

PROJECT FINAL REPORT

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4.1 Final publishable summary report

An executive summary

The overall objective of Multi-PART (Multicentre Preclinical Animal Research Team; www.Multi-PART.org) was to develop the capacity to undertake international multicentre animal studies.

Unfortunately, developing effective therapies to treat neurological disorders has proven challenging. Ischaemic stroke is probably the most studied example of this translational failure. More than 1000 drugs have proven to be effective in animal models of focal cerebral ischaemia but only thrombolysis with tPA has shown to be an effective therapeutic, albeit in a small proportion of patients. A number of explanations have been proposed by the field for such translational failures. Firstly, it has become apparent that many animal studies are confounded by bias that limits their validity. Further, these studies that do not report measures to reduce risks of bias have been shown to overstate treatment effects. There is also evidence from secondary analyses of *in vivo* data of substantial publication bias and selective outcome reporting bias across the modelling of human diseases. Doubts have been cast on the validity and usefulness of the animal models used and substantial differences between animal and human studies have, in part, been faulted. These issues have been exacerbated by the waning enthusiasm of pharmaceutical companies to invest in the search for effective therapies for ischaemic stroke. Our proposal was to address many of the shortcomings in translational stroke research by providing a platform to perform international, centrally coordinated phase-III like preclinical studies. The work was divided into six workpackages (WP):

- i. Project management, training and dissemination
- ii. Scientific coordination
- iii. Experimental design
- iv. Regulation and ethics
- v. Data management
- vi. Statistical analyses.

Our approach was to pair individuals with expertise in each theme with active *in vivo* stroke practitioners to ensure that contemporary solutions were relevant to scientists in the field.

It is neither appropriate nor desirable that every *in vivo* experiment is part of a multicentre programme. We believe that hypothesis-generating and testing experiments can and should remain as single-centre studies. However, we envisage the place of multicentre studies place in the development pipeline as confirming efficacy in robust and intensively monitored experiments with transparent analysis and reporting. Such data will guide whether or not interventions should be taken forward and tested in human clinical trials.

In WP1 our outputs included defining the practicalities of organising multicentre animal studies, a model consortium agreement, a financial costing model, defining the requirements for Multi-PART study sites. In WP2 we established a framework for the scientific coordination of potential multicentre studies, including a mechanism for initiating and approving studies, with a process for pre-trial knowledge exchange on therapy, an agreement and definition of a core set of rodent models, a template for designing the structure of a study protocol and defined the structure and remit of a Quality and Data monitoring committee. In WP3 we present strategies to maximise the internal and external validity of multicentre animal studies. In WP4 we define the ethical and regulatory environments around the conduct of multicentre *in vivo* studies, this included identifying the relevant regulatory authorities and approval processes across countries of consortium members and compiling a common application that was presented to an expert working group of the EC to develop guidance and principles for project evaluation and severity assessment of research using animals. In WP5 we have developed a web based data management system for multicentre studies. In WP6 we curated a dataset of previously performed experiments from consortium members to test and rank statistical analysis approaches for multicentre *in vivo* studies to guide statistical analysis of future studies.

We developed this platform and refined our solutions via a series of teleconferences and face-to-face meetings. Presentations and further details can be found via our website: www.multi-part.org.

A summary description of project context and objectives

The overall objective of Multi-PART (Multicentre Preclinical Animal Research Team; www.Multi-PART.org) was to develop the capacity to undertake international multicentre animal studies to improve the validity, and generalisability of current preclinical research to improve the prospects of success for translation of efficacy to human clinical trials. We proposed a paradigm shift to perform experimental studies with the same rigour, and driven by the same central coordination, as has proven successful in multicentre phase-III randomised controlled clinical trials in humans (Bath et al. 2009a; Dirnagl and Fisher 2012).

Developing new drug treatments for human disease is challenging, and the number of new drugs coming to market continues to fall. This is particularly true for diseases of the central nervous system such as dementia, epilepsy, depression and stroke. Although large numbers of novel treatment strategies for these diseases are being developed in laboratories each year and are shown beneficial in animal models, very few are ultimately proven effective in patients (van der Worp et al. 2010). Reasons for this translational failure include limited internal validity (e.g. inadequate sample size, lack of blinding) and limited external validity (poor generalisability) of many animal studies. The failure to translate drug efficacy in stroke from animal studies to clinical trials (Sena et al. 2007) is probably the best studied example, but the problem is widespread (Perel et al. 2007; van der Worp et al. 2010). Translational research is approaching a crisis, and the enthusiasm within pharmaceutical companies for neuroscience research in general and stroke research in particular continues to wane.

We therefore proposed multicentre animal studies as a means of confirming efficacy in robust and intensively monitored experiments with transparent analysis and reporting – *using stroke as a worked example*. Further, the deliberate introduction of systematised heterogeneity might be used to test the range of situations in which efficacy is seen (Richter et al. 2010; Richter et al. 2009), thereby increasing the generalisability of findings. Thus, multicentre animal studies would provide an explicit confirmation of the circumstances of *in vivo* efficacy to inform the design of clinical trials.

Multi-PART established a platform with the potential to transform preclinical animal research, similar to the tremendous improvements in clinical research that occurred through the introduction of multicentre clinical trials. Through a “worked example” of animal modelling of ischaemic stroke, our consortium sought to define the elements of a successful multicentre animal trial and describe the tools (technical, regulatory, organisational) that allow such studies to be conducted, either by Multi-PART or by other consortia. This will inform the design and conduct of adequately powered multicentre animal studies with improved internal and external validity, not just in stroke but also for other disease models. The purpose here was not to perform multicentre studies but deliver the platform to undertake them.

There were six work packages (WP) covering (1) project management, training and dissemination, (2) scientific coordination, (3) experimental design, (4) regulation and ethics, (5) data management, and (6) statistical analysis. Each work package was jointly led by an individual with expertise in the theme and an *in vivo* practitioner to ensure the practicality of solutions developed. All consortium members contributed to each theme, with involvement of external experts as required.

WP1: Project management, training and dissemination was led by van der Worp and Howells and considered the practical aspects of organising multicentre studies. The objectives of WP1 were:

1. Coordination of the Multi-PART programme: Sena oversaw the progress of the project on a day-to-day basis. She participated in teleconferences with the leaders of the other work packages every month, as well as attending the initial start-up meeting, face-to-face meetings and the final consolidation meeting.
2. To define the requirements for study sites: We used a Delphi technique to ascertain the opinions of international experts in the use of animal stroke models of the most important characteristics of study sites when establishing a multicentre trial.
3. To establish a framework for recruiting and approving new sites: We developed a framework to identify candidate study sites and to assess their potential for collaboration.

4. To develop training materials to support the accession of new sites: Training materials were developed to train investigators in the standard Multi-PART procedures by WP2.
5. To develop a framework for financial management of multicentre studies: We sought to develop a costing model for future multicentre animal studies. This was to address multiple scenarios, including requests for work from members of Multi-PART who develop novel treatment strategies, external academics who lack the resources to assess their new drugs and require our expertise, and SMEs and large pharmaceutical companies where fees will be expected to generate either funds or Intellectual property (IP) sharing to support long-term growth.
6. To develop a framework to attribute intellectual property arising from multicentre studies: IP management will be a critical factor in the success of future multicentre animal studies testing individual treatment strategies. Supported by the legal department at UMC, our aim was to develop a model “multicentre animal study IP strategy” which took into account the appropriate attribution of (i) background IP; (ii) foreground IP (generated through multicentre studies) and side-ground IP (developed by consortium members through other activities). The document was also to address possible strategies for sharing IP with commercial organisations that engage with academia in the assessment of their drug or strategy. Such strategies might include their paying a higher fee for service, or paying the standard fee and sharing the resulting IP. The IP strategy will be an integral part of the model Project Consortium Agreement for future multicentre animal studies.
7. To develop a data dissemination strategy: We sought to develop a data dissemination strategy and an “Authorship Strategy” for Multi-PART publications was also to be developed that will adhere to requirements for authorship established by the International Committee of Medical Journal editors. Equity of authorship commensurate with intellectual input will always be ensured.
8. To establish a model Consortium agreement: Supported by the legal department of UMC, we sought to develop a Consortium Agreement. The Consortium Agreement was to be based on relevant European regulations and on the Grant Agreement with the European Commission.

WP2: Scientific coordination was led by Dirnagl and Macrae to establish a framework which allows consortium members to agree on the mechanism for initiating and approving studies, the disease models used, agreement around a standard set of inclusion and exclusion criteria; agreement around outcome measures; the development of standard operating procedures (SOPs); provisions for monitoring standards of laboratory practice and compliance with the protocol and quality control; and responsibilities and operating procedures for a study data monitoring committee. The objectives of WP2 were:

1. To establish a core set of rodent stroke models and prepare a detailed profile on each (SOPs for induction of stroke).
2. To establish a standard operating procedure (SOP) for designing the structure of a study protocol. Our strategy was that a draft template will be designed by the work package leads which will be used to facilitate preparation of study protocols (& SOPs) for preclinical drug testing. The template will be circulated by email to consortium members with request for feedback and will be followed up at two weekly intervals by a series of tele/video conference and one face-to-face meeting with consortium members to refine and reach a consensus on the study protocol template for inclusion in the preclinical drug testing portfolio.
3. To establish a system for monitoring standards of laboratory practice and compliance with the protocol and quality control. This was to include a proposal for the structure, membership, and remit of a “Quality and Standards Committee” to oversee training and ensure individual centres within the network deliver to the same high quality standards.

4. To establish the structure, remit, membership and powers for a study “Data monitoring committee”. This was to include a proposal of the structure, responsibilities and operating procedures for this committee.
5. To establish the structure, membership, remit and powers for a preclinical trial Steering Committee and the mechanism for initiating and approving preclinical stroke studies.
6. To establish a process for information and knowledge exchange relating to each therapy to be investigated. We sought to establish a mechanism for disseminating information on a new therapy to be tested by the consortium (e.g. investigational new drug (IND) document posted on a secure website with email sent to participants to access all the available information available on the proposed therapy).

WP3: Experimental design was led by Vivien and Würbel to develop strategies to maximise the internal and external validity of studies. Our objectives for WP3 were:

1. To establish common protocols of randomisation.
2. To seek to establish restricted randomisation strategies – blocking, stratification, and factorial designs: Small study populations, highly heterogeneous study populations, or study designs involving several replicates (batches of animals) may require restricted randomisation strategies to achieve balanced study designs. Restricted randomisation strategies are also needed if certain key characteristics (e.g. prognostic variables, baseline covariates) are likely to interact with the experimental treatment. Therefore, we will specify strategies for restricted randomisation (such as blocked randomisation and stratified randomisation) that take these aspects into account. We will also specify factorial experimental designs by which effects of key characteristics may be assessed.
3. To define blinding – treatment and outcome assessment: The practicality of blinding partly depends on the experimental design. Therefore, requirements for appropriate blinding during the experiment, as well as for the blinded assessment of outcome were to be specified, and common protocols for blinding developed.
4. To inform sample size calculations: Sample size calculation requires adequate power analysis. While this is relatively straightforward with simple experimental designs, it may become quite complex with more complex experimental designs or statistical models.
5. To explore systematic variation and external validity: we sought to explore potential strategies for exploiting existing differences between study sites, as well as strategies for deliberate, systematic variation both within and between study sites in view of increasing the external validity of results.

WP4: Regulation & Ethics was led by Allan and Percie du Sert to engage with national and institutional regulators and EU institutions to explore the implications of there being different regulatory environments and jurisdictions. Specifically, WP4 explored whether a single or common application for regulatory approval might be developed; the views of regulators to giving approval to an experiment which is conducted in part outside their jurisdiction; and whether there is scope for multicentre animal studies to have a single sponsor within the EU with a single ethical application. This involved consultation with relevant stakeholders. Our objectives for WP4 were:

1. To identify relevant regulatory authorities across countries responsible for implementing Directive 2010/63/EU and the regulatory approvals required to carry out preclinical research.
2. To examine existing ethical approval processes across participating countries.
3. If appropriate, to establish ethical review process for Multi-PART studies by defining the structure, remit and membership of an Ethical Review Committee (ERC) for multicentre animal studies, the organisation of the ethical review process, and the feasibility for a multicentre animal ethical review committee to be recognised by relevant regulatory authorities, which would potentially waive the need for local/institutional approval.

4. To review the core set of rodent models defined by WP2, models will need to be accepted in each member country from an ethical and regulatory perspective. Replacement, refinement and reduction (3Rs) opportunities were also to be considered to ensure the highest standards.
5. To explore the potential to establish a single point of contact and approval for preclinical studies

WP5: Data management was led by Macleod and Planas to establish specifications for a distributed data management system to allow site management and approvals; a randomisation service; central data management; uploading of outcome and sending to blinded outcome assessors for scoring; central statistical monitoring; provision of data for interim and final analysis; providing reports of activity and of missing data; and provisions for external data sharing. Our objectives for WP5 were:

1. To outline the specification of web based trial management system, including each component of the electronic trial management system.
2. To develop a web based pilot data management system: It will include core functions of centre management; randomisation; details of experimental animals; and uploading and adjudication of outcome data. To maximize feasibility this will be tested in the laboratory situation, with scientists interacting with the web based system during experiments; and the system will be changed in the light of that experience.
3. To test the outcome adjudication system with real data: the outcome adjudication system using real data previously collected by participating laboratories. These will include images of stained tissue sections, radiological images, and digital video clips of animals.

WP6: Statistical analyses was led by Montaner and Bath to develop statistical approaches for (i) sample size calculations for the various outcomes used; (ii) blocked randomisation; (iii) interim analyses for efficacy or futility; (iv) primary and secondary outcome analyses; (v) possibility of adjusting outcomes for observed baseline differences; and (vi) approaches for central statistical monitoring to ensure compliance with the study protocol. Our objectives for WP6 were:

1. To establish data sets for statistical development to test the statistical approaches.
2. To define our primary outcome: The most appropriate primary outcome measure for *in vivo* stroke studies is not fixed, and may differ according to the type of intervention. However, we will assess functional outcome (death or impairment) as the primary endpoint of preclinical studies since it most closely reflects the primary outcome in acute stroke trials (death or dependency, accepting that outcome will be measured days-weeks after stroke in animals and is usually measured at 3 months in humans).
3. To assess the impact of adjusting the primary analysis for baseline covariates.
4. To compare the performance of different possible secondary outcome measures. Again, these analyses will be performed with and without covariate adjustment.
5. To ascertain the influence of considering death in assessment of outcome.
6. To define a procedure for the interim analyses of a prespecified primary outcome (be that functional outcome, infarct volume or brain atrophy or death), to be conducted at different stages as data become available.
7. To develop sample size calculations for the primary outcomes assuming significance (α) 0.05 and a range of powers (1-beta) from 0.6 to 0.9.
8. To develop procedures for the central statistical modelling of site performance and multi-rater assessment of structural and functional outcomes:
9. To create a Statistical Analysis guide of all the procedures and approaches described in tasks above to guide statistical analysis for multicentre preclinical studies.

A description of the main S&T results/foregrounds

WP1: Project management, training, and dissemination

1. Define the requirements for study sites;

Delphi survey of participating site requirements: The opinions of international experts on the most important characteristics of study sites for a multi-centre trial were established via a Delphi technique using 2 rounds of questionnaires sent to established leaders in the field followed by 2 rounds of open discussion at the Multi-PART Barcelona and Utrecht meetings to reach consensus about desirable characteristics. 85 world experts were invited to participate with response rates of 58% and 42% in Delphi rounds 1 & 2 respectively. The following were agreed upon as desirable characteristics for study sites and/or principle investigators (PI):

1. ≥ 3 years of expertise in stroke modelling,
2. ≥ 3 original articles reporting animal stroke studies in the previous 5 years,
3. adherence to ethics regulations, assessed by a review of statements thereof in articles from in the previous 5 years,
4. willingness to be trained in study methodology,
5. ≥ 2 FTE scientific staff,
6. ≥ 1 FTE technician,
7. ≥ 25 stroke inductions in previous 2 years by each participating individual,
8. ≥ 100 stroke inductions in previous 2 years at study site,
9. able to assess: body temperature during surgery & follow-up; blood gases; blood pressure,
10. undergo training in behavioural testing, without exam but with central assessment of quality of testing,
11. PI ≥ 3 years of experience in stroke modelling,
12. PI ≥ 3 articles reporting original stroke research in animals in previous 5 years,

3. Establish a framework for recruiting and approving new sites

After completion of the Delphi procedure a PubMed search was then performed to provide a systematic and unbiased approach to identify research groups likely to have the expertise to join the Multi-PART consortium to model ischemic stroke. To provide a margin for error the search extended over the last 10 years with total publication number required increased to 6 over this period. The search identified that there were 4981 individual first authors and 3751 individual last authors in the 7891 record data set. 233 last authors (judged to be senior authors and thus PI's) had ≥ 6 articles reporting original stroke research in animals in the last 10 years. The most recent addresses for all of these authors have been retained for potential invitation to join Multi-PART.

4. Development of training materials to support the accession of new sites

Training materials have been developed by WP2 to train investigators in the following standard Multi-PART procedures - (a) aseptic technique and general anaesthesia during stroke surgery; (b) induction of stroke in rodents (5 models: 3 in mouse, 2 in rat); (c) Animal welfare measures pre- and post-stroke; (d) monitoring cerebral blood flow to confirm successful stroke induction; (e) setting pre-defined study design inclusion & exclusion criteria; (f) Alzet minipump implantation for drug delivery; (g) brain tissue sectioning, staining and assessment of infarct size; (h) behavioural tests and neurological scoring to assess functional outcomes in

stroke models. These materials are ready for upload to the Multi-PART website at the start of a multi-centre animal study. Training materials will be updated on a continuous basis, for instance in the light of problems identified by individual researchers or in case of new technical developments.

5. Develop a framework for financial management of multicentre studies.

Principle of Operation: Multi-PART is a not-for-profit research consortium. The purpose of Multi-PART is to assess candidate drugs for efficacy before the commencement of clinical trials. To ensure provision of appropriately powered experiments in a timely fashion, workload will be distributed across multinational laboratories subject to centralised monitoring and quality control by the consortium. Efficiency will be ensured by provision of centralised randomisation and blinding schedules and raw data collection for distributed primary outcome analysis using a web-based data engine. Using this data, The Multi-PART scientific Advisory Committee will provide advice to investigators on clinical trial go/no-go decisions.

Funding Sources: Multi-PART is not a contract research organisation (CRO). Its purpose is to advance the research interests of the consortium. As such it is expected that core funding will come from public and philanthropic granting bodies. The competitive advantage for the consortium will be the ability to provide world-leading expertise to address the specific scientific requirements of each research project. Nevertheless, the pharmaceutical industry comprises part of the research environment within which Multi-PART operates. The primary mode of engagement with the pharmaceutical industry will be formation of a multi-PART-PHARMA precompetitive sub-consortium. The level of investment can vary depending on the size of the Pharma (e.g. large multinational vs start-up). Pharma investors will be entitled to propose candidate drugs for testing to the value of 50% of their cash investment. The remaining 50% will contribute to the multi-PART core funding and fund internal research for which precompetitive consortia members will have a "right of first refusal" on ongoing IP and development. Testing of compounds proposed by Multi-PART-PHARMA precompetitive sub-consortia members will be subject to the same prioritisation and experimental planning schedule and application of the same go/no-go criteria as for academically proposed drug candidates. The Multi-PART-PHARMA subcommittee will be entitled to one voting member on the multi-PART Scientific Advisory Committee (SAC). Each Multi-PART-PHARMA pre-competitive sub-consortia member will be required to commit to a 5-year agreement payable annually in advance. The Multi-PART SAC may also allow Pharma who are not members of the pre-competitive consortia one-off access to the Multi-PART research infrastructure for a mutually agreed programme of research at cost + 100%. The Multi-PART SAC will be expected to consider team capacity, reputation and willingness of >70 % of team members to engage with that entity. The entity must agree (as will be the case for pre-competitive consortia members) to full public disclosure of the results. Standard go/no-go criteria will be applied at each stage gate. Academic researchers who lack the expertise to be consortium members can engage with the Multi-PART SAC to develop grant proposals to jointly fund their research. The SAC will accept brief 4 page outlines of proposals for consideration as collaborative research projects.

The SAC will meet quarterly (electronically by default, in person when group attendance at scientific conferences permits) to consider research proposals from consortium members and external sources.

Research Prioritization: It is expected that Multi-PART will receive more research proposals than the consortium has capacity to process. Therefore a system of prioritization will be required. For the ongoing reputation of Multi-PART it is essential that this process be evidence-based rather than dependent on force of personality. This prioritization process is expected to be able to adapt to disease-specific circumstances and to evolve as Multi-PART grows and develops.

A stroke-specific starting point is provided below for illustrative purposes. The preclinical dataset for tissue plasminogen activator (tPA) will be used as a bench-mark. This choice is made because this drug is the only one currently to work in both animals and man. Moreover, the limits of efficacy are similar in both species; the dataset is large enough to illustrate historical trends in data interpretation; is large enough that a statistically stable effect size has been reached and to allow the impact of biases to be assessed and accounted for. The starting point for either a new chemical entity or existing "candidate" drug will be a systematic review of the existing data whether that be published or in-house data to establish what is known

about the molecule. In the case of IP-protected or "commercial-in-confidence" information, this will include full disclosure of unpublished data held by the proponent of the molecule. The purpose of this review will be to establish what relevant data is known and what the gaps in this knowledge are. Where the data volume is sufficient, meta-analysis will be performed. The purpose of this analysis will be to determine firstly whether the range of biological activities reported in the available data are consistent with a biologically plausible therapeutic activity for the disease proposed. For example for all disease types, is there evidence of a clear dose-response relationship? For diseases such as stroke, traumatic brain injury or spinal cord injury with both acute and chronic opportunities for intervention, is the time course of effect realistically matched to the human disease target? For example for an acute stroke neuroprotectant, is there a clear window of time to no effect that corresponds with disease evolution? The second aim of this analysis will be to determine whether the available data is consistent with a biased or an unbiased evaluation of the merits of the compound. This analysis will look explicitly for and estimate the impact of failure to reduce the risk of biases, such as the failure to randomise, conceal allocation, report attrition or blind the assessment of outcome. The size of the difference between effect sizes of biased and unbiased cohorts of data will be compared with examples of positive and negative controls for successful and failed translation for that field. For example, for acute stroke therapies these could be tPA and NXY-059 respectively. Where possible the impact of "small sample size" bias and publication bias will also be assessed. The third aim of this quantitative review will be to rank the point estimate of effect size against known translational successes for the field. The purpose will be to balance magnitude of effect with the confidence that this effect is real while also balancing effect size with the proportion of a patient population who might benefit from eventual therapy. For example, a molecule with a small, precisely known and unbiased effect size which might help every member of a disease population would be favoured over a molecule with a large but was imprecisely determined effect size, contaminated by bias and with a target specific to only a small proportion of patients.

Different drug candidates can enter the research pipeline at different points: Depending on quality and breadth of evidence gathered, a decision will be made to determine where a candidate drug enters the Multi-PART research pipeline. A schema for the potential point of entry for candidate stroke drugs is provided in; Howells, D. W., et al. (2010). "Different strokes for different folks: the rich diversity of animal models of focal cerebral ischemia." *J Cereb Blood Flow Metab* 30(8): 1412-1431. For all drugs, and all diseases, the starting point (except when one of the Multi-PART consortium members has already independently performed this experiment) will be a simple dose response experiment designed to confirm a biological effect rather than estimate the likelihood of clinical utility. This is to ensure that Multi-PART does not proceed with molecules without true activity. Subsequent experiment will be designed depending on the limits of the pre-existing data.

Costing of experiments: For each disease domain, Multi-PART will establish the full cost recovery position of common experimental models and experimental designs. This has now been completed for a range of stroke modelling scenarios and a costing model established in MS Excel. To cover the administrative costs required to maintain centralized ethics applications, centralized randomisation, data accumulation, data analysis and reporting, a surcharge of 20% of the direct experimental costs will be applied to all experiments. For research conducted with commercial funding, the above charges will apply plus a 100% surcharge to offset the reduced capacity for internally instigated projects and reputational risks. Non-salary overheads, utilities costs and PI time will not be charged for internal Multi-PART collaborations but will be added where granting bodies would normally pay these costs when such funds are sought by the Multi-PART collaborators. These additional costs will automatically be applied where the research is to be paid for by commercial rather than governmental or charitable funders. Where commercial organisations are willing to join with Multi-PART in a pre-competitive consortia, those organisations will receive a 50% discount on the 100% surcharge in recognition of their long-term commitment to Multi-PART, with this maximum discount given for a 5 year commitment and a proportionally smaller discounts for shorter durations of commitment down to 3 years.

Distribution of funds: Governmental and charitable funds will be made available to the Multi-PART consortium in accordance with standard granting practices. Funds from pre-competitive consortia members

will be paid to Multi-PART annually in advance with the ability to withdraw from the agreement with 6 months' notice. Other commercial collaborators will pay 6 monthly in advance with the ability to give 3 months' notice of withdrawal from the agreement. Funds held by Multi-PART will be paid to investigators quarterly in advance but with the provision that payment can be withheld if agreed deliverables have not been reached. If agreed deliverables are not achieved for 2 consecutive quarters, planned workload will be redistributed to other consortia members where possible and the team rubes on-going Multi-PART involvement reviewed by the SAC.

6. Development of a framework to attribute intellectual property arising from multicentre studies

Supported by the legal department at UMC, a model "multicentre animal study IP strategy" has been developed which takes into account the appropriate attribution of (i) background IP; (ii) foreground IP (generated through multicentre studies) and side-ground IP (developed by consortium members through other activities). This strategy has been included in the 'Multi-PART Project Consortium Agreement (Deliverable 1.1). The document also addresses possible strategies for sharing IP with commercial organisations that engage with academia in the assessment of their drug or strategy (Clause 8.5).

7. Development of a data dissemination strategy

- A data dissemination strategy has been developed based on at least the following preambles. Multi-PART will not enter into agreements that prevent the academic reporting of results.
- Bodies who seek Multi-PART's assistance in drug or therapeutic strategy evaluation will be required to develop a "Study Protocol" with Multi-PART which will be published online on the Multi-PART website. For the protection of IP, specific details of the treatment under study may be masked. Subsequent study results will be published in full in a peer-reviewed journal.
- Decisions on authorship will be based on the requirements established by the International Committee of Medical Journal editors. Equity of authorship commensurate with intellectual input will always be ensured.
- An individual-animal dataset will be deposited in public repository within 18 months after the last follow-up of an animal in the study.

With the assistance of Professor Terrance O'Brien and Professor Michele Simonato representing The International League Against Epilepsy (ILAE) and the American Epilepsy Society (AES) Working Groups for Preclinical Epilepsy Therapy Development, a memorandum of understanding was established and executed as a pilot to facilitate interaction with researchers working in other disease models. Work with this working group has now progressed to training of a cohort of epilepsy researchers in the processes of systematic review and meta-analysis. This will provide an unbiased assessment of the epilepsy preclinical dataset and establish a working group for the epilepsy arm of Multi-PART.

8. Establishment of a Consortium agreement

Supported by the legal department of UMC, a model Project Consortium Agreement (Deliverable 1.1) has been developed which defines a range of processes and rights, including but not limited to confidentiality, publication rights, intellectual property rights, access rights, and liability and indemnification. The model Project Consortium Agreement is governed by and construed in accordance with the laws of the Netherlands, but this may be changed where appropriate. This agreement sets the framework to be applied to future multicentre programmes that will assess individual drugs or other treatment strategies in animal models through a collaboration of Multi-PART members and additional key players such as other academic institutes, SMEs or pharmaceutical companies. This model Project Consortium Agreement include chapters on IP management mentioned under 7 above.

WP2: Scientific coordination

This WP led by Dirnagl and Macrae has established the framework for consortium members to reach agreement on the following:

- 1) the mechanism for initiating and approving multi-centre pre-clinical (animal-based) stroke studies.
- 2) the disease models and the outcome measures to be used, a standard set of inclusion and exclusion criteria and the development of standard operating procedures (SOPs).
- 3) provisions for monitoring standards of laboratory practice and compliance with the protocol and quality control;
- 4) responsibilities and operating procedures for a study data monitoring committee.

This framework has been built in consultation with the other work packages and is described in detail in the following deliverables

D2.1 Agreement around and definition of a core set of animal models.

Collaborating with WP5, a survey was developed, and data collected to capture key information from the consortium members on the expertise and range of animal stroke models available within the network. This was presented at the second face-to-face meeting where participants agreed on a core set of 5 rodent stroke models (3 in mouse, 2 in rat). Since there is expertise & capacity to build in additional models available within the consortia (including models with comorbidities, and non-human primate models) the core set will expand as Multi-PART develops.

A template, to generate the standard operating procedure (SOP) for each stroke model was developed. A consortium member with the required expertise was then identified to draft the SOP for each of the 5 core model. WP2 leads circulated these draft SOPs round consortium members, collected feedback and produced refined SOPs with regional variations agreed for each stroke model to facilitate maximum participation. A knowledge exchange workshop was organised in one of the consortia sites (Barcelona), practical techniques and skills were shared, and final refinements to the SOPs made which were then approved by all consortium members. These have now been uploaded on the Multi-PART website and will be shared via open access publication to raise the standards of pre-clinical stroke research throughout the EU and beyond.

WP2 leads also expanded this deliverable to generate SOPs for the procedures which are required to complete a pre-clinical stroke study design:-

1. Aseptic technique and anaesthesia
2. Maximising animal welfare pre- and post-stroke
3. Cerebral blood flow (CBF) monitoring to confirm successful stroke induction using laser Doppler flowmetry (rat & mouse)
3. Inclusion & exclusion criteria
4. Alzet mini-pump implantation for drug delivery
5. Outcome measures – TTC staining in rodent brain for infarct assessment
 - Measurement of infarct volume
 - Extended neuroscore (mouse)
 - Inverted grid test (mouse)

These have also been circulated around the consortia for comment and approval and are now in final form. All SOPs will be held within a **study design portfolio** with protocols for randomisation, blinding, data

collection, analysis etc. prepared within other WPs. There is significant expertise amongst the group, in additional sensorimotor outcome measures, behavioural assessment for successful stroke induction, MRI imaging to confirm successful stroke induction, infarct volume assessment etc. in rats and mice. SOPs for these are being developed and refined using the same process as Multi-PART develops.

WP2 have also worked closely with WP4, Drs K Ryder (external advisor board) of the UK Home Office and D Anderson (Expert Working Group of the EC), to prepare a proposal to the EU for an animal licence template which would allow multi-site approval for pre-clinical stroke trials across Europe. This has involved careful definition of humane end-points in consultation with the UK NC3Rs (<https://www.nc3rs.org.uk/>) and in alignment with the EC Directive 2010/63/EU on protection of animals used for scientific purposes – “Caring for animals, aiming for better science”.

D2.2: Template for designing the structure of a study protocol approved.

The template design has been discussed and refined in consultation with the consortium members at the face-to-face meetings. This will comprise:

- 1. Overall study protocol document:** with details of models, outcome measures, drug/therapy treatment plan, sample size calculations for planned experiments, detailed plan of statistical analysis and any defined GO/NOGO points as data are being generated.
- 2. Folder containing the appropriate SOPs,** selected from **the study design portfolio** (rodent stroke models, experimental protocols, and outcome measures from WP2, Randomisation and Blinding protocols from WP3 and statistical analysis protocols from WP6). These will be the most appropriate for the therapy under test and will be selected by the Steering Committee who would also assign specific sites within the consortium to conduct the research.
- 3. A pre-clinical investigator’s brochure (IB)** on the drug or therapy being considered for a multi-centre trial. This will contain all the available background data and publications on the therapy under consideration: chemistry, pharmacokinetics, pharmacodynamics, safety, toxicology, in vitro and in vivo pre-clinical stroke data. All multi-PART sites will be sent this document (in confidence) and asked to feedback comments to the steering committee on whether there is sufficient evidence of efficacy to proceed with a multi-centre trial. The steering committee will use the IB and comments from multi-PART members to decide if sufficient positive data exists to approve translation to a multi-site pre-clinical trial. If they are not convinced by the available data, they will advise funders of the positive data that would be required to move the therapy to multi-site investigation status. Single site studies could be offered within the Multi-PART framework to provide missing data if the committee think the therapy has potential to translate to the clinic. If no meta-analyses and/or systematic reviews are available, the committee could also advise on whether these should be commissioned. Multi-PART members have significant expertise in this field and could prepare these if funding was available.
- 4. Site-specific study protocol document:** Each site involved in a multi-site pre-clinical trial will be sent a document (with a reference ID) detailing the study(s) to be undertaken, SOPs to use with details of species, strain, sex & age, stroke model(s), drug/therapy treatment plan, group sizes, randomisation & blinding procedures, outcome measures, reporting instructions and (agreed) deadline for delivery of results.
- 5. Site-specific costs documentation:** The Centre will calculate the costs for each multi-PART site’s studies from the costing database (prepared by WP1) and send a costing agreement document (with the same reference id) for site PI signature prior to study commencement. This will detail the agreed funding, number of experiments, data sets to be generated & deadline for completion & uploading of data.

6. Site-specific results summary: Each multi-PART site will be sent a template report form and instructions for a short narrative report of any unexpected findings, problems etc. to be uploaded on the central database along with the data.

D2.5: Steering Committee (SC) established along with the mechanism for initiating and approving preclinical stroke studies.

1. Structure of SC:

The steering committee will comprise 5-6 members and include at least one clinician, external members independent of the investigators, their employing organisations, funders or sponsors. It can include investigators and a representation of the sponsor. The chair should not be involved in the trial.

2. Remit: To decide if there are sufficient positive data to authorise a multi-site study, select participating laboratories, monitor trial progress and conduct and advise on scientific credibility. Decide on amendments to the protocol, consider and act, as appropriate, upon the recommendations of the Data Monitoring & Quality Standards Committee and ultimately carry the responsibility for deciding whether a trial proceeds, and/or needs to be stopped on grounds of animal welfare or efficacy. Be responsible for designing the study, maintaining the quality of study conduct, ethical review, ongoing monitoring and writing study reports and publications.

3. Powers: The SC can delay (until sufficient positive data are available), halt or stop a study altogether (animal welfare, futility). The SC can exclude centers that violate protocol or are not adhering to guidelines.

D2.3&4 Data Monitoring & Quality Standards Committee (DM&QSC) established

Deliverable 2.3 & 2.4 have been combined to streamline the committee structures within the Multi-PART platform. A DM&QSC will be established for each multi-site pre-clinical stroke trial undertaken by Multi-PART

1. Structure of DM&QCC: Members of this committee will be selected and appointed by the steering committee, paying attention to any potential conflict of interest. It will comprise 5 members with experience in animal stroke studies who are not directly involved in the trial, an external representative, a clinician, a vet/animal welfare representative and a statistician. The committee must operate under a written charter that includes well-defined SOPs.

2. Remit: Animal welfare, 3Rs, responsible for monitoring for excessive mortality with the power to halt the trial early.

3. Monitoring: DM&QSC members will perform site monitoring to assure high quality trial conduct. For example, "on site" monitoring of individual experiments, assess adherence to protocols, ensure ongoing implementation of appropriate data entry and quality control procedures, and in general assess adherence to good laboratory practices. These monitors will remain blinded to study arm assignment.

4. Powers: The DM&QSC in consultation with the SC, can delay, halt, or stop a study altogether (animal welfare, futility) and exclude centres that violate protocols or are not adhering to guidelines.

D2.6 Process for pre-trial information and knowledge exchange on therapy to be established.

1. All available data on the candidate drug/therapy (chemistry, in vitro and in vivo data including all PK, PD, safety & toxicology data) collected & loaded onto the multi-PART site in the form of an IB.

2. Follow up of corresponding authors to ask for any additional data (e.g. negative or neutral data) which could be made available

3. Include search of Dryad and Figshare databases for any additional data.
4. In vivo studies will be assigned a quality score to facilitate assessment of available data.
5. There will be an option to commission a meta-analysis / systematic review [Multi-PART publication]
6. Multi-PART members will be send the IB and invited to communicate any comments to the Steering Committee charged with making the final decision on whether or not to proceed with a multi-centre trial.
7. The Steering Committee will prepare a report providing the evidence on which the decision was based (which would also be used within applications for funding).
8. Funding for personnel to search & collect data, prepare IB, reviews and reports will be included in multi-site study costings as described by WP1.

MULTI-PART WORKSHOP: Research on Cerebral Ischemia Models

The workshop was held in Barcelona on 20th-23rd April 2015 and organized by IIBB-CSIC-IDIBAPS and the VHIR (Vall d'Hebron Research Institute), both partners of the Multi-PART Consortium.

The Objective was to bring together researchers performing experimental stroke procedures in rodents to discuss a list of stroke model SOPs written by the consortium members, exchange experiences, methodologies and difficulties when performing surgeries. The discussion sessions were combined with three experimental sessions at the surgery rooms of both Research Institutes.

A total of 20 participants (from 9 Multi-PART Centers) including technicians, laboratory managers, pre-/post-doctoral researchers and principal investigators attended the workshop.

Two scientific sessions were organised to discuss the following: Session 1- rodent stroke models (middle cerebral artery occlusion, MCAO) and SOP documents: 1. Distal MCAO in mice using diathermy (electrocoagulation), 2. Distal MCAO in mice using direct compression of the distal MCA, 3. Intraluminal filament MCAO in mice, 4. Distal MCAO in rats using diathermy, 5. Embolic stroke in rat and 6. Intraluminal filament MCAO in rat. Session 2- discussion on stroke outcome measures used by the different centres and other protocols required for a multi-site study: 1. Inclusion and Exclusion Criteria for animal inclusion in a pre-clinical stroke trial, 2. Pre-and Post-Surgical Care of rodents undergoing experimental stroke, 3. Neuroscore assessment of stroke severity, 4. Inversed grid test/grip test, 5. TTC stain for infarct assessment, 6. Mini Pump implantation for drug delivery.

The first experimental session at VHIR Animal Facilities included a demonstration of the permanent and transient distal MCAO in mouse, the performance of the grip test plus a TTC stain of brain slices and the surgical preparation of brain capping (for laser Doppler flowmetry) followed by the embolic MCAO in rat. The second experimental session at IDIBAPS-CSIC Animal Facilities consisted of a demonstration of the intraluminal filament MCAO in rat, the permanent distal MCAO in mouse and the intraluminal filament MCAO model in mouse.

After three days of fruitful discussions on best practice and experimental sessions a series of SOP documents were edited and modified offering alternative methods and describing the pros and cons of different modifications of the models.

WP3: Experimental Design

Objectives

The purpose of WP3 was to develop potential strategies to maximise both the internal and external validity of multicentre preclinical studies performed within Multi-PART or other multi-centre research consortia.

With respect to the internal validity of preclinical multi-centre studies, we first conducted a survey of *in vivo* stroke scientists with the aim to ascertain current practice, including measures to reduce the risk of bias, to inform the central coordination of multi-centre animal studies. Based on the results of this survey, we explored strategies to minimise bias, focusing on (i) centralised randomisation, (ii) blinding of experimental conduct and outcome assessment (including off-site assessment of structural or functional outcomes), and (iii) a priori sample size calculations.

With respect to the external validity of preclinical multi-centre studies, we explored ways to exploit differences between study sites to maximise the external validity (generalisability) of findings. In particular, we reviewed the existing literature for (iv) evidence on the external validity of single-centre studies compared to multi-centre studies (taking into account the evidence for publication bias). Furthermore, we explored the literature as well as available data from previous preclinical multi-centre studies to assess (v) within-study variation relative to between-study variation as a basis for estimating optimal trade-offs between the number of centres to be included in a multi-centre study, and the number of samples to be obtained from each centre.

Internal validity – avoiding bias

Survey on risk of bias among *in vivo* stroke scientists

We conducted an online survey of *in vivo* ischaemic stroke scientists identified through membership of the Multi-PART consortium or those that have expressed interest in the consortium. We asked respondents to state whether they randomly allocate animals to treatment groups and if applicable, the method used to perform randomisation. We also asked respondents if they blinded the conduct of surgery, animal handling, and assessment of infarct volume and behavioural outcome. Separately, we assessed the reporting of randomisation, allocation concealment and blinded assessment of outcome in *in vivo* stroke studies identified in systematic reviews, published after 2010 and curated in the CAMARADES database (www.camarades.info).

We invited 59 laboratories to participate in the survey of which 32 (54%) responded; 23 were European-based laboratories and nine from the rest of the world. Of the 32 participating laboratories, 29 (91%) stated that they allocated animals to treatment groups in a random manner. However, 6 of these ‘picked animals randomly from the cage’ or used ‘alternate allocation’, not considered true randomisation. Most (25) respondents induced focal ischaemia blinded to treatment allocation, and of these 90% maintained blinding for the duration of the experiment. All respondents blinded the assessment of infarct volume and behavioural outcomes. Of 80 experiments in the CAMARADES database published after 2010, 23% reported allocation concealment, 36% reported randomisation, and 44% reported blinded assessment of outcome.

We identified that self-reporting of measures to reduce the risk of bias in leading stroke laboratories was substantially higher than in published reports of stroke studies. We purport that either (i) those responding to our survey may not be typical of the *in vivo* stroke scientist population or (ii) published reports do not adequately reflect experimental conduct and may actually underestimate the measures taken by researchers to reduce the risk of bias. In addition, our survey raised concerns of different interpretations of what is meant by randomisation. Protocols for multi-centre animal studies must therefore take care to ensure that all aspects of experimental design are clearly defined and where appropriate provide training in experimental design.

Centralised randomisation and restricted randomisation strategies (Tasks 3.1 and 3.2)

Randomisation is one of the most critical determinants of the internal validity of experimental results. It ensures that each subject has an equal probability of being assigned to any treatment group so that groups do not vary in a systematic way other than treatment (Suresh, 2011). However, as our survey revealed, adequate randomisation strategies are not even the rule among all *in vivo* stroke researchers – one of the scientific communities most sensitised to the risks of bias and translational failure in preclinical research.

Adequate randomisation protocols for multi-centre studies are well established and readily available from platforms developed for the design and conduct of randomized clinical multi-centre trials (e.g. CONSORT, sealed envelope). There are only some general recommendations that can be made since in most cases, randomisation strategies need to be tailored to the specific needs and characteristics of individual studies. However, centralised treatment allocation and distance randomisation (via www, phone, text message, etc. instead of envelopes) is generally recommended to avoid bias most effectively.

Furthermore, because simple randomisation is likely to result in imbalance when trial size is smaller than 2000 subjects, more refined randomisation strategies (blocking, stratification, and factorial designs) are generally recommended for preclinical multi-centre studies. Sample size, heterogeneity of study populations, and inclusion of prognostic variables, baseline covariates and stratification variables are the main determinants of the randomisation strategy. In most cases, blocked randomisation with random block sizes (Efird, 2011) or minimization (especially, in small trials with multiple stratification variables) provide the best solutions. These can be easily implemented and managed centrally.

Blinding – treatment and outcome assessment (Task 3.3)

Together with randomisation, blinding represents one of the most important precautions against bias (performance bias and detection bias) in experimental results. Blinding here refers to “keeping the persons who perform the experiment, collect data, and assess outcome unaware of the treatment allocation” (van der Worp et al. 2010).

The practicality of blinding partly depends on the experimental design and the specific needs and characteristics of individual studies. Therefore, only some general recommendations can be made for adequate blinding during experimental conduct and outcome assessment.

Since knowledge of treatment condition may affect experimental conduct subconsciously, blinding should ideally extend to all persons interacting with the animals or being involved with outcome assessment, including the personnel responsible for animal husbandry. Unblinded experimental conduct or outcome assessment can lead to exaggerated estimates of effect sizes, as shown for clinical studies (e.g., Hróbjartsson & Boutron, 2011; Hróbjartsson et al., 2012) as well as preclinical animal studies (e.g. Bello et al., 2014; van Luijk et al., 2014). Blinding is particularly important when outcome measures involve some subjectivity, e.g. rating scales (Boutron et al. 2006). For preclinical multi-centre studies, central blinded drug supply and off-site blinded outcome assessment are strongly recommended, while blinded data analysis is less important if a data analysis plan has been specified before study onset.

However, blinding may not be possible in all cases, e.g. when surgeons perform surgery vs. sham surgery or when treatment groups differ visually (e.g., strains differing in fur coats). The blinding of people involved in an experiment is not a *sine qua non* for the internal validity of a study (Schulz et al. 2002). In accordance with the CONSORT statement for clinical trials, preclinical trialists are recommended not to use terms such as ‘single blind’, ‘double blind’ or ‘triple blind’, but rather to explicitly report the blinding status of all persons involved in a study (Moher et al. 2010), “how they achieved blinding”, and if possible even “whether they tested the successfulness of blinding” (Karanicolas et al. 2010). This includes animal caretakers, experimenters, data collectors, outcome assessors, data analysts, and other involved personnel whose knowledge of the treatment assignment might bias the experiment.

Sample size calculations (Task 3.4)

Sample size calculation requires adequate power analysis. While this is relatively straightforward with simple experimental designs, it may become quite complex with more complex experimental designs or statistical models. However, software for sample size calculations with adjustments to experimental design are readily available (e.g. PASS (NCSS), G*Power).

In the simplest form, the statistical design includes treatment as a fixed factor and centre as a random factor. If between-centre variation is unknown, the proportion of variance explained by centre (intraclass correlation coefficient, ICC) may be estimated. The clinical trial literature suggests using an ICC of 0.1 (assuming that 10% of total variance is explained by between-centre variation; Vierron and Giraudeau 2009).

Given the higher level of within-experiment standardisation in preclinical animal studies compared to clinical studies, the proportion of variance explained by between-centre variation would be expected to be higher. However, current evidence based on meta-analyses of preclinical animal studies also indicates an ICC of about 0.1 (unpublished results), which may thus be used if no preliminary data are available. However, further multi-centre studies are needed in view of better estimates of within-centre vs. between-centre variation in results.

External validity – avoiding spurious results

Systematic variation and external validity (Task 3.5)

It is increasingly acknowledged that small scale single-centre studies are prone to produce spurious results. One important reason for this is that small studies often lack the necessary power to detect true effects, resulting in a large proportion of false negative effects. Perhaps even more importantly, however, low power also reduces the likelihood that a statistically significant result reflects a true effect, and even if a true effect is detected, the effect size is likely to be inflated (Button et al. 2013).

The problems of underpowered studies for estimating true effect sizes are exacerbated by the likely presence, but uncertain extent, of publication bias. Although there are a number of ways to test and/or statistically compensate for publication bias (e.g. Egger's test, trim and fill, test for excess of significant findings, p -uniform), these rest on specific assumptions and are often themselves prone to a lack of power and consequently false negative results or distorted effect sizes (Nuijten et al. 2015). These authors therefore conclude that "until there are ways to eliminate publication bias or correct for overestimation because of publication bias, researchers are wise to only incorporate and perform studies with high power".

The other important reason for spurious results is that single-centre studies are prone to produce study-specific or centre-specific results that do not generalise to replicate studies in the same or other centres, let alone translate to clinical trials (Crabbe et al. 1999, Richter et al. 2009, 2011). This problem is further exacerbated by rigorous standardisation of study conditions. Thus, laboratory animal science textbooks advise experimenters to standardise the conditions of their experiments as much as possible by using genetically homogenous animals (e.g. inbred lines, F1-hybrids) and carefully equating husbandry and test conditions for all animals (Beynen et al. 2003). The aim of such standardisation is to minimise variation in the data to increase test sensitivity, assuming that this will also "improv[ing] comparability of results within and between laboratories" (Beynen et al. 2003). However, Würbel (2000, 2002) and Richter et al. (2009, 2010, 2011) have shown that standardisation reduces the external validity of the results and this compromises reproducibility across replicate studies in the same or different centres.

Taken together, both sufficient statistical power and sufficient heterogeneity in study populations are crucial for obtaining externally valid and reproducible results, thus avoiding spurious results. The best way to guarantee reproducibility across different centres is to incorporate the variation between centres in the study design by designing them as multi-centre studies. Alternatively, and in particular for exploratory or proof-of-concept studies, heterogeneity of study populations may be achieved within single-centre studies by systematically varying (i.e. by heterogenising instead of standardising) either genotype (e.g. by using multiple strains) or environmental conditions (e.g. by using multiple housing conditions) as part of the experimental design (cf. Richter et al. 2010). However, further studies (both computational and experimental) are needed to identify ways in which between-centre variation can be modelled within single-centre studies by systematic heterogenisation of conditions, before it may guarantee reproducible results (Richter et al. 2011).

Concerning the trade-off between the number of centres to be included in multi-centre studies, and the number of independent samples per centre, there are no strict rules. In general, external validity increases with the number of centres, and external validity is maximized by maximising the number of centres while minimising the number of samples per centre. Multiple samples per centre are only needed for assessing within-centre vs. between-centre variance. However, a relatively small number of animals per centre may be sufficient to obtain a good estimate of within-centre variability (i.e. individual variability between samples), unless within-centre variability is expected to vary greatly between centres and one wants to obtain

estimates of within-centre variability for each centre, in which case the best solution may be to equate the number of centres and the number of samples per centre (i.e. to choose the square-root of the total sample for both). However, because for reasons of feasibility a small number of centres, and unequal samples per centre, may be desirable, further studies (both computational and experimental) are needed to find optimal solutions to these trade-offs.

WP4: Ethics and Regulation

Overview

The primary aim of WP4 was to identify current practice in participant's countries and to liaise with relevant local, national and international bodies to establish whether single level ethical and/or regulatory approval is attainable for preclinical multicentre studies.

To achieve this aim a number of tasks and deliverables were defined (as above) and the results/findings in relation to these are reported below. It should be noted that a WP4 task was identified as one of only two major risks associated with the whole Multi-PART project, in that engagement with national regulatory bodies in an attempt to establish a single ethical approval system for multicentre animal studies operating within the EU may not be forthcoming. However, through EU directive 2010/63/EU we did manage to have engagement of member states, as described in more detail below.

Results - Ethical review process between countries

We conducted a survey within the Multi-PART consortium to examine the ethical review and regulatory approval processes across eight participating centres from the following countries: Spain, Germany, France, UK and Australia (see Table).

All centres needed both regulatory and ethical approval to conduct in vivo stroke studies in rodents and most centres (6/8) required separate ethical and regulatory approvals. The ethical approval is provided first, at a local level, for example by the institutional AWERB (Animal and Welfare Ethical Review Board) in the UK or CEEA (Ethical Committee of Animal Experimentation) in Spain. Then once ethical approval is obtained, regulatory approval is sought at a regional (Spain) or national (France, UK) government level. In two centres, regulatory and ethical approvals were combined and provided at a regional level (e.g. State Government of Berlin for Germany) or institution level (e.g. Australia).

Between centres, the length of application varied from 10-15 pages detailing a particular study to close to 100 pages describing a five-year programme of work. The length of time taken to obtain full approval for a new application varied from 2 months up to 1 year.

As a follow up to the survey we explored further what common information was required across participating centres and where differences existed in the level of detail required. As a starting point we used the UK Project Licence template. This revealed for all participating centres, bar the UK, that detailed experimental protocols were required with approval predominantly on a study-by-study basis. As a result much of the information required for the UK project licence form was not applicable to other centres and vice versa in that information considered essential for approval in non-UK member states was not specified. These findings helped in the subsequent development of a common application (see below).

Question	Barcelona (CSIC)	Barcelona (Vall d'Hebron)	Berlin	Caen	Glasgow	Manchester	Melbourne	Nottingham
1. Do you need regulatory approval for conducting stroke studies?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Do you need ethical approval for conducting stroke studies?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Do you obtain approval for your stroke studies from the same body?	No	No	Yes	No	No	No	Yes	No
4. If yes, who gives this approval:	n/a	n/a	State Government of Berlin	n/a	n/a	n/a	Austin Health Animal EC	n/a
5. If no, who gives ethical approval?	Ethical Committee of the University of Barcelona (CEEA)	Ethical Committee of the University of Barcelona (CEEA)	n/a	local authority (council)	Local Animal and Welfare Ethical Review Board (AWERB)	AWERB	n/a	AWERB
6. If no, who gives regulatory approval?	local Government (Generalitat de Catalunya) (DAAM number)		n/a	national level (ministère de l'agriculture)	UK Home Office	UK Home Office	n/a	UK Home Office
7. On average, how many pages does an application for conducting a stroke study contain, and how long does it take to put an application together (in total if several applications needed)?	~10 pages. 2-3 months.	12-15 pages.	45-50 pages, 1-2 weeks	Not defined	Current licence 91 pages. Valid for 5 years. Amendments~1 page.	Current licence 49 pages. ~2 months.	Ethics applications 40-60 pages.	A new application might take couple of months to write.
8. On average, how long does it take to obtain full approval for a stroke study from the time of submission?	Up to 6 months	1-2 months	4-6 months	~2 months	Amendments <2 month.	Amendments <2 months. Full application up to 1 year.	6-8 weeks.	For an amendment 1-2 months. For a full licence – up to 1 year.
9. Can you make an application ad hoc or are there annual deadlines?	Ad hoc	Ad hoc - monthly deadline.	Ad hoc	Ad hoc submission of study protocols.	Ad hoc - but AWERB only meets certain times.	Ad hoc - but AWERB only meets certain times.	Ad hoc but EC only meets bimonthly.	Ad hoc

Involvement with regulatory authorities in EU countries

With the introduction of EU directive 2010/63/EU, animal research in each of the EU Member States is regulated under the same legal framework. Every member state has a National Contact Point within each of the Member State authorities responsible for implementing the directive and current efforts are directed towards harmonising the implementation of the directive across countries.

As part of this initiative, the European Commission has convened an expert working group (EWG) composed of individuals nominated by all member states and main stakeholder organisations, to develop guidance and principles for project evaluation and severity assessment of research using animals. In respect of project evaluation the EWG is tasked with developing exemplar applications, that allow the evaluation process to be demonstrated and that would obtain regulatory approval in all member states countries.

Following a meeting with members of the EWG we were invited to submit a common application describing a typical multi-centre study testing the effect of an intervention in ischaemic stroke models in mice. This would subsequently undergo several rounds of evaluation by the EWG, culminating in a project proposal that should contain the information necessary for regulatory and ethical approval in each of the Member States. In compiling the common application we firstly identified the requirements and information necessary for an ethical review application in each of the countries participating in the consortium: Spain, Germany, France, UK, Netherlands, Switzerland and Australia. These details were then combined such that information common to all participants was included in the main body of the application, with details specific to only one or two centres added as footnotes. The latter information largely related to prescriptive details of the experimental protocol e.g. exact anaesthetic used, dose and timings of drug administration etc. The common application then underwent review by the EWG with clarification sought on a number of different points and additional information requested e.g. details of number of participating centres, sample size calculations, severity assessment. A revised version of the common application was then submitted for further review by the EWG with the intention that it will be selected for inclusion in the set of exemplar applications published as part of a guidance document on Project Evaluation by the European Commission.

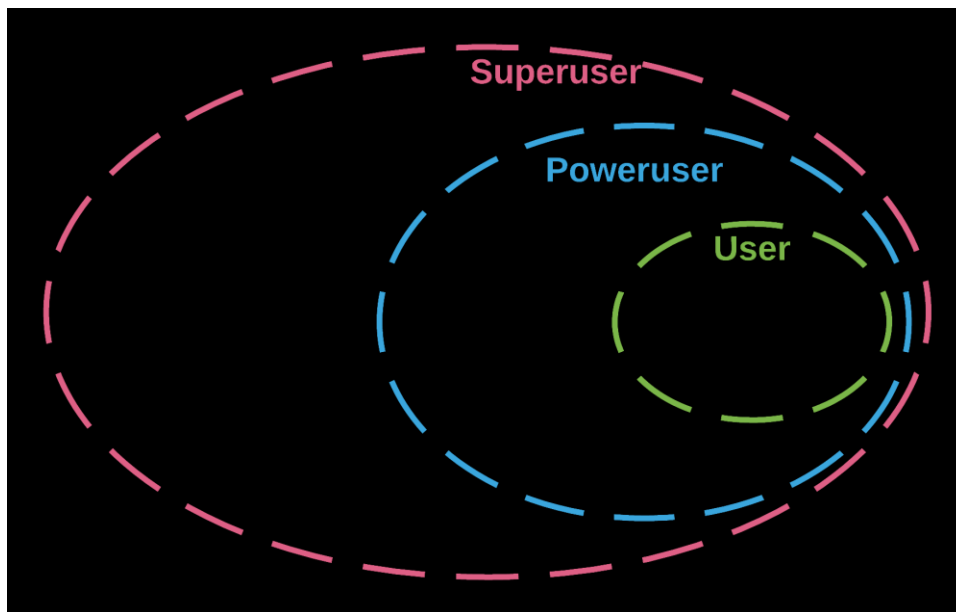
In developing the common application we had the opportunity to discuss and refine various protocols widely used in the stroke research field, including functional assessments of outcome, pre- and post-operative care and humane endpoints. This process included input from participants in a techniques workshop involving researchers from across all participating centres where best practice was agreed upon.

WP5: Data Management

Work package 5 is data management package. Our deliverable is an interactive web application that can be used to store all data associated with a project from project profile and design to animal husbandry and experimental data. It helps regulate the flow of the project design and provides flexibility in project description.

The Multi-PART application is a web based password protected application with a relational database built on SQL server. The application is available to the public. A simplified sitemap of the application is attached below. The functions described on the sitemap are limited to those that can be accessed by external users after added to a recognized research group by current users with higher power.

Anyone with internet access can register with username, password, and email address, and set up an account. They may also input their forename, surname and institute and extra notes to describe themselves to the central administration. We will now refer to an online registered person as a **user** in the later context. Users need to complete a requested research history in user profile page, which links the user to an institution and provides information about the user's research experience. There are different ranking roles for the application users: "administrator", "superuser" and "poweruser".



The Administrator role is only assigned to the administrator of the application, who has access to all features of the application.

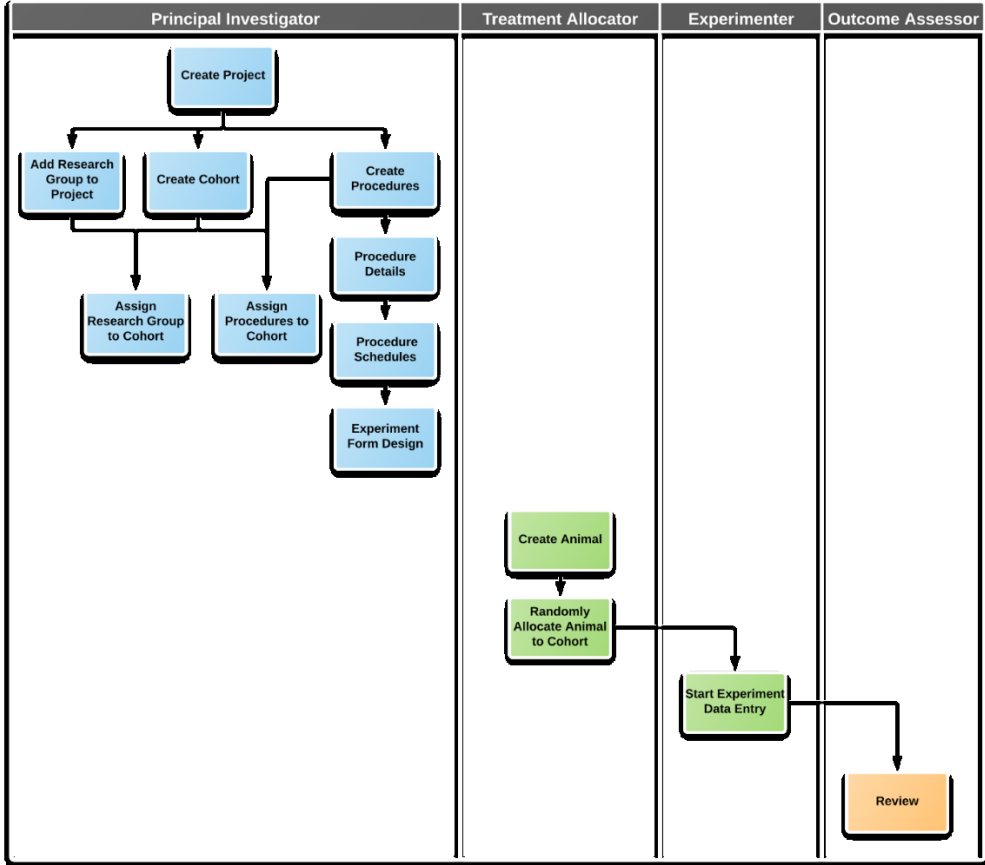
Superusers can access the user list, all users' role assignment pages, all users' profile (viewing and editing) pages. Superusers can also access the lookup table editor page, which allows the superuser to add new lookup table items, or to edit the current lookup table items. Currently, our lookup tables include: species, strain, animal husbandry field, animal husbandry options, procedure detail field, procedure detail options, data entry field, data entry options. Superusers are allowed to view and delete any research group and any project. Superusers can also access all functions that powerusers can. Due to the high power of superuser, the role is only assigned to the coordinators of the Multi-PART project.

Poweruser can be assigned by the superuser of the application. Typically, only research group leader and senior group members will be assigned as poweruser. Poweruser can manage their own research group. Powerusers can introduce institutions, research groups and add other users to the research group. It is worth noting here that powerusers can only add users within the same institution can be added to the research group. Hence, users have to complete the research history section in user profile first, so they can be added - by the poweruser - to the research group.

Only users within a research group can generate projects. Basic project information is required to generate a new project. After generating a project, the user is automatically assigned as principal investigator (PI) role for this particular project while the user's group is assigned as wet lab for this project. Only the PI of a project is able to add more research groups and researchers as participants to the project. A research group can be assigned either a dry lab or a wet lab role. Dry lab refers mostly to the groups that coordinate the project or perform purely remote reviews of the animal data, or only work on statistical analyses. Wet lab, on the other hand, performs experiments and provides data to the system. Animal cohorts in a project can only be assigned to a wet lab.

Only personnel within a research group that has been added to the project, can be assigned the role of user of the project by the PI. Adding a user will require specification of the user's role in the project. User roles includes: principal investigator, treatment allocator, experimenter, remote outcome assessor, and analyst. Different roles of a user in a project allow the user access different functions within the project. This assignment in a particular project has no effects regarding the role of each user in other projects.

- Principal investigator (PI): can design and edit a project, add new research groups and new users to the project.
- Treatment Allocator: can generate an entry for new animals that will be randomly assigned to a cohort. Current randomisation is a simple randomisation engine with a nudge factor that increases the complexity. The code has been made in a way that further more complicated randomisation engine can be easily adapted if is required by the end user. The randomisation engine is shown in annex A below.
- Experimenter: can input experiment recordings and outcome result.
- Outcome Assessor: can assess the behaviour score videos remotely, or remotely assess other parameters (e.g. obtain measures of infarct volume from scanned histological sections or MRI files).



Project PI may edit the project description, and design the project any time after generating the project. Project PI will need to perform the work on the following steps in order to complete the project design:

1. Generating cohorts - Basic information is needed at the time of generating a new project (e.g. animal species, strain ...).
2. Generating procedures - Different experiment procedures can now be introduced. This is the key step for project design. Currently, procedures include model induction, anaesthetics, treatment, analgesia, co-morbidity induction, outcome assessment, and mortality report. Each procedure will have a procedure label, and will specify the types of procedures (developmental, behavioural, pharmacological, observational, surgical, cell injection, transplant). The PI should also complete the following forms, which can be reached after the procedure has been generated.
 - a. complete procedure details -- Different procedure details are required depending on the specific procedures. A new procedure detail field can be added by a superuser through lookup table editor.
 - b. generate the schedule design for the procedure -- The temporal schedule of interventions in each procedure must be specified, assuming that the model induction time corresponds to time zero. All necessary time slot can be generated for each specific procedure depending on the number of required sequential interventions (e.g. inject drug, introduce a diet, etc.).
 - c. design the experiment form -- the project experimenter will use this form at the time of the experiment performance to enter data. This is a standardized form equivalent to the surgery form or post-op assessment form currently used in the lab. The PI may choose specific fields for the form for each procedure, and decide whether any field is mandatory or can have multiple inputs. In the event that additional fields were required than currently provided, the superuser will be able to generate more fields through the lookup table editor.The procedure design is flexible and adaptable. It also provides a structured way to describe the interventions performed by the researchers when carrying out work with experimental animals. To get the system started, the model induction should be defined. Certain parameters, such as co-morbidity, should be recorded as a procedure only when they are experimentally used. However, other parameters, such as mortality report, are mandatory and should always be included.
3. Assign procedures to cohorts - it defines all the procedures that will be performed to each cohort
4. Assign research group to cohorts - it assigns cohort animals to research group (wet lab only)

Users from a wet lab in a project can record animal husbandry for each species used in each particular project by their laboratory. Animal husbandry is recommended to be filled up at the beginning of the project design, although this information can be filled up any time during the course of the project design.

After the design of the project is completed, treatment allocators can start to generate entries for animals by clicking the "experiment" button on the project list page. This will bring the user to the experiment cover page. Here she/he may choose the corresponding model induction and select her research group.

The system will validate the user's role for each project. Only treatment allocators can generate new animal entries and view the animal list with the assigned cohort. When generating animal entries, the treatment allocator needs to input an animal label that is recognised in the lab and then the animal will be randomised to a cohort within induction model. The cohort label needs labels of all procedures assigned to the cohort,

including treatment labels. The treatment allocator can generate a few animal entries in advance and view the list of animal entries in the animal list page. This function allows the labs to either randomize animals live on site or prepare randomisation ahead of the experiment. The PI will generate the procedure label and will have to identify codes to name treatment procedures, e.g. "treatment A" and "treatment B", for blinding purpose. All information, including drug name and drug dose, of the treatments is saved as the procedure details.

After an animal entry is carried out, experimenters can go to the data entry page through the experiment page. Again, only users assigned with an 'experimenter role' in the project can enter data. The data entry cover page has all the animal entries that have been generated, with the procedure assigned to them. By clicking on the procedure name, one can see the schedules of the particular procedure. The experimenter can then access the data entry page for this animal during the performance of this procedure.

The field on the form is designed by the PI in the project design stage. The experimenter is required to fill up all the mandatory fields, and fill up the optional fields if applicable. All fields come with a time property, which is set by default as the current time in that timezone. The time picker on the top of the page is the procedure time. When completed, the experimenter can submit the form. It is important to notice that the form cannot be edited by the experimenter any more once submitted.

For the project with outcome assessment procedures with number of remote assessment greater than 0 and users with outcome assessor role added, the data entry records for those procedures will be sent to outcome assessor for assessment. Checklist is selected during the project design stage as well. Outcome assessor will get an email with the link to the Outcome assessment task every time a task has been generated. They can also access it later with the "Outcome assessment" link on the top menu. All completed tasks cannot be seen any more.

The poweruser role in the application can view the progress of their research group in their project, while superusers can view the progress of all projects.

Annex A

Function Randomization take an array of *Cohort* class and return the id of the chosen cohort. The probability depends on the remaining number of animals in each cohort. A two-step randomization

```
class Cohort
{
    int CohortID;
    int RemainNumberOfAnimals;
    float probability;
}

function Randomization (Cohort[] cohorts)
{
    // new random seed
    var random = new Random();
    // sum up all remain number of animals. If there is no
    var rtotal = cohorts.RemainNumberOfAnimals.Sum();
    if(rtotal == 0) return -1; // return -1 if there is not remaining animal

    var cohortid = 0;
    var min = 0;
    var max = 0;
    var rand1 = random.Next(0,100); //1st random number
    //search through the cohorts finding the location
```



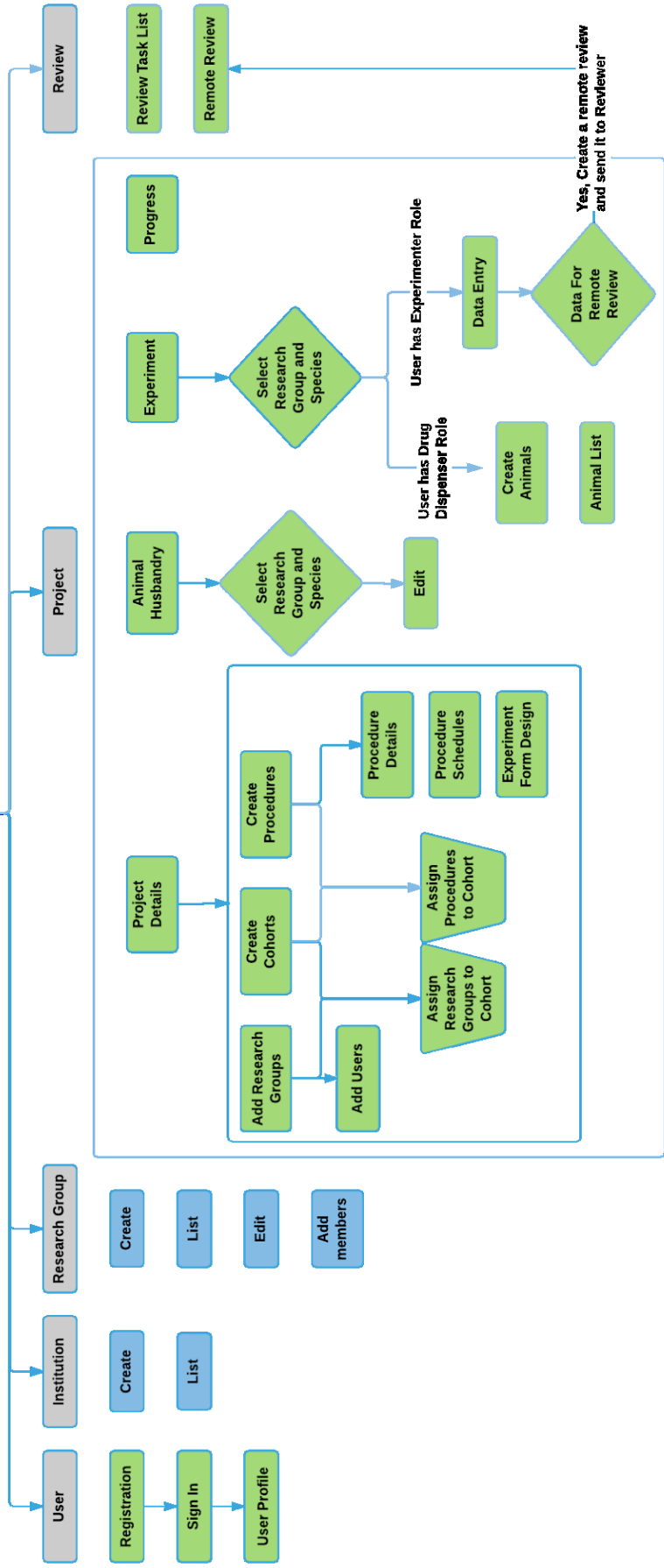
```

        foreach (var cohort in cohorts)
        {
            cohort.probability = Convert.ToInt32((cohort.RemainNumberOfAnimals * 1.0) /
rtotal * 100); // probability times 100.
            max = max + cohort.probability;

            if (rand1 >= min && rand1 < max)
            {
                cohortid = cohort.CohortID;
                break;
            }
            min = max;
        }
// a nudgefator add an extra level of randomisation. This nudgefator of 90% provides a
probability of 10% that the previously chosen cohortid will not be used.
const int nudgefator = 90;
var rand2 = random.Next(0, 100); //2nd random number
var lastid = cohortid;
if (rand2 < nudgefator) { return cohortid; }
else
{
    var newcohorts = cohorts.where(c.CohortID != cohortid);
    rtotal = newcohorts.Select(c => c.RemainNumberOfAnimals).Sum();
    if (rtotal == 0) return lastid;
    var rand3 = random.Next(0, 100); //3rd random number
    min = 0;
    max = 0;
    foreach (var cohort in newcohorts)
    {
        cohort.probability = Convert.ToInt32(cohort.RemainNumberOfAnimals * 1.0
/ rtotal * 100);
        max = max + cohort.probability;
        if (rand3 >= min && rand3 < max) cohortid = cohort.CohortID;
        min = max;
        lastid = cohortid;
    }
    if (cohortid == 0) cohortid = lastid;
}
return cohortid;

```

Multi-PART Web Application



WP6: Statistical Analysis

Survey on statistical practice in pre-clinical studies

A questionnaire was designed using Survey-Monkey with 25 questions. 11 sets of answers were received from MULTI-PART APRTNERS: Charité – Berlin Germany; CSIC- Barcelona, Spain; Florey Institute of Neuroscience and Mental Health – Melbourne Australia; University of Edinburgh – Edinburgh UK; INSERM U919 – Caen France; SANISYS AG – Bienne; University of Bern – Bern Switzerland; University of Glasgow – Glasgow UK; University of Manchester – Manchester UK; and Vall d’Hebron Research Institute – Barcelona Spain.

In a second call another 12 top researchers on cerebral ischemia/Stroke field from Europe and North America responded the survey (names are kept confidential as indicated when inviting those researchers). The invitation to answer the survey was sent whenever a researcher contacted the coordinator interested in the Multi-PART activities.

The 25 questions were classified into four categories: 1) Experimental and Statistical design, 2) Collection of the data, 3) Analysis of the data and 4) Data representation.

The results of the survey are summarized in the following table and presented below where appropriate.

High Consensus (>80% of survey responders)	Medium Consensus (60-80% of survey responders)	Low Consensus (<60% of survey responders)
Pre-definition of the Statistical Analysis before unblinding (yes)	Sample size calculation when designing experiments	Performing Interim analysis to stop/continue
Performing randomizations (yes)	Describing secondary endpoints	Calculate the statistical power of obtained data
Blinding for data acquisition (yes)	Testing all variables for normal/non-normal distribution	Blinding during statistical analysis
Describing the primary endpoint (yes)	Method to assess normality (most K-S)	Adjustment considering co-variates
Level of significance (0.05)	For related measures non-parametric, n=5 is needed to reach significance	Describing normally-distributed data as mean SEM/SD/CI
Performing Post Hoc after ANOVA (differences, most Bonferroni)	Ipsi/contra analysis (Paired)	State the number of animals/measures represented in graphs

Choosing different tests for repeated vs. non-repeated (yes)	Description of non-normally distributed variables as median (IQR)	How to report functional outcome measures
Adjustment for Multiple comparisons (yes, most Bonferroni)	Non-normally distributed data represented in Box-Plots	
	Discard extreme/outlier values	
	Representing individual values in graphs (60.87%)	
32% of Total	40% of Total	28% of Total

Establishment and initial analysis of pooled experimental stroke data

This is described below.

WORK-PACKAGE COMPONENTS

6.1 Establishment of data sets for statistical development

Members of the consortium and guests attending Multi-PART meetings were invited to share individual animal data from experimental stroke studies. Data were received in Excel or other data formats for 54 studies involving 2501 animals (mice 708, rats 1756, marmosets 27). Studies involved 14 interventions: citicoline, endothelial progenitor cells (EPCs), hypothermia, interleukin-1 receptor antagonist (IL-1ra), induced diabetes, nitric oxide donors, NXY-059, progesterone, semicarbazide, simvastatin, recombinant high density lipoprotein (rHDL), and three compounds that were not identified for patent reasons.

6.2 Primary outcome

Lesion size is the outcome most commonly used in animal studies although it is less relevant to human studies than other outcomes such as neurological impairment and functional measures.

Survey

90% of respondents routinely describe their primary endpoint.

Pooling project

The primary outcome was lesion size and analyses were standardised since brain and lesions size varies by species. Animals that died following treatment were assigned a supra-maximal score (mice 150 mm, rats 475 mm, marmosets 650 mm).

Overall, treatment was associated with reduced standardised lesion volume: 0.29 (1.76) vs 0.80 (1.78), mean difference -0.51 (95% confidence intervals -0.36 to -0.67; $p < 0.001$). Treatment with hypothermia, IL-1ra, NXY-059 and semicarbazide were associated individually with reduced lesion size; trends to a reduced lesion size were also present for citicoline, NO donors, and one of the three unnamed compounds.

6.3 Adjustment of primary analysis for baseline covariates

Adjustment for covariates improves statistical power in clinical stroke trials.

Survey

40% of respondents adjusted their analyses for baseline covariates.

Pooling project

Few baseline variables were present in all preclinical studies but adjustment was possible for species, sex, weight (standardised), stroke model and anaesthetic agent. Using the pooled data, co-variate adjustment did not materially alter analyses whether analysed using multiple linear regression or multi-level modelling.

6.4 Secondary outcomes

Most studies collect information on additional outcomes in addition to the primary outcome. These allow testing of internal validity (i.e. is the primary outcome result replicated in other outcomes) and hypothesis generation.

Survey

60% of respondents described results for secondary endpoints or outcomes.

Pooling project

Data for neurological deficit were present in 22 studies (1130 animals); since these involved a number of different scales, data were standardised. Far less data were present for other outcomes and these were not analysed. Pooled treatment was associated with less neurological deficit at 24 hours: 0.06 (1.43) vs 0.48 (1.46), mean difference -0.42 (95% CI -0.25 to -0.59; $p < 0.001$). Once again, covariate adjustment did not materially alter results.

Pooled treatment did not affect rates of death in unadjusted analysis (binary logistic regression): 8.2% vs 9.8%. No treatment effect was seen following covariate adjustment: OR 1.01 (95% CI 0.73-1.41; $p = 0.94$).

6.5 Influence of considering death in assessment of outcome

Clinical outcome scores typically include values for death, e.g. modified Rankin Scale, death=6; health utility status as derived from Euro QoL-5D, death = 0. This approach allows for interventions which might improve functional outcome whilst increasing death ('kill or cure'), as was observed in preclinical studies of progesterone.

When comparing the effect of pooled treatment on standardised lesion volume, the mean difference was similar when including death -0.51, and excluding death -0.45. The same was observed for standardised neurological deficit: mean difference including death -0.42, and excluding death -0.38.

6.6 Interim analyses – harm/futility

Interim analyses are standard in phase III clinical trials and would be appropriate in large preclinical studies. Interim analyses allow for a study to be stopped early for reasons of hazard, or extreme efficacy. Futility due to expectation of a neutral result may also be assessed, as is often done in commercial phase III trials.

Survey

Interim analyses are routinely performed by 50% of respondents, and occasionally by 10%.

Pooling project

The pooling project was not relevant to test the role of interim analysis.

6.7 Sample size calculations

Sample size calculations are readily available for continuous, ordinal and binary statistical analyses. Which analysis to perform depends on the primary outcome. For example, lesion volume might be analysed using a t test (or multiple linear regression of covariate adjustment is to be performed) or Mann-Whitney U test, or following dichotomisation.

Survey

The vast majority of respondents use a significance level of $p < 0.05$ (or 5%) in their statistical analyses. Sample size calculations were performed routinely by 70% of respondents, and occasionally by 20%. 30% of respondents calculate statistical power after a study.

Pooling project

When assessing the pooled animal data, analysis approaches that used all the data (multiple regression, Mann-Whitney U test) were more efficient than those that dichotomised the data (e.g. median test). Sample size calculations (assuming a power of 90% and significance 5%) suggested that similar sized studies would be needed to assess lesion size when analysed using a t test (N=510) or Mann Whitney U test (N=534).

6.8 Central statistical monitoring

Central monitoring of accruing study data is needed to detect (in near real time) extreme data values or 'unusual' data patterns. Value and logic tests were performed in the pooling project to identify and correct data errors prior to the above analyses.

6.9 Protocol for multi-rater assessment of outcomes

This was not possible in the pooling project.

The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results.

The multi-PART consortium is a translational network that supports the innovation of a novel translational research paradigm that is open and actively collaborative. Multi-PART has created a blueprint for and develop the capacity for international multicentre animal studies. Our platform includes frameworks for central randomisation, monitoring of laboratory practice; planned variation of experimental conditions between sites to increase generalisability; data sharing that will give the capacity to deliver large studies in a short timeframe. Data derived from such studies will be more reliable than those obtained from isolated research groups, reducing the need for further animal studies. Because clinical trials will be founded on better evidence the chance of benefit to patients participating in clinical trials will be higher, and their risks lower. Multicentre animal studies offer the real prospect of providing the route for the development of effective treatments for diseases of substantial public health and economic importance to improve the health of European citizens and beyond. We acknowledge that the human related socio-economic impact is unlikely to be realised in the short term, but we do believe that these measures are necessary and require an investment now because of the long-lead times necessarily involved. If we are to realise these benefits in the future, we must start sometime.

Effective and translational brain research could have a major impact on associated health care costs. Developing the capacity to undertake multicentre preclinical studies has the potential to deliver effective agents that translate to patients with ischaemic stroke and will act as a blueprint for other neurological disorders and beyond. We have already initiated a collaboration with AES/ILAES to test the applicability of our platform for use in animal models of epilepsy.

Improving the quality and methodology of preclinical studies is likely to reduce the number of ineffective interventions being taken forward to clinical trial, and thereby improve research efficiency. This will reduce potential harm to trial participants and ultimately benefit patients in Europe and beyond. The economic and social costs of translational failure are substantial. Stroke is the second cause of death in Europe, with over 500,000 deaths each year. Annually, immediate healthcare costs and long-term disability costs in the developed world have been estimated at between €202.4bn and €790.1bn. It currently takes industry investment of around €11bn for each successful stroke drug brought to market, and it has been estimated that the introduction of multicentre animal studies would reduce this by €1.4bn. In human terms the impact of developing an effective intervention has been estimated at around 15 patients saved from death or dependency per 1000 strokes, equivalent to 22,250 persons per year in Europe, and twice the potential benefit of thrombolysis.

Demonstrating neuroprotection or neurorepair in stroke, a prototypical acute CNS disorder, would have substantial implications for the development of treatments in many other acute and chronic neurological and neuropsychiatric diseases. Furthermore, such a trial paradigm could serve as a blueprint to overcome similar translational roadblocks in other disease areas.

This project was driven by the demand to reduce health care costs of ischaemic stroke and the need to transform translational stroke research, currently characterised by a failure to translate the effects from the bench to the bedside. Current therapy for acute ischaemic stroke is extremely limited. Thrombolysis with tPA is the most biologically effective intervention but is not applicable to the majority of patients, and the recanalisation rate in patients who receive thrombolysis is less than 50%. Novel therapies are urgently required.

We paired *in vivo* scientists with specialist methodologists of the theme addressed to co-lead each work package to ensure the tools proposed to enable international animal studies were piloted and validated by the researchers intended to benefit from the framework developed. Further, integration with more junior postdoctoral scientists and the end-users likely to be directly undertaking such research and was supported by hosting bi-annual open meetings to discuss our findings and progress to date.

Participating in multi-PART studies has the potential to increase the standard of research undertaken by participant sites and will widen participation by smaller and less experienced laboratories, with appropriate levels of support and supervision to undertake research with a rigour that would otherwise not be possible. Establishing rigorous experimental designs, procedures and statistical analyses requires resources (intellectual and other) not available in a single country or programme; for this reason, a European consortium is required. Our approach allows smaller laboratories with limited personnel and experience to contribute to high-quality research. Central provision of study platforms (randomisation, data management, outcome adjudication, statistical analysis) will reduce costs for national funding agencies and will allow new centres (and new countries) to join the field in a supported, mentored and monitored way. In this way, Multi-PART will increase high-quality research capacity by reducing entry costs. Further, both members of the consortium, and external partners who submit requests, will have access to high quality datasets generated from international multicentre studies. This approach might be characterised as the national equivalent of pre-competitive partnership, creating tools to bring about health gains for all.

International cooperation, both within and outwith Europe, is a key objective of Multi-PART. The inclusion of an Australian participant has contributed to the innovative potential by demonstrating validity outside of Europe for a topic that is of international interest.

Basic and clinical stroke researchers from North America, Europe, and the Asia-Pacific regions have convened in various workshops and meetings (Barcelona, Spain, May 2011; Potsdam, Germany, May 2012; and Boston, US, February 2013) to provide research priorities to address the reasons for translational failure in stroke research. The overarching take home message was that the development of international stroke research initiatives was required and this would lead to quality standards with cross-validation and reproduction of results prior to decision-making regarding clinical development (Dirnagl et al. 2013). The United States NIH/NINDS also recognised the need to “Establish and implement a platform for international, multi-centre preclinical stroke trials using the repertoire of randomised clinical trial design and the complexities of a multi-centre, multimodal paradigm. Develop a trial design which exploits the potential for randomized stratification to enhance generalizability” to accelerate the translation of stroke research in preclinical animal models into clinical studies of highly promising treatments. Further, they are considering calling for preclinical phase III type trials, potentially in collaboration with the EC, Canada, and Australia. Multi-PART provides the conceptual basis for such international trials, and allows swift action. Additionally, the strong European representation within the Multi-PART consortium places Europe at the forefront of this exciting and important change in research strategy.

Multi-PART defined the regulatory needs and ethical considerations for multicentre preclinical studies. To sustain competitiveness we explored the scope of single or common regulatory approval of multicentre animal studies across Europe with the relevant stakeholders tasked with the implementation of directive 2010/63/EU and are still working with an expert working group (EWG) convened by the EC composed of individuals nominated by all member states and main stakeholder organisations, to develop guidance and principles for project evaluation and severity assessment of research using animals. In respect of project evaluation the EWG is tasked with developing exemplar applications, that allow the evaluation process to be demonstrated and that would obtain regulatory approval in all member states countries. Following a meeting with members of the EWG we were

invited to submit a common application describing a typical multi-centre study testing the effect of an intervention in ischaemic stroke models in mice to guide this process.

Preclinical multicentre trials are likely to have a substantial 3Rs impact, primarily in refinement and reduction. The traditional assumption is that standardisation of experimental conditions and procedures guarantees reproducibility and the external validity of results. However, environmental homogeneity within laboratories together with unavoidable environmental differences between laboratories (e.g. staff, room architecture and noise) has been shown to lead to spurious results with limited external validity (Richter et al. 2009). By incorporating environmental variability in the experimental design, multicentre trials provide more robust findings, and there is now good evidence supporting systematic heterogenisation between animal studies. Improving the validity of data from animal experiments is likely to reduce the requirements for animal use in research; for any given number of animals, a small number of adequately powered and externally valid studies are likely to be more informative than a large number of small underpowered studies.

A potential impact relates to research capacity. *In vivo* research capacity is unevenly distributed through member states, and the costs for new entrants are large. Without training and mentoring programs, and a means to externally validate the quality of research conducted, it is difficult for new entrants to become competitive for either academic funding applications or externally funded contract research work. By providing such training, mentoring and validation, and by providing fully funded research activity, Multi-PART will allow new centres to reach the critical mass required to develop the required expertise to pursue both Multi-PART projects and their own scientific projects, thus increasing the *in vivo* research capacity in their countries.

The deliverables presented in this project provide a framework for multicentre animal studies. These deliverables include policy documents relating specifically to Multi-PART, but also include guidance documents supported by our data management system web application that are applicable to multicentre preclinical studies more generally.

We have successfully raised awareness of Multi-PART through our website, presentations, workshops and publications and propose to continue this dissemination. Our primary target audience are scientific researchers (academic and industrial), funding agencies, regulators and SMEs.

Our presentations have used the following format: describing the problems associated with the current translational paradigm, the impact of bias on animal studies, the differences between animal and human studies, the place of multicentre animal studies in the drug development pipeline, and the impact of such trials. In addition to these convincing arguments related to the purpose of multicentre animal studies we have described the practicalities of how such trials are to be performed and putative costs. A list of these dissemination activities are described in section 4.2.

The deliverables are freely available on the Multi-PART website after receiving login details, which are received via using the contact details of the project coordinator on the website. The protocols and SOPs developed in this project are also be available on the Multi-PART website so that peer-reviewers of articles written using the Multi-PART model can be referred to where desired.

The structure of the work packages was developed to allow specific attention to demonstration, piloting and validation of the proposed procedures within the project. This has been addressed by giving joint leadership the work packages to an individual with expertise of the theme and to an *in vivo* practitioner both supported by their postdoctoral staff. Time has been allocated to allow verification of feasibility of the procedures and testing of prototypes. In addition, the consortium has an extensive network of collaborators, both within and outwith (EUROPAIN, MS-START, National Toxicology Programme/NIH) the field of stroke. Our coverage is clearly already wide and many collaborators have already requested participation in future multicentre trials.

The address of the project public website, if applicable as well as relevant contact details.

www.multi-PART.org

Photos and presentation from our face-to-face meeting can be found:
<http://www.dcn.ed.ac.uk/multipart/meetings.html>



4.2 Use and dissemination of foreground

A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

The plan should consist of:

- Section A

This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers ² (if available)	Is/Will open access ³ provided to this publication?
1	Results of a preclinical randomized controlled multicenter trial (pRCT): Anti-CD49d treatment for acute brain ischemia	Dirnagl	Science Translational Medicine	Vol 7, Issue 229	American Association for the Advancement of Science	USA	2015	299	http://stm.sciencemag.org/content/scitransmed/7/299/299ra121.full.pdf	yes/no
2	A cross-laboratory preclinical study on the effectiveness of interleukin-1 receptor antagonist in stroke.	Allan	Journal of Cerebral Blood Flow & Metabolism	DOI 10.1177/0271678X15606714	Nature Publishing Group		2015		http://jcb.sagepub.com/content/early/2015/09/16/0271678X15606714.full	Yes
3	Refinement of Experimental Design and Conduct in Laboratory Animal Research	Würbel	ILAR Journal	Vol 55, issue 3	Institute for Laboratory Animal Research	USA	2014	383-391	http://ilarjournal.oxfordjournals.org/content/55/3/383.long	Yes
4	A concerted appeal for international cooperation in preclinical stroke research.	Dirnagl	Stroke A Journal of Cerebral Circulation	Vol. 44/Issue 6	Lippincott Williams and Wilkins	USA	2013	1754-1760	http://stroke.ahajournals.org/content/44/6/1754.long	Yes

² A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

³ Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

N°	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Dendritic cells in ischemia-induced inflammation	15/01/2014	Caen, France	Scientific community (higher education, Research)	40	Europe
2	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Status report P06/moderation WP5-WP6	4/02/2014	Amsterdam, the Netherlands)	Scientific community (higher education, Research)	86	Europe
3	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Stroke in the XXI century	12/02/2014	Barcelona, Spain	Civil society	50	Spain
4	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Brain inflammation and immunity in stroke	19/02/2014	Barcelona, Spain	Scientific community (higher education, Research)	100	Spain
5	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"What goes wrong if blood does not reach the brain?	12/03/2014	Barcelona, Spain	Civil society	50	Spain
6	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"The marker of leukocyte activation CD69 plays beneficial functions in stroke.	9/04/2014	Magdeburg, Germany	Scientific community (higher education, Research)	250	Europe
7	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Advances in basic science on stroke: potential translations from experimental setting to clinical trials	22/05/2014	Nice, France	Scientific community (higher education, Research)	250	Europe
8	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Wireless networks between the brain and the immune system	17/03/2015	Berlin, Germany	Scientific community (higher education, Research)	50	Germany
9	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Neutrophils in Stroke	8/04/2015	Edinburgh, UK	Scientific community (higher education, Research)	70	UK
10	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Design and results of the first multicenter trial in experimental stroke research (multi-trial): anti-cd49d treatment in acute brain ischemia	23/04/2015	Glasgow, UK	Scientific community (higher education, Research)	70	UK
11	Oral presentation to a scientific event	CHARITE - UNIVERSITAETSMEDIZIN BERLIN	International, multicenter randomized preclinical trials in translational stroke research: The MULTI-PART experience	11/03/2014	Vancouver	Scientific community (higher education, Research)	100	Canada

12	Oral presentation to a scientific event	CHARITE - UNIVERSITAETSMEDIZIN BERLIN	Design of Multicentre Animal Studies	11/02/2014	San Diego	Scientific community (higher education, Research)	200	International
13	Oral presentation to a scientific event	CHARITE - UNIVERSITAETSMEDIZIN BERLIN	The Moor has done his work the Moor may go: Beyond STAIR	12/02/2015	Nashville	Scientific community (higher education, Research)	200	International
14	Oral presentation to a scientific event	CHARITE - UNIVERSITAETSMEDIZIN BERLIN	Multicentre preclinical animal research team (MULTI-PART): A European Commission funded international effort to to develop the capacity to undertake international multicentre animal studies	16/04/2014	Leipzig	Scientific community (higher education, Research)	150	International
15	Oral presentation to a scientific event	UNIVERSITAET BERN	The forgotten tenets of behavioural neuroscience: power, reliability and reproducibility	6/09/2013	Munich, Germany	Scientific community (higher education, Research)	100	International
16	Oral presentation to a scientific event	UNIVERSITY OF GLASGOW	Multi-PART: An international collaborative approach to overcoming the translational roadblock in neuroprotection and neuroregeneration research	12/02/2015	American Heart Association International Stroke Conference 2015, Nashville Tennessee, USA	Scientific community (higher education, Research)	200	International
17	Oral presentation to a scientific event	UNIVERSITY OF GLASGOW	"Pursuit of Relevant Preclinical Studies of Stroke in Rodent Model: Overview of preclinical models of brain injury	28/06/2015	17th International Symposium on Cerebral Blood Flow, Metabolism and Function Vancouver, Canada	Scientific community (higher education, Research)	100	International
18	Oral presentation to a scientific event	UNIVERSITY OF GLASGOW	Where next for pre-clinical neuroprotection research	2/12/2014	Annual UK Stroke Forum Harrogate UK	Scientific community (higher education, Research)	100	UK
19	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Brain leukocyte infiltration in stroke	21/01/2014	Lund , Sweden	Scientific community (higher education, Research)	100	Sweden
20	Posters	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"IL-4 expression after stroke and alternative microglia/macrophage activation	9/04/2014	Magdeburg, Germany	Scientific community (higher education, Research)	500	Europe
21	Posters	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Response of monocytes to brain ischemia in mice	9/04/2014	Magdeburg, Germany	Scientific community (higher education, Research)	500	Europe

22	Posters	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Il-4 induces an acute pro-inflammatory burst and activates an alternative gene program mediated by the jak1/jak3/stat6 pathway in microglia	9/07/2014	Bilbao, Spain	Scientific community (higher education, Research)	1000	Spain
23	Posters	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Cd163+ macrophages in human ischemic stroke	9/07/2014	Bilbao, Spain	Scientific community (higher education, Research)	1000	Spain
24	Posters	THE UNIVERSITY OF NOTTINGHAM	Multi-PART: A Framework For Multicentre Preclinical Trials	28/06/2015	Vancouver, Canada	Scientific community (higher education, Research)	1000	International
25	Posters	THE UNIVERSITY OF EDINBURGH	Multi-part ² eta-test: testing the feasibility and functionality of multicentre preclinical animal studies	28/06/2015	Vancouver, Canada	Scientific community (higher education, Research)	1000	International
26	Posters	THE UNIVERSITY OF EDINBURGH	Multi-part ² eta-test: testing the feasibility and functionality of multicentre preclinical animal studies	28/06/2015	Vancouver, Canada	Scientific community (higher education, Research)	1000	International
27	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	Cd69 plays a beneficial role in ischemic stroke potentially via the modulation of leukocyte recruitment and secondary microthrombosis	28/06/2015	Vancouver, Canada	Scientific community (higher education, Research)	1000	International
28	Oral presentation to a scientific event	UNIVERSITAET BERN	Refinement of housing conditions and experimental design in lab rodent research	10/10/2013	Guelph, Canada	Scientific community (higher education, Research)	100	Canada
29	Oral presentation to a wider public	UNIVERSITAET BERN	Refinement im Tierversuch	17/09/2013	Basel, Switzerland	Industry	100	Switzerland
30	Oral presentation to a scientific event	UNIVERSITAET BERN	Refinement of rodent research	6/03/2014	Helsinki, Finland	Scientific community (higher education, Research)	100	Finland
31	Oral presentation to a wider public	UNIVERSITAET BERN	"Revisiting Refinement in Care and Use of Laboratory Rodents"	24/06/2015	Varese, Italy	Industry	200	International
32	Oral presentation to a scientific event	UNIVERSITAET BERN	Improving the validity of animal research	6/04/2015	Bern, Switzerland	Scientific community (higher education, Research)	50	Switzerland

33	Oral presentation to a scientific event	UNIVERSITAET BERN	On standardisation and other fallacies in behavioural phenotyping	23/01/2015	Tel Aviv, Israel	Scientific community (higher education, Research)	50	International
34	Oral presentation to a scientific event	FUNDACIO HOSPITAL UNIVERSITARI VALL D'HEBRON - INSTITUT DE RECERCA	New diagnostic and therapeutic strategies for vascular cognitive impairment (VCI): learning from biomarkers and Multi-Part approaches in stroke	10/07/2014	Copenhagen, Denmark	Scientific community (higher education, Research)	100	International
35	Articles published in the popular press	THE UNIVERSITY OF MANCHESTER	A cross-laboratory preclinical study on the effectiveness of interleukin-1 receptor antagonist in stroke	3/08/2015	Journal of Cerebral Blood Flow and Metabolism	Scientific community (higher education, Research)		International
36	Articles published in the popular press	UNIVERSITAET BERN	Results of a preclinical randomized controlled multicenter trial (pRCT): Anti-CD49d treatment for acute brain ischemia	5/08/2015	Science Translational Medicine	Scientific community (higher education, Research)		International
37	Articles published in the popular press	CHARITE - UNIVERSITAETSMEDIZIN BERLIN	A concerted appeal for international cooperation in preclinical stroke research	1/06/2013	American Heart Association	Scientific community (higher education, Research)		International
38	Articles published in the popular press	UNIVERSITAET BERN	Refinement of Experimental Design and Conduct in Laboratory Animal Research	20/12/2014	ILAR Journal	Scientific community (higher education, Research)		International
39	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Inflamatory signalling	22/09/2014	Bressanone, Italy	Scientific community (higher education, Research)	30	International
40	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Immunity in stroke	22/09/2014	Bressanone, Italy	Scientific community (higher education, Research)	30	International
41	Oral presentation to a scientific event	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	"Neuroimaging in stroke	14/07/2015	El Escorial, Madrid, Spain	Scientific community (higher education, Research)	50	Spain
42	Oral presentation to a wider public	UNIVERSITAET BERN	Workshop Science-based Refinement, European Refinement Initiative	19/09/2013	Basel, Switzerland	Policy makers	50	International
43	Organisation of Conference	THE UNIVERSITY OF EDINBURGH	Multi-PART Kick-Off meeting	16/09/2013	Edinburgh	Scientific community (higher education, Research)	40	International

44	Organisation of Conference	THE UNIVERSITY OF EDINBURGH	2nd Multi-PART Meeting	14/04/2014	Berlin	Scientific community (higher education, Research)	40	International
45	Organisation of Conference	THE UNIVERSITY OF EDINBURGH	3rd Multi-PART meeting	2/10/2014	Barcelona	Scientific community (higher education, Research)	42	International
46	Organisation of Conference	THE UNIVERSITY OF EDINBURGH	4th Multi-PART Meeting	12/03/2015	Utrecht	Scientific community (higher education, Research)	42	International
47	Organisation of Conference	THE UNIVERSITY OF EDINBURGH	Final Multi-PART Meeting	28/08/2015	Edinburgh	Scientific community (higher education, Research)	43	International
48	Organisation of Workshops	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	MULTI-PART WORKSHOP: Research on Cerebral Ischemia Models	21/04/2015	Barcelona	Scientific community (higher education, Research)	20	International
49	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	THE CONCEPT OF INTERNATIONAL, MULTICENTER, RANDOMIZED AND CONTROLLED PHASE III PRECLINICAL TRIALS EXPECTED IMPACT ON STROKE RESEARCH	23/05/2013	Brain 2013, Shanghai	Scientific community (higher education, Research)	199	International
50	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	DOES EXPERIMENTAL BIAS CONTRIBUTE TO POOR BENCH TO BEDSIDE TRANSLATION OF NOVEL PAIN THERAPEUTIC AGENTS?	25/05/2013	NeupSig, Toronto	Scientific community (higher education, Research)	300	International
51	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Restoring faith in the research enterprise - a call to action	5/06/2014	ILAR Roundtable, Washington DC	Scientific community (higher education, Research)	50	USA
52	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Designing Animal Studies for Better Human Translation	28/05/2015	Experimental Biology, Boston	Scientific community (higher education, Research)	30	International
53	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	How can we optimise the reproducibility of research using animals?	1/04/2015	UK Academy of Medical Science Reproducibility, London	Scientific community (higher education, Research)	100	UK

54	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Pursuit of Relevant Preclinical Studies of Stroke in Rodent Models Brain Course	27/06/2015	Brain 2015, Vancouver	Scientific community (higher education, Research)	100	International
55	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	"What do we know about the credibility of research in the biomedical sciences?"	3/07/2015	Research Credibility, Southampton, UK	Scientific community (higher education, Research)	50	European
56	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	When do I have enough information to proceed to a clinical trial?	2/09/2015	ECNP Targeted Network, Amsterdam, the Netherlands)	Scientific community (higher education, Research)	25	European
57	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Multicentre Preclinical Animal Research Team	9/09/2015	International League Against Epilepsy Translational Force, Istanbul, Turkey	Scientific community (higher education, Research)	50	European
58	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	When do I have enough information to proceed to a clinical trial?	14/09/2015	Janssen, Beerse, Belgium	Scientific community (higher education, Research)	40	Belgium
59	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Impact of poor experimental design	22/09/2015	Science Europe, Brussels, Belgium	Scientific community (higher education, Research)	40	European
60	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	The Rationale of Preclinical Phase-III Multicentre Trials	11/04/2014	Magdeburg, Germany	Scientific community (higher education, Research)	500	Europe
61	Oral presentation to a scientific event	THE UNIVERSITY OF EDINBURGH	Using evidence-based data aggregation to inform multi-centre preclinical phase III trials	14/11/2014	Washington DC, USA	Scientific community (higher education, Research)	50	International

Section B (Confidential⁴ or public: confidential information to be marked clearly)

Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights ⁵ :	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

⁴ Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

⁵ A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table hereafter:

Type of Exploitable Foreground ⁶	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application ⁷	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	<i>Ex: New superconductive Nb-Ti alloy</i>			<i>MRI equipment</i>	<i>1. Medical 2. Industrial inspection</i>	<i>2008 2010</i>	<i>A materials patent is planned for 2006</i>	<i>Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC</i>

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- Its purpose
- How the foreground might be exploited, when and by whom
- IPR exploitable measures taken or intended
- Further research necessary, if any
- Potential/expected impact (quantify where possible)

¹⁹ A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

⁷ A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.3 Report on societal implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

A General Information <i>(completed automatically when Grant Agreement number is entered).</i>	
Grant Agreement Number:	603043
Title of Project:	Multicentre Preclinical Animal Research Team
Name and Title of Coordinator:	Dr Emily Sena
B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? <ul style="list-style-type: none"> If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? <p>Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 'Work Progress and Achievements'</p>	0Yes 0No
2. Please indicate whether your project involved any of the following issues (tick box) :	YES
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	No
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	No
• Were those animals transgenic small laboratory animals?	No
• Were those animals transgenic farm animals?	No
• Were those animals cloned farm animals?	No

• Were those animals non-human primates?	No
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	No
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	No
DUAL USE	
• Research having direct military use	0 Yes 0 No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	0
Work package leaders	3	9
Experienced researchers (i.e. PhD holders)	10	7
PhD Students	7	2
Other	6	0

4. How many additional researchers (in companies and universities) were recruited specifically for this project?

Of which, indicate the number of men:	1 (0 men)
---------------------------------------	-----------

D Gender Aspects

5. Did you carry out specific Gender Equality Actions under the project? Yes No

6. Which of the following actions did you carry out and how effective were they?

	Not at all effective	Very effective
<input type="checkbox"/> Design and implement an equal opportunity policy	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="checkbox"/> Set targets to achieve a gender balance in the workforce	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="checkbox"/> Organise conferences and workshops on gender	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="checkbox"/> Actions to improve work-life balance	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/>
<input type="radio"/> Other: <input type="text"/>		

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

Yes- please specify

No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

Yes- please specify

No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

Yes- please specify

No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

Main discipline⁸:

Associated discipline⁸: | Associated discipline⁸:

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14) Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

No

Yes- in determining what research should be performed

Yes - in implementing the research

Yes, in communicating /disseminating / using the results of the project

⁸ Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	<input type="radio"/> <input checked="" type="radio"/>	Yes No
12. Did you engage with government / public bodies or policy makers (including international organisations)		
<input checked="" type="radio"/> No <input type="radio"/> Yes- in framing the research agenda <input type="radio"/> Yes - in implementing the research agenda <input type="radio"/> Yes, in communicating /disseminating / using the results of the project		
13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers? <input checked="" type="radio"/> Yes – as a primary objective (please indicate areas below- multiple answers possible) <input type="radio"/> Yes – as a secondary objective (please indicate areas below - multiple answer possible) <input type="radio"/> No		
13b If Yes, in which fields?		
Agriculture Audiovisual and Media Budget Competition Consumers Culture Customs Development Economic and Monetary Affairs Education, Training, Youth Employment and Social Affairs	Energy Enlargement Enterprise Environment External Relations External Trade Fisheries and Maritime Affairs Food Safety Foreign and Security Policy Fraud Humanitarian aid	Human rights Information Society Institutional affairs Internal Market Justice, freedom and security Public Health Regional Policy <u>Research and Innovation</u> Space Taxation Transport

13c If Yes, at which level?

- Local / regional levels
- National level
- European level
- International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?

4

To how many of these is open access⁹ provided?

How many of these are published in open access journals?

3

How many of these are published in open repositories?

To how many of these is open access not provided?

Please check all applicable reasons for not providing open access:

- publisher's licensing agreement would not permit publishing in a repository
- no suitable repository available
- no suitable open access journal available
- no funds available to publish in an open access journal
- lack of time and resources
- lack of information on open access
- other¹⁰:

15. How many new patent applications ('priority filings') have been made?
("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).

Trademark

Registered design

Other

17. How many spin-off companies were created / are planned as a direct result of the project?

Indicate the approximate number of additional jobs in these companies:

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

- | | |
|--|--|
| <input type="checkbox"/> Increase in employment, or | <input type="checkbox"/> In small & medium-sized enterprises |
| <input type="checkbox"/> Safeguard employment, or | <input type="checkbox"/> In large companies |
| <input type="checkbox"/> Decrease in employment, | <input type="checkbox"/> None of the above / not relevant to the project |
| <input checked="" type="checkbox"/> Difficult to estimate / not possible to quantify | |

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:

Indicate figure:

Difficult to estimate / not possible to quantify

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

- Yes No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

- Yes No

22. Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

- | | |
|---|--|
| <input checked="" type="checkbox"/> Press Release | <input type="checkbox"/> Coverage in specialist press |
| <input checked="" type="checkbox"/> Media briefing | <input type="checkbox"/> Coverage in general (non-specialist) press |
| <input type="checkbox"/> TV coverage / report | <input checked="" type="checkbox"/> Coverage in national press |
| <input checked="" type="checkbox"/> Radio coverage / report | <input type="checkbox"/> Coverage in international press |
| <input type="checkbox"/> Brochures /posters / flyers | <input checked="" type="checkbox"/> Website for the general public / internet |
| <input type="checkbox"/> DVD /Film /Multimedia | <input type="checkbox"/> Event targeting general public (festival, conference, exhibition, science café) |

23. In which languages are the information products for the general public produced?

- | | |
|--|---|
| <input type="checkbox"/> Language of the coordinator | <input checked="" type="checkbox"/> English |
| <input type="checkbox"/> Other language(s) | |

Question F-10: Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

- 1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
- 1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
- 1.3 Chemical sciences (chemistry, other allied subjects)
- 1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
- 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2. ENGINEERING AND TECHNOLOGY

- 2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)
- 2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]
- 2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised

⁹ Open Access is defined as free of charge access for anyone via Internet.

¹⁰ For instance: classification for security project.

technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

- 3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immuno-haematology, clinical chemistry, clinical microbiology, pathology)
- 3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)
- 3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

- 4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)
- 4.2 Veterinary medicine

5. SOCIAL SCIENCES

- 5.1 Psychology
- 5.2 Economics
- 5.3 Educational sciences (education and training and other allied subjects)
- 5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary, methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

- 6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)
- 6.2 Languages and literature (ancient and modern)
- 6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

2. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1.UEDIN	
2.NSRI	
3.UNIMAN	
4.CHARITE	
5.UMC Utrecht	
6.CSIC	
7.INSERM	
8.BERN	
9.UGLA	
10.VHIR	
11.UNOTT	
Total	