasing the amount of plasmin formed on the target surface. Further studies are necessary to determine the role of this cross-talk in the pathophysiology of the neurovascular unit.

WP02 – Inflammation in stroke

The main aim for WP2 "Inflammation in stroke" was to investigate the role of central and systemic inflammatory mechanisms in brain injury, to identify novel therapeutic targets and to test novel interventions against brain injury in clinically relevant models of cerebral ischaemia.

Main achievements

We have established and developed clinically relevant models of comorbidities in stroke (e.g. rodent models of aging, obesity, atherosclerosis or infection) in order to study mechanisms by which inflammatory mechanisms contribute to brain injury. We found that common comorbidities in stroke are associated with elevated systemic inflammatory burden that was evidenced by altered levels of inflammatory mediators in several peripheral organs and in the circulation. Systemic inflammation is associated with brain inflammation (microglial activation, vascular activation, etc.) both in patients at risk of stroke and in experimental animals prior to any acute cerebrovascular event takes place. We found that interleukin 1 (IL-1) is a key mediator of peripheral inflammatory actions and also brain inflammation after infection or diet-induced atherosclerosis. In addition, we have shown how brain injury drives inflammatory responses throughout the body that could contribute to further injury in the central nervous system.

We have investigated the effects of systemic inflammation and common stroke comorbidities on outcome following experimental cerebral ischaemia. Acute or chronic infection, obesity/atherosclerosis and old age were generally associated with increased neuroinflammation, larger brain injury and impaired functional outcome after experimental stroke. Different risk factors have additive effects on outcome after stroke: for example, the effects of preceding chronic infection on brain injury induced by cerebral ischaemia were more severe in aged animals compared to young; in aged rats obesity and atherosclerosis were associated with more severe blood brain barrier breakdown compared to lean animals.

We demonstrated that inflammatory mechanisms contribute to brain injury even in the absence of comorbidities, since blockade of inflammatory responses (such as blocking IL-1 actions by IL-1 receptor antagonist, IL-1Ra) is protective in rodent models of experimental stroke. We have also shown that comorbidities lead to worse outcome after stroke through inflammatory mechanisms: by blocking proinflammatory actions, we could reverse the effects of comorbidities and systemic inflammation on impaired outcome (increased brain injury, inflammation and impaired neurological function) after experimental stroke. These experiments also demonstrate that is it possible to protect against brain injury even in aged animals or in those having multiple risk factors for stroke, and beneficial effects of blocking proinflammatory responses are maintained for a long time (at least up to 28 days as tested).

Based on our results, and in accordance with the recommendations by the Scientific Advisory Board, we selected our most promising candidate, IL-1Ra that has been subjected to rigorous testing in a translational context. IL-1Ra has been found to protect against brain injury in young and aged rodents, with or without systemic inflammation and has shown no negative interaction with plasminogen activator (tPA), which eligible stroke patients would receive as a standard therapy. In addition, we have initiated (to our knowledge) the first preclinical multicenter phase II trial to investigate the beneficial effects of IL-1Ra on brain injury and functional outcome following cerebral ischaemia. IL-1Ra is now being also tested in stroke and subarachnoid haemorrhage patients in phase II trials in Manchester. We have also identified several new candidate approaches to block brain injury and neuroinflammation, which will be subject to further investigations.

Future perspectives

We will aim to get a deeper understanding into the molecular mechanisms of brain injury, with special respect to how inflammatory actions contribute to brain injury. Identification of new therapeutic targets could lead to more powerful and site-specific interventions that are likely to be relevant for several other brain diseases in addition to stroke. Several new targets and candidate approached we have identified will be subject to further investigations, funding permitting. Specifically, we are aiming to understand molecular mechanisms of IL-1-induced injury in the brain, and to pave the way for IL-1Ra as a leading therapeutic option in stroke.

WP03 – Brain-immune interactions after stroke

In the acute course of the disease, stroke patients often develop complications, such as pneumonia, increasing morbidity and mortality. Susceptibility to infection is facilitated by systemic immunosuppression, described as stroke-induced immunodeficiency syndrome (SIDS) (Prass et al., 2003; Meisel et al., 2005; Chamorro et al., 2012). WP03 was focused in investigating consequences of brain-immune interaction after stroke for CNS parenchyma, in

establishing sensitive immune marker for predicting post-stroke infection and identifying antibiotics for prevention and treatment of post-stroke infections in stroke patients.

The crosstalk between CNS and immune system is mediated mainly by the sympathetic nervous system (SNS), the hypothalamic-pituitary-adrenal axis (HPA), and the parasympathetic nervous system (Meisel et al., 2005). Stroke induces a hyperactivation of neuroendocrine stress pathways including the SNS and HPA leading to a rapid and long-lasting systemic suppression of cell-mediated immune responses causing spontaneous systemic bacterial infections within the first days after stroke (for a review Meisel et al. 2005; Chamorro et al. 2012). Lymphopenia and lymphocytic dysfunction mediated by the overactivation of both stress axes has been demonstrated to be essential causes of post-stroke infections. Although lymphocytes belonging to the innate immunity, such as NK cells as well as invariant natural killer T cells (Wong et al., 2011) have been described to be involved in SIDS, the main effects in were contributed by T and B lymphocytes belonging to the adaptive immunity (Meisel et al. 2005). However, the first line of antibacterial defence is organized by cells of the innate immunity such as macrophages. The cholinergic anti-inflammatory reflex, which is mediated by the parasympathetic nervous system (PNS), has been identified as an adaptive response preventing a potentially harmful excessing systemic inflammatory response to a local inflammatory challenge (Rosas-Ballina and Tracey, 2009). Thus, innate immunity seems to be mainly controlled by cholinergic pathway. Experimental stroke activates the PNS, thereby breaking down antibacterial host-defence in lung causing post-stroke pneumonia. The local immune response in the lung is impaired via stimulation of the vagus nerve activating α7 nicotinergic acetylcholine receptors of lung macrophages and alveolar epithelial cells (Engel et al. in preparation).

Main achievements

In our multi-center PREDICT study (N=486; clinicaltrials.gov: NCT01079728), we prospectively validated our A2DS2 score to predict stroke-associated pneumonia (SAP) based on simple clinical parameters (Hoffmann et al. Stroke 2012). PREDICT demonstrated for the first time that stroke induced immunodepression (marker: monocytic HLA-DR expression) and dysphagia independently causes of post-stroke pneumonia (Hoffmann et al. in preparation). Immune markers such as moncytic HLA-DR expression improve the predictive capacity of the prognostic score for identifying patients at risk of SAP (Meisel et al. 2012).

Data from patients suggest that stroke induces an antigene specific immune response in the CNS (Prüss et al. Arch Neurol 2012). In experimental stroke (mice MCAo model) lymphocytes mainly T cells infiltrates into the ischemic brain 7 to 14 days after stroke onset. T cell infiltration into brain after stroke is CNS antigen-specific and stroke primes CNS antigen-specific T cell responses (Klehmet, Engel et al. in preparation). Although the site of priming is unknown, our recent data from experimental models and stroke patients demonstrated that CNS antigens drain to cervical lymph tissue. Already one day after stroke onset the frequency of CNS antigen-laden cells in deep cervical lymph nodes is increased. Brain derived antigens were presented by CD68+ MF in lymphoid tissue tissues (van Zwam J Mol Med 2008; Planas et al. J Immunol 2012). These data suggest that T cell priming occurs in the cervical lymph tissue.

The delayed brain infiltration of T cells affects all subsets including regulatory T cells (Tregs). Tregs proliferate in the ischemic hemisphere but were not induced de novo. Foxp3-positive Tregs contact with MHCII expressing myeloid cells thereby modulating local inflammatory response in the ischemic brain. However, delayed depletion of CD25+ Tregs, which are believed to be involved in recovery, does not worsen stroke long-term outcome (Stubbe et al. JCBFM 2013).

Post-stroke pneumonia enhances brain leukocyte infiltration and alters the phenotype of CNS antigen-specific T cells, increasing detrimental Th1 and Th17 cell responses and reducing protective regulatory T cell responses. Enhanced priming of inflammatory CNS antigen-specific T cells due to post-stroke bacterial infection worsen outcome after stroke (Klehmet, Engel et al. in preparation). Modelling the current clinical standard care, we demonstrated that prevention of post-stroke pneumonia improves neurological outcome compared to standard antibiotic treatment in experimental stroke. Neuroprotection by antibiotics in the mice MCAO model is facilitated by prevention of detrimental post-stroke infections but not by direct neuroprotective properties (Mergenthaler & Meisel, DMM 2012; Hetze et al. J Neurosci Meth 2012; Hetze, Engel et al. JCBFM 2013).

The biological function of stroke-induced immunodepression (SIDS), however, remains elusive. According to our main hypothesis, SIDS represent an adaptive response preventing autoreactive CNS antigene specific immune responses after cerebral ischemia (Meisel et al. 2005, Dirnagl et al., 2007) we demonstrated that reversing SIDS increases delayed CNS antigene specific CD4+ T cell responses in the brains after experimental stroke. However,

blocking SIDS by inhibiting body's main stress axes does not worsen functional long-term outcome after experimental stroke (Roemer et al. manuscript in preparation).

Investigating signalling mechanisms and modulation of brain-immune cell interactions we demonstrated that the Asc-mediated inflammasome rather than IL-1β processed by NALP3 contributes to stroke outcome. Deficiency in the anti-inflammatory cytokine IL-10 exacerbates the inflammatory response and worsens brain damage in experimental stroke (Perez-de-Puig et al. JCBFM, 2013). Deficiency in mannose-binding lectin (MBL) is protective against stroke brain damage in mice and humans and does not increase the risk of post-stroke infection (Cervera et al. PLoS ONE 2010; de la Rosa et al. in preparation). Stroke alters not only the peripheral inflammatory responses in the spleen, thymus, blood, lung, and liver but also in the bone marrow. Activation of granulocytes in the bone marrow following stroke is mediated by NFkB- and P38 MAPK-dependent pathways. Neuronal cues are likely to regulate bone marrow trafficking of neutrophils (Denes et al., JCBFM 2010). The release of granulocytes from the bone marrow is reduced by CXCR2 antagonist treatment, but it has no major impact on circulating leukocyte numbers and outcome after brain injury (Denes et al., manuscript to be submitted).

WP03 unraveled novel targets for modulating immunity after stroke in order to prevent detrimental post-stroke infections and to diminish cerebral inflammation following stroke. Moreover, WP03 identified immune markers predicting patients at high risk for developing post-stroke infections. Based on these findings we need to develop biomarker-guided immune modulation in order to prevent post-stroke infections and diminishing post-stroke inflammation thereby improving long-term outcome after stroke.

WP4 – Endogenous Neuroprotection

Workpackage 4 explored strategies to induce mechanisms of endogenous neuroprotection after stroke. Unfortunately our studies on the interaction of combining the thrombolytic agent rtPA and G-CSF (an endogenous neuroprotectant) were overshadowed by the neutral results of a randomized controlled clinical trial sponsored by one of our partners (SYGNIS) testing G-CSF in acute stroke (AXIS-2). Consequently, G-CSF will not be further developed as a stroke therapeutic.

Main achievements

However, we have elucidated a novel 'Janus'-faced mechanism related to neuronal cell survival (involving TAK1, MAP kinase pathway, NF-κB and ASK1), potentially explaining some of the discrepant results concerning the role of NF-κB in neuroprotection and neurodegeneration. We also identified a novel pathway of Hypoxia inducible factor 1 (HIF1) mediated neuroprotection and demonstrated the functional relevance of the interaction between Hexokinase II and Pea15 for mediating protection from hypoxic cell death. More generally, HKII-mediated apoptosis may represent an evolutionarily conserved altruistic mechanism to eliminate cells during metabolic stress to the advantage of a multicellular organism. The exciting finding that Exendin-4 induces hypothermia in the rat and lowers body temperature to therapeutic levels unfortunately did not lead to clinical testing as we found that in monkeys, dogs and pigs, hypothermia was not induced by exendin-4. However, we were able to demonstrate the efficacy and safety of a novel nasopharyngeal cooling device developed within the consortium in healthy volunteers. Testing of the device in patients will be integrated into the EuroHYP-1 trial. EuroHYP-1 is a European multicenter hypothermia in stroke trial funded by the EU, which is about to start recruiting. Several partners of ARISE are members of the EuroHYP-1 consortium (Dirnagl, Wieloch, Meisel, Quickcool).

WP5 – Recovery and Repair

Work package 5 aimed at elucidating the processes in the brain associated with recovery of lost brain function after brain damage induced by experimental stroke in rodents. The focus has been on repair and regenerative mechanisms, investigating the contribution of all major classes of cellular element: neurons, glia, vascular components and their precursor cells and their interactions to the spontaneous or treatment-promoted recovery of brain function. The approach has been broad with a major aim to identify new treatment regimens, including pharmacological and cell therapies that promote recovery of brain function when instituted days after stroke occurrence in patients.

Main achievements

WP5 has informed that after stroke all cellular elements participate in the repair and recovery of lost brain function. The processes involve the damaged brain tissue, the surviving tissue in the vicinity of the infarct as well as in remote regions of the CNS, including the spinal cord and the non-injured hemisphere. The recovery processes comprise highly dynamic sequence of events that remodel the brain tissue (brain plasticity) and that go on for days to several

months after stroke. This includes growth of new vessels (angiogenesis), extension of new nerve fibres (axonal outgrowth) connecting and forming new synapses (synaptogenesis) and generation of new neurons (neurogenesis). In addition, and in intimate association with repair over the entire recovery process, is the activation of the local brain inflammatory response and invasion of blood borne inflammatory and immune cells. WP5 has also provided evidence for various new therapeutic approaches that target particular repair processes and enhance recovery of various modalities of brain functions and that can be of importance for future stroke therapies.

Spontaneous recovery of lost motor function after experimental stroke involves the remodelling of brain connectivities. We found that following stroke to the forelimb motor cortex, cells in the hindlimb sensory–motor area reorganize and become functionally connected to the cervical spinal cord, i.e. take over the function of lost cells in the fore brain. Also behavioural and anatomical results demonstrate a side switch, anatomically and functionally, in the projection of corticospinal neurons induced by destruction of one sensory- motor cortex and neutralization of the CNS growth inhibitory protein Nogo-A.

Spontaneous recovery can be enhanced by training or by housing animals in an enriched environment. We found that this is associated with a depression of brain inflammation, including the levels of particular cytokines and chemokines (CXCL12) and up-regulation of lipid trafficking and associated regulatory proteins (sigma-1 receptor). The dopamine system is activated by enriched housing and we found that treatment with L-DOPA enhances functional recovery concomitantly with depression of brain inflammation and modulation of the extracellular matrix.

Interleukin (IL)-6 is a pleiotropic cytokine which plays a complex role in brain injury. We found that there was a significant up-regulation of angiogenesis-associated gene transcription and circulating VEGF levels after stroke which appears to be IL-6 regulated. After stroke, higher numbers of newly generated endothelial cells along with macrophage/microglial cells and increased density of perfused microvessels was found in the brain, which was blunted in genetic deletion of IL-6.

Following stroke, neural stem/progenitor cells (NSPCs) migrate into injured brain and differentiate to neurons. Treatment with the novel neurotrophic factor Meteorin (METRN), stimulated proliferation in SVZ, promoted neuroblast migration, and increased number of neurons in the ischemic striatum. Human fetal NSPCs implanted into the striatum after stroke, increased the numbers of proliferating cells and new neurons, and improved recovery. Transplanted neuroepethilial-like stem cells, generated from adult human fibroblast-derived induced pluripotent stem cells, into stroke-damaged brain survived, improved recovery, and formed functional neurons. This is the first evidence that transplantation of reprogrammed human skin cells can promote recovery and supply the cortex with new neurons after stroke.

The speed by which brain damage develops after a stroke and the problems associated with bringing the patients into care, makes less likely that a majority of stroke patients will be provided with an efficient neuroprotective therapy. Most stroke patients have to rely on rehabilitative therapies. Results from WP5 have identified novel therapeutic targets, pharmacological entities that hold promise for treatment of stroke in a not too distant future. Importantly these effective treatment modalities can be initiated several days after stroke preferably in combination with rehabilitative training:

Formation of new functional neuronal fiber projections of adult corticospinal neurons are enhanced by anti Nogo-A treatment. The positive effect can be observed when the anti-Nogo-A treatment is combined with rehabilitation therapy.

Treatment with SA4503, a Sig1R agonist enhances functional recovery after stroke.

Inhibiting the CXCR4 receptor by AMD3100 during the first 14 days after stroke in rats diminishes brain inflammation and enhances recovery.

Treatment with L-DOPA after stroke enhances recovery, depresses brain inflammation and releases the growth factor GDNF.

Intracerebral transplantation of human fetal neural stem/progenitor cells promotes several steps of neurogenesis after stroke. Combination of NSPC transplantation and stimulation of neurogenesis may become a valuable strategy for restoration after stroke. Reprogrammed human skin cells improve recovery and differentiate to functional neurons after transplantation into stroke-damaged mouse or rat brain.

IL-6 is an essential factor for post-stroke angiogenesis that affording long-term histologic and functional protection.

Future perspectives

The dynamics and extent of recovery of sensori-motor functions in stroke patients is in many aspects mimicked in experimental rodent models of stroke. Also, the positive results of recovery enhancing treatment, several days after stroke, holds promise that an effective stroke therapy can by developed within a not too distant future. Further research is needed along the lines initiated by WP5, with a focus on basic mechanisms of the recovery processes, on therapeutics of brain rehabilitation, as well as on planning of translation of the new treatment modalities discovered in experimental setting into the clinic.

WP6 – Brain targeting of stroke therapeutics

Most substances and drugs that are potentially useful for treatment of stroke cannot be applied due to their inability to penetrate the blood-brain barrier (BBB). In particular, large-molecule agents such as monoclonal antibodies, recombinant proteins, or gene therapeutics do not cross the BBB. Currently, no clinical approach is available to open the BBB for local drug delivery. Localized drug delivery can be accomplished only by injecting an agent through a needle or catheter directly into the targeted brain area, which may be useful in preclinical experiments, but will be of limited clinical use. Clearly, targeted delivery of therapeutic agents through the BBB is highly desirable in the treatment of stroke. This would allow approaches that normally fail due to lack of adequate delivery across the BBB. Thus, a primary objective of the ARISE project was to develop new approaches to overcome this barrier to drug delivery.

Recent studies have shown that high-intensity focused ultrasound (US) can allow selective and non-destructive disruption of the BBB. If microbubbles are introduced to the blood stream prior to focuse US exposure, the BBB can be transiently opened at the ultrasound focus. This is because the microbubbles can act as cavitation nuclei that confine the ultrasound effects to the vasculature and reduce the intensity needed to produce BBB opening.

Main achievements

The ARISE project has made significant contributions towards establishing this new technology for clinical applications, in particular for stroke therapy. Extensive work was undertaken to first establish the safety of this procedure. Adverse effects were investigated histologically by documenting hemorrhage, apoptosis rates and expression of heat shock proteins and ubiquitin. Through application of a wide range of US parameters suitable for BBB opening, a number of important findings were made regarding the molecular effects of BBB opening with US and microubbbles:

- At the upper thresholds of acoustic pressure for safe BBB opening a reorganization of gap-junctional plaques in both neurons and astrocytes may occur. This is important because gap junctions allow transfer of information between adjacent cells and are responsible for tissue homeostasis.
- Focused ultrasound-induced opening of the BBB in the presence of ultrasound contrast agents can lead to increased ubiquitinylation of proteins in neuronal cells, indicating that brain molecular stress pathways are affected by this treatment.

Thus, although we were able to show that this novel delivery method can be performed without hemorrhage, apoptosis or expression of heat shock proteins, its merits for stroke patients must always be weighed against possible deleterious effects of transient BBB opening.

Following up on these results for optimization of BBB opening with US and microbubbles, ARISE researchers were able for the first time to effectively deliver chimeric adeno-associated virus 2/1 (AAV2/1) particles containing the coding region for the LacZ gene into the rat brain upon intravenous (IV) administration after BBB opening by focused ultrasound in the presence of microbubbles. We showed that the transgene is correctly and efficiently expressed in cells located in the neighborhood of the insonated focus, especially in the vicinity of small vessels and capillaries. Using double immunofluorescence (IF) with antibodies against tubulin III and bacterial LacZ, we identified these cells to be mostly neurons. These results demonstrate that our approach allows a very specific, localized, and efficient expression of intravenously administered transgenes in the brain of rats upon ultrasound-induced BBB opening.

The use of nano-sized drug carriers has emerged as a promising approach to transport drugs through the BBB. This is because nano-complexes demonstrate added functionality over conventional pharmaceutical approaches. This functionality, besides the ability to deliver the drug across the BBB, includes other key functions such as shielding the drug from producing systemic side effects, preventing premature drug clearance or metabolism, and targeting specific cells after the drug has gained access to the CNS.

A number of novel nanocarrier delivery strategies were developed and evaluated in the ARISE project. Adenovirus loaded microbubbles were prepared via chemical modification of which enabled microbubble loading via avidin-biotin strategy. Using this concept, we were the first group to show that intracellular localization of adenoviruses after ultrasound treatment of adenovirus loaded microbubbles is independent of endocytosis, which has important consequences for the activity of several nanoparticles. To evaluate the possibility to selectively delivery BDNF over the BBB, we succeeded in preparing lysozyme loaded microbubbles by loading lysozyme encapsulated liposomes on the surface of lipid microbubbles. Due to its size and isoelectric point this is a comparable but much cheaper alternative to BDNF. However, further experiments pointed out that only a small part of the lysozyme becomes encapsulated within the liposomes and is released from the microbubbles. Finally, as an alternative tPA loaded microbubbles were designed. Ultrasound triggered localized delivery of tPA at the blood clot can assure that tpA is locally delivered and systemic side-effects and neurotoxicity can be avoided.

It was recently demonstrated that nerve growth factor (NGF) can be delivered across the blood-brain barrier using poly (butyl cyanoacrylate) PBCA nanoparticles coated with polysorbate 80 in rodents. In line with the goals of the ARISE project we evaluated this promising avenue for drug delivery in rodents subjected to middle cerebral artery occlusion (MCAO). The results of this work demonstrate that a single dose of nanoparticle-bound NGF can effectively cross the BBB to cause up-regulation of a number of molecules playing a central role in regeneration of brain tissue. Nestin, an intermediate filament protein, is a marker for migrating and dividing neuronal precursor cells of the subgranular zone. Tie-2 is a cell-surface receptor that binds and is activated by the angiopoietins, (Ang1, Ang2, Ang3, Ang4), which are protein growth factors that promote angiogenesis. Up-regulation of Nestin and Tie-2 indicates increased neurogenesis and angiogenesis, respectively. These up-regulations are triggered by a single dose of nanoparticle-bound NGF 3 days after MCAO.

WP07 – Stroke model platform

Given the devastating failure rates of potential new interventions in patients, animal models of stroke have quite rightly been questioned for their relevance. As a general criticism, most work performed in animal models has not reflected clinically relevant endpoints in stroke patients. Further mechanisms and interventions have not taken into account the range of paradigms covering the diversity of human disease including the impact of co-morbidities. Therefore within WP07 we aimed to overcome this criticism by establishing, validating, and disseminating the following objectives and tasks:

Main objectives

To develop assays for long-term functional outcome and neurological consequences after stroke, models of senescence, co-morbidity, atherosclerosis, and associated risk factors and study impact on stroke susceptibility as well as models of clot occlusion, small vessel disease, lacunar stroke, and spontaneously occurring stroke

Main achievements

Within WP07 we successfully developed and validated a whole battery of tests to evaluate functional neurological deficits, following focal ischemic brain injury in mice and rats. The tests have been evaluated in different stroke pathologies as well as for assessing short term and long term outcome. Further we established and validated a number of tests for assessing behavioural and emotional abnormalities following stroke.

Together with WP02 important new models for senescence, co-morbidity, atherosclerosis, and associated risk factors have been established and characterized extensively. These models have been disseminated to labs within the consortium and tested in the context of different stroke paradigms. Results from all these models show a detrimental effect of systemic inflammatory conditions, aging and chronic stress on stroke outcome, with multiple pathophysiological mechanisms involved. Finally new models of clot occlusion, small vessel disease, hemorrhage, and spontaneously occurring stroke have been developed and tested. Especially the thromboembolic stroke model established in Caen has been disseminated to a number of consortium partners via training courses in Caen.

Results and protocols have been disseminated within the ARISE Consortium and made public by numerous publications including the book “Rodent Model´s of Stroke” as well as an educative MCAO video from Berlin for the Journal of Visualized Experiments (JoVE), which has been viewed more than 45.000 times since its release in October 2011. In addition together with WP09 a number of educative courses on cerebral ischemia were realized covering the topics mentioned above.

Overall the work of WP07 together with other work packages marked a very successful and productive period. We clearly profited from the expertise of the different consortium partners and could build new synergies. Modelling

stroke with high clinical relevance and testing for clinically relevant endpoints is an important step to overcome the "translational roadblock" between pre-clinical and clinical testing. In summary our results regarding functional and behavioural testing have the potential for setting new standards for testing the effect of interventions and pathophysiological mechanisms far beyond infarct volume and histology. In addition especially the impact of co-morbidities and risk factors underline the urgent need for using appropriate co-morbid models in experimental stroke research to develop new clinically relevant therapeutic protocols. In the future, we will further characterize long-term behavioural outcome in stroke models. Importantly, we will compare this to endpoints in clinical trials to increase predictability of pre-clinical findings for patient trials.

WP08 – Brain imaging platform

WP08 used a variety of non-invasive imaging technologies to image the brain after stroke. Imaging techniques, particularly MRI, but also PET and SPECT, are realistic tools for application in stroke patients due to its availability at the clinic. In experimental animals, non-invasive imaging has several interesting features to study the ischemic brain that we have considered in the course of this project, as follows:

- Reperfusion injury can be assessed with imaging methods
- Molecular imaging to study brain inflammation
- Cellular imaging to investigate leukocyte infiltration
- Multimodal and multiparametric longitudinal studies in the same subjects to follow the progression of the brain lesion, monitor the effects of treatments, and imaging the integrity of white matter using DTI
- Imaging dynamic functional parameters such as cerebral blood flow and brain activity

Main achievements

Reperfusion is the main objective to prevent neuronal cell death after ischemic stroke. Thrombolysis with rtPA is currently the only approved treatment for thromboembolic stroke. In spite of the demonstrated benefits of thromolytic therapy, this bears risks of unwanted side effects, such as hemorrhagic transformation, that globally are recognised as reperfusion injury. Signs of reperfusion injury are more prominent when rtPA is administered late compared with early rtPA treatment. A main goal of this project was to find strategies for preventing thrombolysis-associated reperfusion injury that could be achieved with treatments accompanying rtPA. Reperfusion triggers an oxidative stress burst that might be combated with antioxidant agents. This principle has been proved in animals but remains to be demonstrated in humans. In rodents, we showed that antioxidant treatment with a synthetic vitamin E analogue (CR-6) can prevent side effects of reperfusion such as matrix metalloproteinase-9 (MMP-9) activation and blood-brain barrier (BBB) breakdown. Also, an antibody directed against the N-terminal end of NR1 subunit of the NMDA receptor (ATD-NR1) developed in this consortium prevented reperfusion injury effects of late rtPA turning this treatment virtually as safe as early rtPA administration. Imaging MMP activation and increased BBB permeability are two parameters that can inform on reperfusion injury. In this consortium we carried out studies to image MMP-9 and BBB breakdown using several imaging strategies. Near-infrared fluorescence activatable probes were successfully used to image MMP-9 activation in vivo by optical imaging technology. BBB alterations were assessed also by optical imaging using near-infrared fluorescence imaging with fluorescently labelled albumin, by MRI using contrast agents (gadolinium-DTPA), and by SPECT technology with ^{111}In-DTPA-HAS. Although the later SPECT study was not very promising, successful imaging of BBB breakdown was achieved with optical imaging and with MRI in experimental models of stroke, and was used to show benefits of treatments. Some of these strategies are readily applicable to humans and should be more widely used in stroke patients to monitor the effects of thrombolysis.

Neuroinflammation was imaged in vivo in experimental rodent models of stroke by PET using classic tracers, such as [^{11}C]PK11195, and the newly developed tracer, [^{18}F]DPA-714. Both PET radioligands bind TSPO, a receptor located in the mitochondrial membrane. Studies with [^{3}H]DPA-714 demonstrated ex vivo that the cellular targets of radiolabel binding were mainly microglia/macrophages. In vivo, PET studies in stroked rodents with [^{18}F]DPA-714 demonstrated a better performance of this new tracer over [^{11}C]PK11195 to image inflammation in the brain after stroke. Future studies with this PET tracer are guaranteed, as well as its use in stroke patients. Using PET with these TSPO tracers we assessed inflammatory changes in the brain in rodent models of chronic, systemic inflammation and showed that this technology allows identifying exacerbated brain inflammation under these conditions. Likewise, preliminary studies in patients at risk of stroke (multiple risk factors for stroke, with chronically elevated C-reactive protein, but negative MRI for brain pathology) showed that they exhibited increased inflammation in the brain, as indicated by PET imaging. Besides PET, the new developments in SPECT technology allowing high-resolution studies, as we proved in this WP, encourage the development of DPA-714, or related compounds, radiolabeled with SPECT tracers to imaging neuroinflammation. Inflammation after stroke involves the induction of cytokine and adhesion molecule

expression. In this consortium we imaged post-stroke ICAM-1 and VCAM-1 up-regulation using functionalized antibodies directed against these molecules that were labelled with paramagnetic micron-sized iron oxide particles (MPIO). This study was partly carried out in collaboration with our EUSTROKE partners. We showed that MRI and or PET and SPECT can image several aspects of the inflammatory process and we proved that these technologies are useful to assess neuroinflammation after stroke in rodents with a clear translational power for application in stroke patients.

Labelling circulating leukocytes is a putative way to image cells recruited to the ischemic brain. Previous reports provided images attributed to infiltrated cells after i.v. injection of various paramagnetic particles after induction of stroke in rodents. Nevertheless, passive passage of free particles after the BBB is damaged cannot be excluded in these studies. To overcome this problem several laboratories in this consortium and in EUSTROKE undertook studies intended to label circulating cells before stroke, with the aim to avoid brain labelling due to BBB breakdown rather than to cell infiltration. We used either various types of paramagnetic particles or fluorescent beads. All the laboratories came out with the same finding, which is that the majority of the particles were retained in peripheral organs (mainly the spleen) but did not label circulating monocytes. We concluded that macrophages in peripheral organs phagocyte free particles injected in the circulation in the hours that follow i.v. administration while the phagocytic activity of circulating cells was very low. Therefore, this strategy does not allow imaging infiltration of monocytes in the stroked brain.

Multiparametric imaging studies highlight different tissular features: The stroke brain lesion evolves dynamically with time and changes its physical characteristics in terms of water content and distribution, energy consumption, cell viability, cellularity and nerve fibre integrity. Alterations in some of the above features can be detected with different MRI sequences that are specifically suited to highlight cytotoxic or vasogenic edema, glial reaction, or alterations in nerve fibre structure. In the ARISE consortium together with EUSTROKE, structural brain tissue alterations have been followed up longitudinally in stroked rodents providing information that has some predictive value in terms of lesion volume as demonstrated using specific algorithms. Imaging in the very acute phases allows carrying some approximate prediction of the magnitude of the final lesion to study how the expected lesion might be modified by treatment. For this purpose we used treatments such as thrombolysis, antioxidants and anti-inflammatory drugs. These approaches were also used to standardize animals before treatments and to show that the same initial lesion evolved differently in certain genetically modified mice. Besides its use in acute stroke studies, we used multiparametric MRI imaging in experimental situations of chronic vascular deficits caused by hypoperfusion, as induced by bilateral common carotid occlusion in rats, where by longitudinal imaging studies we could distinguish acute from delayed brain damage and adaptive changes, such as compensatory arteriogenesis. Also, we showed in animals that multiparametric MRI, including DTI, might help to respond to clinical questions such as the condition of wake-up stroke that is currently excluded of thrombolysis due to uncertainty about the time of stroke onset. Multi-parametric brain imaging with MRI is feasible in acute and chronic patients with vascular diseases since it can be performed within short time frames and with widely available equipment, and should be considered at the bedside as a tool to stratify patients that should lead to more accurate diagnostics and personalized therapy.

Imaging cerebral blood flow (CBF) and brain function in a non-invasive manner is carried out in humans. Interestingly, it is easier to apply these technologies in humans than in rodents for several reasons, including its low spatial resolution that requires stronger magnetic fields to image very small brain regions in rodents, and the need to keep the animals anesthetized, which is a terrible drawback in as much as it alters brain function. Quantification of CBF after non-invasive imaging in mice is not a standardized procedure. In this consortium we studied CBF by several imaging modalities. CBF imaging using flow sensitive alternating inversion recovery (FAIR)-MRI was compared to the standard invasive ^{14}C-Iodoantipyrine (IAP)-autoradiographic technique in a mouse model of acute stroke. Although FAIR-MRI overestimated CBF compared to autoradiography, it provided repetitive quantitative measurements of hemispheric CBF. CBF quantification requires knowledge of the brain–blood partition coefficient (BBPC) for water, which is normally assumed as a fixed value. Here we determined the BBPC for water in mice from proton density measurements of brain and blood, calibrated with deuterium oxide/water phantoms. This information will contribute to provide more accurate measurement of CBF in mouse models of stroke. Besides CBF studies, we also carried longitudinal fMRI in stroked rats in long-term studies using BOLD contrast after forepaw electrical stimulation. Statistical parametric activation maps showed that rats housed in an enriched environment recovered brain function 3 months after stroke significantly better than rats housed under standard conditions. fMRI has translational power and could be used in stroke patients for unbiased monitoring of stroke recovery of function in clinical trials aimed to assess regeneration after stroke.

Future perspectives

Perspectives for the future include the use of multiparametric/multimodal brain imaging in acute and long-term studies of experimental stroke to obtain non-invasively structural and molecular information that can be combined with the study of other biological parameters, contributing to stratify subjects and reducing the number of animals per study. Some of these imaging approaches are readily translatable to humans and should be used more often in the clinics as imaging biomarkers aiming for a more personalized therapy in stroke patients.

WP09 – Training in stroke models and research techniques

The purpose of ARISE training activities in WP-9 was to contribute to the training of a new generation of basic- and clinician-researchers in the stroke field. We aimed at (1) standardisation/harmonisation of laboratory practices; (2) training in scientific and technological key competences of the consortium; (3) to implement a translational approach; (4) to foster staff mobility and exchange among all partners in order to reinforce the research capacities of each partner institution and to stimulate complementarity of the research teams and their mutual specialization, and to overcome duplication and/or fragmentation of research efforts.

The WP-9 was a great success. We organised a total of seven courses on different aspects of stroke research, including biometry and ethical principals and practices. Regarding the technological competences, two of these courses concentrated on animal models of transient, permanent and TE models of focal ischemia (MCAo), including the aspects of co-morbidity and aging; three courses on focal ischemia models in general with the comparison to in vitro stroke models and behavioural analyses; and one course specialized on non-invasive imaging, mainly MRI analysis of stroke outcome. All these courses had participants from several ARISE member laboratories representing several countries. We also organized a large 3-day course with 15 invited expert speakers, including three Canadian specialists, on the impact and research tools of neuroinflammation and its clinical aspects. The course was attended by 63 participants, representing 7 ARISE member labs. An attempt was made to include clinical aspects to the courses and thereby diminish the translational barrier between laboratory animals and human patients. A definite progress in training was achieved by publishing a book “Rodent models of Stroke (Dirnagl U, Ed.)” in 2010 and a tutorial video “Modeling stroke in mice - middle cerebral artery occlusion with the filament model. J Vis Exp. by Engel et al in 2011.

Main achievements:

- Dissemination of hands-on and theoretical instructions for generation of rodent stroke models
- Dissemination of hands-on and theoretical instructions for physiological, histological and behavioral analyses as well as non-invasive imaging of rodent stroke models
- Dissemination of instructions for planning stroke experiments, including measured to prevent bias, statistical power, and clinical relevance
- Understanding the importance of relevant therapeutic time window, confounding factors and long-term functional outcome of stroke.
- Promotion of collaboration and researcher exchange

Along with the progression of WP9 it became apparent that while theoretical teaching can be sufficient during short-term courses, the hands-on training required more time and practice than could be offered within one or two days. Thus, the courses served as triggers for making longer lasting visits to those labs, which were able to train researchers more individually and efficiently. For example, the method of thromboembolic stroke in mice, a very relevant but complicated stroke model, was thoroughly learned by more than 10 researchers while making short-term, separate visits to the lab of partner 10.

Training on experimental stroke will remain crucial in future research programs of stroke. This is important not only for maintaining and disseminating the already achieved expertise in rodent stroke models, their imaging and behavioural testing, but also extending the training to novel mouse models, such as white matter stroke, tPA-resistant thromboembolus model, multi-infarction and recurrent infarct models. Other important aspects to be learned and disseminated are methods for measuring long-term functional recovery, gender differences and aging-related changes in brain and periphery. Stem cell technologies offer now possibilities for modelling stroke that could not be foreseen last decade. A substantial part of training could be “tailor-made” personal teaching that can be achieved by short-term researcher exchange program. Training is more rewarding when clinical aspects are brought up by neurologists specialized in treating stroke and by keeping the training as open and intercontinental as possible. Finally, quality aspects of experimental stroke will remain as key elements of future stroke training.

WP10 – Clinical Trial Platform

The Clinical Trial Platform (a joint WP of EUSTROKE and ARISE) provided a clinical trial network of centers associated with preclinical sites to allow for swift transition into clinical phase II trials, and assisted the coordination of the ongoing clinical study and trial activities of the partners, such as data entry and management and data sharing. It is coordinated by the University of Heidelberg and the Karolinska Institute. The Trial Platform also helped with the coordination of the design, fund raising, ethical and regulatory issues of phase II studies emerging from ARISE and EUSTROKE associated researchers and their collaborators (e.g. EUROHYP-1, PREDICT, WAKEUP).

Main achievements

The Trial Platform responded to several new opportunities for enhanced clinical stroke research in Europe. These include:

Increased opportunities of translating pre-clinical research to clinical practice/trials. There are several European driven pre-clinical research projects that soon could enter the clinical stage. The Trial Platform offered opportunities for faster and more cost-effective translation of pre-clinical research to clinical experience regardless if initiated from academic institutions or from industry.

Increased opportunities to run investigator driven trials. The industry-independent Trial Platform facilitated investigator driven trials. Examples include, but are not limited to: provision of cost effective monitoring procedures in line with GCP, procedures for submitting trial concepts, and clinical study protocols online, peer review systems, educational and certification modules adapted to GCP and individual trial settings, publication of study protocols on line and data ownership by investigators.

Increased demand to optimize conditions for collaboration with industry and SMEs. The Trial Platform was a strong partner to industry (SYGNIS, PAION, QUICKCOOL, etc.). By having such a Trial Platform, the scientific community took a more active role for study protocol development and transparency, data management and publication. The industry does not have access to clinically well-characterized biobanks, and it is therefore the task of the academic community to investigate this material and provide novel targets for drug development.

Opportunity for enhanced knowledge sharing among stroke practitioners interested in participating in clinical trials. The Trial platform played an important role in promoting knowledge sharing on front-line stroke research and trial logistics, educational support and support in contacts with ethics committees.

SITS: The Trial Platform was based on the SITS Network (Safe Implementation of Treatments for Stroke). The SITS Network is the world's largest stroke treatment network with more than 500 participating stroke units in over 30 countries. Participating clinics are primarily located in Europe but also in Asia, the Middle East, Australia and South America. More than 2,000 researchers and MDs are currently active in this network. The objective of SITS is broad implementation and quality development of evidence-based stroke treatments.

ARISE and EUSTROKE adapted the SITS network and database to support the Trial Platform. SITS supported pre-clinical trial design and pre-clinical data acquisition to facilitate the transition from pre-clinical results into clinical application. We discussed the results of the preclinical testing of innovative therapies within the consortium and selected promising candidates for clinical trial (e.g. IL-1RA). The Trial Platform helped coordinate ongoing clinical trial activities of the partners, and assisted in coordinating the design and fund raising of II studies emerging from the research.

1.4 The potential impact

Socio-economic impact and the wider societal implications of the project

Contribution to Community and social objectives

ARISE lead to a number of tangible results benefiting European citizens. ARISE specifically delivered on all the targeted medical needs and priorities, as formulated in our initial submission:

'Improving the health of European citizens (including improving the health of the ageing population)'

ARISE activities had direct impact on the health of European citizens. Via public campaigns, and through collaboration with SAFE (Stroke Alliance for Europe) we contributed to an increased awareness of risk factors for stroke and the urgency of medical attention in case of a suspected stroke.

Importantly, through

'Development and validation of new therapies' ARISE was able to demonstrate

- the existence of safer thrombolytics and adjuvant therapies to thrombolysis.
- that inducers endogenous neuroprotection can be therapeutically exploited and fostered (e.g. via hypothermia)
- that immunomodulation can protect the brain from the consequences of immune cells, and harness the regenerative capacity of immune cells.
- that drugs or physical measures (e.g. exercise) that stimulate adaptive brain plasticity and enhance functional recovery after stroke exist and can act via neurogenesis, axonal growth and recovery of function of surviving neurons.

'Better understanding of brain dysfunction'

ARISE research has made major contributions to our current understanding of stroke pathophysiology, which is the basis for the development of novel treatments. For example, through the interdisciplinary collaboration of vascular biologists, preclinical as well as clinical stroke researchers we were able to refute an old dogma regarding the invasion of the brain by granulocytes after stroke. This is highly relevant, as these findings may partially explain why certain antiinflammatory strategies have failed in clinical testing (e.g. Enlimomab), but also point to alternative strategies to modulate the inflammatory process towards brain protection. Another example concerns the discovery of a masterswitch of cell death or cell survival (PEA15/HKII) which opens up novel therapeutic avenues for stroke, but also for tumor therapy. Pharmacological interventions in this signaling pathway are currently further explored with the pharmaceutical company SANOFI.

'Translation of basic discoveries into clinical applications including scientific validation of experimental results'

Bench to beside translation was and still is the major bottleneck of stroke research. ARISE research (together with EUSTROKE) was internationally leading the efforts to improve translational success by improving the positive predictive value of preclinical stroke research.

- We established and used predictive stroke models, which included clinically relevant confounders such as comorbidities, gender and age
- exploring clinically relevant issues (bedside to bench), such as infection before and after stroke
- identified biomarkers that predict complications of stroke and hence allow preventive treatment, first in animal models, then validated the approach in clinical studies (e.g. PREDICT study).
- established novel non-invasive imaging approaches to obtain 'fingerprints' of disease processes in experimental models which can now be used in clinical trials for therapeutic stratification, pathophysiological investigation, outcome monitoring, and as surrogate parameters.

'Increasing European competitiveness'

Stroke research in the EU is highly competitive on the international level. ARISE and EUSTROKE were further contributing to this outstanding role of European research in this area. ARISE researchers were invited by the National Institutes of Health/National Institutes for Neurological Disorders and Stroke to participate in their quest to define US research priorities in the stroke field for the next decade (SPRG-process).

Competitiveness is the basis for collaboration: Through a collaboration with the Canadian Stroke Network we were able to pool resources, generate mutual training opportunities and exchange of research expertise. Complex issues can be broken down and distributed in a coordinated fashion between partners. The vast experience of the European and Canadian Stroke Networks suggest that further benefits can be obtained from enhancement of their previously developed avenues of collaboration. We established a unique transatlantic pilot cooperation, the first of its kind in stroke research, to bundle expertise of some of the best researchers across the Atlantic. Six joint projects utilizing expertise from 22 research centers were chosen for this unique cooperation, the results of which could set the stage for further international efforts to overcome the translational roadblock.

'Boosting the innovative capacity of European health-related industries and businesses'

'High-tech' SMEs were important contributors to ARISE. The innovation and the integration of SYGNIS, NSGENE, QuickCool and PAION ensured that new knowledge is disseminated and translated into new therapies and clinical practice. Development of new pharmaceuticals has a high attrition rate. SYGNIS, our partner testing a hematopoeitic cytokine as a novel stroke therapy (G-CSF) was unable to demonstrate clinical benefit of the compound. Consequently, SYGNIS is no longer developing pharmaceuticals, but has instead switched to developing DNA multiplication and sequencing techniques. However, other industrial partners were highly successful. For example, QUICKCOOL was able to demonstrate the safety and efficacy of its novel, minimally-invasive and portable techniques for early, and safe whole-body cooling and preferential cooling of the brain. Clinical testing is under way. PAION is clinically developing the novel strategies to improve the safety of thrombolysis which were developed within the consortium. It is remarkable that in a field, which due to massive failures in clinical trials has been largely abandoned by the pharmaceutical industry, ARISE and EUSTROKE were able to involve pharmaceutical companies in joint projects which are currently being scaled up and roled out towards clinical testing.

Main dissemination activities and exploitation of results

ARISE results were disseminated in scientific articles, brochures for lay persons, conferences, workshops, books, TV-appearances and public awareness campaigns throughout Europe. ARISE results led to a number of patents, some of which have already been licensed for further development by SME and industrial partners.

The results of the ARISE research were made available to the scientific community at large via a large number of publications (>170) in highly esteemed journals, including Nature family journals, PNAS, Brain, Lancet Neurology, J Neurosci, etc.. Several of these articles were featured in editorials and press releases with subsequent coverage in news media (e.g. Enzmann et al. 2012). Many more articles describing ARISE research are currently in preparation or in submission.

Members of the ARISE consortium organized numerous scientific meetings and symposia or took part in such meetings as invited and keynote speakers (>140). Of note, at the European Stroke Conferences (e.g. Nice, Barcelona, Lisbon, London), we organized dedicated and very well attended symposia highlighting the results of our collaboration (together with EUSTROKE).

Internet home-page: The ARISE (= ESN, www.europeanstrokenetwork.eu) home page served as an important communication platform. The website facilitated regular and programmed, dissemination of data and promoted dialogue between investigators. The website had have ‘open’ and ‘restricted’ areas (that are accessible only to consortium members). The website will also include regularly updated ‘consensus statements’ that represent the views of the consortium on study design and reporting. The website also played an important role in targeting the general public.

An important dissemination activity was the training of researchers, key staff, including managers and industrial executives, even beyond the ARISE consortium. ARISE and EUSTROKE organized and advertised these courses in such a way that they were open to all professionals interested even if they were not members of the consortia. External participation included neurologists and neuroscientists, students, but also technicians from academia and industry. A particular focus with regular courses held was on issues of modelling of stroke (techniques, limitations, quality), on brain imaging (in particular harmonization of protocols), brain inflammation, and behavioural assessment in models of stroke. Together with the publishing house Springer, ARISE and EUSTROKE researches produced a widely read book on Modelling Stroke, which is a current standard source of information for basic stroke research laboratories world-wide ('Rodent models of stroke'; http://www.springer.com/biomed/neuroscience/book/978-1-60761-749-5). We also produced instructional videos available via the internet (e.g. J Vis Exp. 2011 Jan 6;(47), making expert know how of the consortium available to the research community at large.

Numerous activities targeted increasing stroke awareness in the general public: In stroke treatment, 'time is brain', and the ARISE consortium was well positioned to participate in the propagation of this knowledge. In addition, since a number of stroke risk factors can be prevented or ameliorated (hypertension, smoking, diabetes, etc.), educating the public about these risk factors and strategies to decrease personal stroke risk are highly important. ARISE (together with EUSTROKE) improved European stroke awareness by participating in national as well as international health campaigns, such as the 'World Brain Awareness Day', the 'World Stroke Day' etc. ARISE scientists gave public lectures, appeared on TV or other news media, and organized local awareness campaigns (e.g. 'Berlin gegen den Schlaganfall', Berlin against stroke). ARISE and EUSTROKE produced a widely disseminated brochure explaining how to prevent a stroke and what to do if one suspects that a stroke has occurred.

Exploitation

Work of the ARISE consortium has led to 8 international patent applications, all of which concern novel treatments for stroke. Several of those have already been licensed (e.g. a patent on immunotherapy of stroke) by pharmaceutical companies and are in clinical development. Unfortunately, one industrial partner (SYGNIS) had to abandon clinical development of a putative neuroregenerative agent (G-CSF) due to neutral results of the phase II trial. On the other hand, our partner QuickCool is currently enrolling patients in clinical trials in Sweden and Denmark. These studies investigate the safety and efficacy of the novel QuickCool Intranasal Brain Cooling System in the following clinical areas: cardiac arrest, traumatic brain injury and subarachnoid hemorrhage. Although PAION has sold its rights to the thrombolytic agent Desmoteplase to H. Lundbeck A/S, remains active in the field by collaborating with partner 7 (INSERM Caen) in developing adjunctive therapy for thrombolysis.

Outlook and future research

Improving the predictiveness of preclinical stroke research

A central question in drug development, not only in stroke, relates to whether and when the researcher, investor, or pharmaceutical company should venture from preclinical testing into clinical development. Clinical development programs are exceedingly expensive, take a long time, and carry the risk of potential harm for both volunteers and patients. At present, no firm criteria exist for such go/no go decisions. In many prior translational stroke research programs the decision to proceed was based on limited experimental evidence and personal opinions of stakeholders in the process. ARISE (and EUSTROKE) were spearheading the the notion that the decision to move from animal experiments to patients should be based on robust, high quality data. We have proposed that preclinical translational stroke research should learn from the experience of clinical stroke research. Our consortia were ideally placed for this approach, as preclinical and clinical researchers were working side by side. In our joint research we have established measures to improve the quality of clinical trials and hence the robustness of our results. This included strategies to minimize bias (randomization, blinding, allocation concealment), a priori Power analysis and other biostatistical advances, careful definition of the primary and secondary endpoints, data monitoring and auditing, internationalization and inclusion of many centers, external steering committees and safety monitoring, rigid publication standards, trial registries, among others. Not only have we applied these measures within our consortium, we have propagated them into the scientific community at conferences and through articles and editorials (e.g. Dirnagl and Fisher, jointly published in Stroke and J Cereb Blood Flow Metab). A future quest will be to continue a process by which preclinical researchers are enabled to understand the limitations of their approaches, to limit bias, and to improve the predictiveness of their research.

Conducting international, multicenter preclinical ‘phase III’-type studies

We have also called for international, multicenter preclinical ‘phase III’-type studies of promising, novel ischemic stroke therapies before moving from animal modeling to clinical trial. Such trials would not replace basic research targeted at discovering or investigating pathophysiological mechanisms of drugs (preclinical phase I), or initial preclinical trials by individual scientists (preclinical phase II). Rather, 'phase III' preclinical trials would be based on such prior studies, and only those compounds or treatment modalities would enter phase III that were highly promising in phases I and II. In fact, within ARISE (and EUSTROKE we have already been conducting such international phase III type studies, for example crossvalidating the results concerning IL1RA of Partner 4 (University of Manchester). This has led to an international drive for such studies, and a successful application (MULTIPART, Multicentre Preclinical Animal Research Team, Grant agreement 603043) in the FP7 call [HEALTH.2013.4.1-4] 'Preparing the future for health research and innovation'. Several members of ARISE are part of this consortium, which is engaging all partners to build consensus around the feasibility, structure, composition and operation of multi-centre preclinical phase III consortia. Issues include the role of industry and regulators; whether the capacity to

deliver such studies exists; the statistical analysis to be used; and ethical, legal and governance issues. This consensus will be achieved through a series of themed meetings involving the applicants and others; the development of a detailed plan for such a consortium; and the validation of that plan with a specially constituted Scientific Advisory Board. MULTIPART will then seek funding for the delivery of multi-centre animal studies based on this plan to allow its delivery.

Stroke outcome depends on medical complications

Outcome of stroke patients is not only dictated by non-modifiable factors, such as severity of stroke, age, or premorbidity, but also by modifiable factors, largely related to medical complications, such as infections and possibly sarcopenia. The highly successful concept of stroke units attests to the benefits of treating stroke comprehensively. Breakthroughs in improving outcome after stroke will only result from approaches that target not only the brain, but also systemic effects of stroke. ARISE had two workpackages regarding the interplay of immune system, infection, and stroke. While further research on specific molecular and cellular pathways of ischemic damage is absolutely warranted, ARISE research has shown that we should not expect to find the golden bullet that can substantially benefit stroke patients by acting on a magical, single mechanism. ARISE and EUSTROKE results has had a major impact on the stroke research community worldwide in the insight that further research needs to address the complex interactions between the 'super systems' (nervous, cardiovascular, immune, endocrine, metabolic, etc.), and target CNS as well as systemic effects of stroke.

Demonstrating the clinical efficacy of therapeutic strategies investigated in ARISE

ARISE researchers have investigated a number of promising therapeutic targets, for several of those, clinical testing is imminent, or already under way. To this end, EuroHYP-1 trial is a pan-European, EU funded (Grant agreement 278709), open, randomised, phase III clinical trial which will assess the benefit of therapeutic cooling in adult patients with acute ischaemic stroke. In addition to efficacy and safety, the economic impact of therapeutic hypothermia will be also evaluated. The EuroHYP-1 consortium brings together leading European experts in statistical design and analysis, therapeutic hypothermia, imaging, health economics, ultrasound, biomarkers, and trial execution (implementation and monitoring) as well as European patient and family advocacy groups and Small and Medium-size Enterprises. Several ARISE partner were involved in kickstarting the initiative, developing the study design, and are now actively recruiting patients for this promising therapy for which ARISE has investigated pathophysiological mechanisms and developed devices.

The multicenter PREDICT study (N=486; clinicaltrials.gov: NCT01079728), sponsored by ARISE partner 1 and involving several other ARISE partners, prospectively validated a score to predict stroke-associated pneumonia. PREDICT demonstrated for the first time that stroke induced immunodepression and dysphagia independently causes of post-stroke pneumonia. Immune markers improved the predictive capacity of the prognostic score for identifying patients at risk of stroke associated pneumonia. Currently interventional stroke trials are under way to test the efficacy of stratifying patients based on the score to preventive antibiotic therapy.

A number of other clinical trials of ARISE based therapeutics are in planning, for example for IL1RA and immunotherapeutic modification of thrombolysis.

Extending European collaboration to transatlantic/transpacific, multidisciplinary collaboration

Establishing the European Stroke Network (with EUSTROKE) was a major step forward to bring together preclinical and clinical stroke researchers in Europe, and has generated huge synergies and increased the efficiency of the research enterprise. The inclusion of researchers of related fields (cardiovascular, immunology, vascular biology) has generated tremendous interaction, and was responsible for some of the most relevant and surprising results of our work. By teaming up with the Canadian Stroke Network which led to a number of ongoing translatlantic projects we have demonstrated in a bottom up manner that transcontinental research is feasible and productive. We have also had numerous talks with representatives of the National Institute of Health to partake in this European- North American initiative, but budgetary constraints have so far prevented concrete projects, despite tremendous interest and positive signals. We are currently talking to stroke researchers and research organizations of the Asia - Pacific region, where there is a great openness and interest in joining translational transnational stroke research. Australian groups are already members of the European MULTIPART project (see above). We will continue our quest to bring together the leading researchers worldwide to combine efforts to increase the productivity of our research to tackle the immensely complex issues of preventing stroke, reducing brain damage when it has occurred ('neuroprotection') and to foster regeneration of lost function ('neurorepair'). Hopefully the enthusiasm of the research community will be matched by national and international programs providing the funds for such collaborative work.

Section 2 – Use and dissemination of foreground

2.1 Plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research)

Section A

List of Scientific Publication

For more information please see the ECAS system.

List of Dissemination Activities

For more information please see the ECAS system.

Section B

For more information please see the ECAS system.

Section 3 – Report on societal implications

For more information please see the ECAS system.