Joint Programming Neurodegenerative Disease:

European research projects for the optimisation of biomarkers and harmonisation of their use between clinical centers

Acronym: SOPHIA

Title of the transnational collaborative project:
Sampling and biomarker **OP**timization and **H**armonization **I**n **A**LS and other motor neuron diseases

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1. Short progress summary of the collaboration, major achievements

SOPHIA aims to develop optimally informative biomarkers for ALS and establish stakeholder agreement regarding their use. This will be achieved by defining, validating and harmonizing optimal methodologies that can be reliably implemented within a pan-European framework program.

The provision of a common European strategy for the prioritization and selection of candidate biomarker domains for optimization and harmonization will in turn provide a long term platform by which existing collaborative structures that are relevant to neurodegenerative disease biomarkers (including academic initiatives, co-funding strategies, biobanks, industrial efforts, private-public alliances) are integrated within an inclusive web-based virtual biobank. The established platform will act as an important communication channel between this consortium and the broader international ALS/Neurodegenerative Diseases field, to ensure that the optimization efforts are consistently applied.

The work to be done for SOPHIA is subdivided in work packages (WPs). WP1-3 relate to the optimization and harmonization of data management (WP1), sampling and molecular biomarkers (WP2) and imaging and neurophysiologic biomarkers (WP3a – Imaging Biomarkers; WP3b - Neurophysiological Biomarkers). WP4 is dedicated to the two-way communication with the entire ALS/ND field, to ensure that all input is acquired for efficient and effective optimization, and that all results are effectively disseminated for use within the wider neurodegenerative diseases field. WP5 entails project management and clinical coordination.

In general the SOPHIA project is well underway and each WPs is on track with its tasks and deliverables.

1.1 WP1 Data & Sampling Infrastructure

The web-based data repository for SOPHIA (Progeny database) has been set up, a core clinical dataset has been defined and data templates have been created for collection of the core clinical data, imaging biomarkers data (MRI and MUNIX) and neuropathology data. SOPs for collection and storing of biological material including serum, urine, CSF, DNA, RNA, fibroblasts, DNA purification, brain dissection, core clinical data collection and ECAS have been generated and are available on the SOPHIA website.

1.2 WP1 Neuropathology

An optimized protocol for the collection and storage of CNS and other tissues has been developed and a template for collection of the neuropathological data has been set up in the Progeny database, ready for data import and upload of anonymised autopsy reports. Early results with respect to early bio-informatic analysis of already collected neuropathology data with clinical and genetic data have been reported and various bioinformatics projects are ongoing or being written up.

1.3 WP1 ECAS

The validation and harmonisation of cognitive screening of ALS patients through SOPHIA has continued and an official ECAS SOP was created and an ECAS data template was been set up in the Progeny database. Currently a certification system for ECAS usage is being set up, supported by ENCALS. The ECAS was successfully translated into 8 different languages, other translations are underway. Data is being collected in several centers internationally and the screen has been validated against extensive neuropsychology in the UK, and against other screening instruments in Italy and Germany. Early analysis results have demonstrated that the screen is sensitive to the types of cognitive and behaviour change prominent in ALS across centers and the findings have been related to disease and genetic variables.

1.4 WP2 Molecular Biomarkers

WP2 has collected optimally processed CSF and blood samples from patients using the established SOPs for biosampling. A first common basic neurochemical data set for CSF was defined and a first integration into a round-robin system has been implemented. First results were accepted for publication. A two round-robin process for the potential biomarkers neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) in CSF was set up with the aim to evaluate inter-laboratory variations and in the longer-term

potentially improve the industry-assays. Results were discussed with the company Instand e.V. and an application has been filed to perform the round-robins under this society as legal person.

In parallel, a monitoring and teaching system for biosampling was created and collaboration with other initiatives (e.g. JPND-BiomarkerAPD project) evolved. The webpage www.neurochem.info was created, the start of a common IT-structure for a decentralized biomaterialbank (virtual biobank) according to NEALS, Euro-HD, FTLDc and the German ALS registry.

Blood and isolated RNA have been collected from 8 patients and 7 aged matched controls under 5 different set conditions (LeukoLOCK, PaxGene, PaxGene + GlobinClear, Tempus and Tempus + GlobinClear). Analysis revealed that LeukoLOCK was the preferred method due to be more cost effective and time efficient. This has led to the production of an SOP for blood collection for transcriptomics, which is to be used in future projects.

1.5 WP3a Imaging Biomarkers (MRI)

In WP3a the central repository system for MRI data has been developed and opened up for SOPHIA members. Quality control algorithms have been developed and implemented to automatically identify systematic and centre specific deviations in T1 images, as initial data analysis identified that the datasets were grossly aberrant. A beta version of the T1 quality control toolbox has being used in the overall analysis of the first cohort of data (quantification of the amount of noise, geometric distortion, contrast variability and overall quality). Publication of results and an optimised protocol for MRI (VBM and DTI analysis) in ALS followed. For comparison of group-level results from different analysis platforms (SPM versus FSL versus TIFT), the T1 and DTI data sets are being analysed at group level. The T1 and DTI analysis progress and results were presented on several meetings and symposia, a publication will be submitted in 2015 and current recommendations for optimisation of the MRI protocol are continuously fed to the community through the NISALS network and at NISALS meetings.

A bidirectional interface to the core clinical data management in WP1 to obtain harmonization of the MRI acquisition with the clinical dataset (WP1) and other sample procedures (WP2) has been established.

With the establishment of the NISALS repository, the Neuroimaging society in ALS has established governance rules for the sharing and use of MRI data in ALS and the necessary minimum quality requirements as developed in SOPHIA. This repository can be utilized by all ALS researchers worldwide, and has recruited more than 1.000 datasets of patients and controls from 40 centers in 30 countries (http://nisals.org).

1.6 WP3b Neurophysiological Biomarkers

WP3b has been providing MUNIX investigator training to all participating SOPHIA centres over 4 courses. The detailed MUNIX protocol including SOPs has been disseminated to all participating centres and they all received ethical approval for longitudinal data collection from their local ethics committees. All of the trained centres have passed the test-retest variability test where they receive feedback on their individual MUNIX performance. They are currently in the process of collecting longitudinal data. In the Progeny data repository a datasheet has been created for entering longitudinal MUNIX data.

Analysis of test-retest variability of MUNIX measurements is almost completed: Analysis of inter-rater and intra-rater reliability across centers and identification of factors influencing reliability of measurements being finalised. Analysis of multicenter longitudinal data on the rate of motor unit loss in individual patients is ongoing.

1.7 WP4 Open Innovation Platform

As per December 2013 the SOPHIA website is up and running (www.sophiaproject.eu) to serve as a first vehicle to support implementation of the pan-European methodology on ALS biomarker optimization and harmonization. Integration with the data repository Progeny (WP1) will be done, however, the data repository is accessible to all SOPHIA partners already through a secure link.

Discussions about the continuation of the SOPHIA effort beyond its timeframe have led to the development of a new consortium of ALS centers for clinical ALS research (trials) and biomarkers. The effort is called TRICALS (Treatment Research Institute for the Cure of ALS) and has launched an online platform in May 2014 in The

Netherlands for a test phase.In 2015 TRICALS hopes to start its first clinical trial in The Netherlands together with a Dutch biotech company. (A Horizon 2020 grant application has been submitted mid-2014 to partly fund this trial as well.) Also, TRICALS will extend to include more countries in the course of the year, e.g. Italy, UK, Ireland, etc.

In 2014 three project meetings were held: An interim meeting in Leuven (Belgium) during the ENCALS symposium in May, the second annual meeting in London (UK) in October (together with other JPND-sponsored ALS project meetings – ALS-CarE, STRENGTH and Needs in ALS) and a short update meeting in Brussels (Belgium) in December during the International MND Symposium and ENCALS interim meeting.

1.8 WP5 Management, Clinical Coordination & Ethics

Achievements in overall project management (WP5) in 2014 were one newsletters, the organisation of an interim and an update meeting (23 May, 4 December) and a full annual meeting (6 & 7 October) and general arrangement of other meetings and teleconferences, management of budget, scheduling and project objectives, etc. The general project timeline is presented in Figure 1.

In 2015 there will not be much time for another annual SOPHIA meeting before project finish in June. But, as a second JPND ALS projects meeting will be held for ALS-CarE, STRENGTH and Needs in ALS in September/October in any case, it is envisioned most SOPHIA members will be present anyway for a last wrap-up. The SOPHIA PC will organise time for this for the SOPHIA project. The final report of the SOPHIA project is due after termination of the project on 1 June 2015, and will be delivered by the project coordinator to JPND.

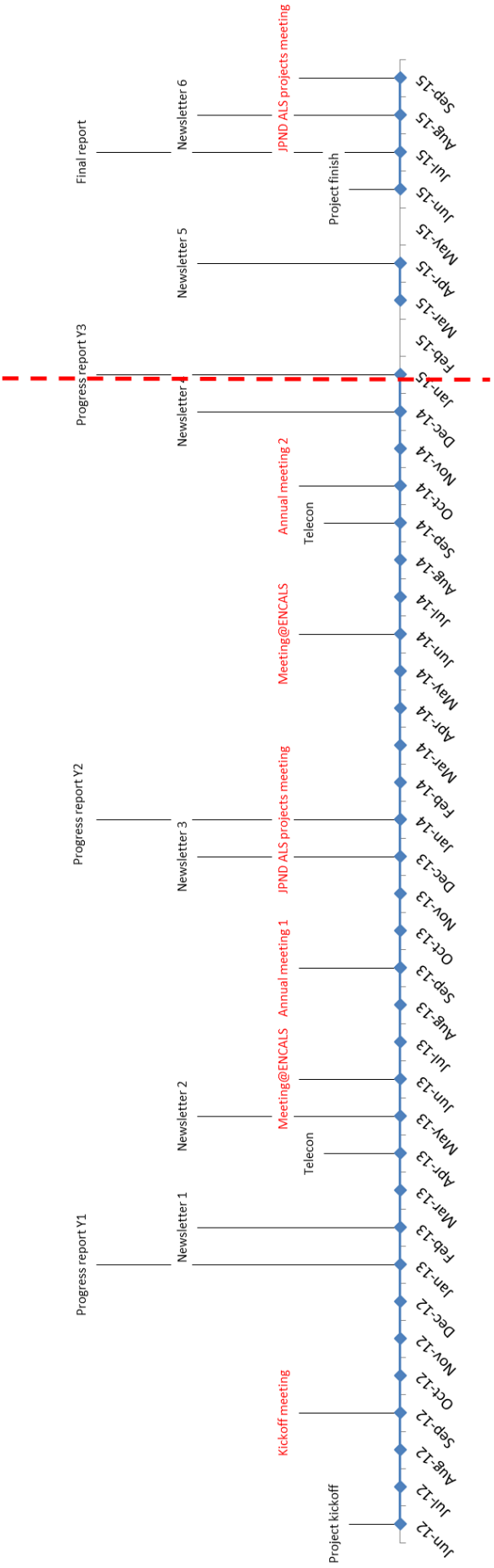

Figure 1 The SOPHIA project timeline and major milestones

2. Short progress summary of the respective subprojects

2.1 WP1 Data & Sampling Infrastructure

WP1 aims to ultimately provide optimized and harmonized SOPs of patient biomarker assays, analytic methods, and neuropathological techniques mapped to defined pathological and genetic subtypes of ALS, based on input from the different workpackages of SOPHIA. Utilizing existing technologies, including a web-based data repository provided by the EuroMOTOR consortium (www.euromotorproject.eu), this work will provide an integrated virtual biobank from which biological samples and clinical, imaging, neurophysiologic and neuropathological (biomarker) datasets provided by participating members can be utilized to enable state of the art collaborative analyses.

Key deliverables of WP1 are the establishment of a template for collecting core clinical data of ALS patients (deliverable 1.1) and establishment of common IT-platform/a virtual biobank (deliverable 1.2).

Relevant clinical variables have been defined and chronological-progression markers based on validated functional scores have been included in a database template for collection of core clinical data, which has been reviewed by all SOPHIA members (deliverable 1.1.1). Core clinical data is defined as the minimal data to be collected on ALS patients for various studies. The core clinical dataset contains general personalia (DOB, gender), diagnosis (revised El Escorial scoring), co-morbidity and disease onset & symptoms, respiratory function tests, endpoint data, cognitive status (ECAS), short familial history, genetic screening and ALS-FRS-R.

The dataset is fairly short and simple (only a few key items (= “minimal”)), as the goal is compliance in data entry, and not an over-detailed dataset. The core clinical dataset is clearly defined, to enhance compliance in data entry. A standard operating procedure (SOP) has been created, to make sure all researchers collect the data in a similar fashion. More complex clinical data will be part of separate projects (e.g. what data best stratifies patients, Awaji validation, etc.).

A web-based interface to a database for all partners to collect and access data has been developed (deliverable 1.2.1). The data repository as set up for the EuroMOTOR project with Progeny Software has been used and is well suited for this purpose. The Progeny system has clinical data security at its core and meets the robust compliance required by the US HIPAA standards for managing sensitive clinical data. The WEB system separates the application server that is exposed to the outside world and the database server that remains behind the UMC Utrecht (SOPHIA project coordinating partner) firewall. All data transferred between the database, WEB application and the outside world is encrypted and secured using SSL connections (https). In addition Progeny has software-specific security measures like user identification and authentication, auditing capability, etc.

With respect to the development of a web-based logistical interface for tracking biological samples (deliverable 1.2.2) and the establishment of real time tracking of clinical data and biological samples across the consortium (deliverable 1.2.3), the consortium is on track:
The approach on clinical data and bio-banking is focussed on being lean, accessible and traceable. Due to the many European collaborations the system requires to be accessible through the internet. The Progeny database currently services ALS centres in 16 countries for several different projects, having started off with EuroMOTOR and SOPHIA. All users within the system access the database through a secure web client and with specific permissions so they are able to only look into and add data they are allowed to handle. There are two types of user roles within the system which each come with specific permissions. The researcher role does see the data but it is all anonymous whereas the assistant role is able to see all the data and is mostly used to generate the data in the system.

The data itself can be differentiated between clinical research data and bio-samples. With the bio-sampling the aim is to have a single way of working. The data of samples needs to be lean and needs to give insights on where the samples are, how much is left of them or where they sent out to. All these actions are handled, tracked and traced with mobile terminals so there is high confidence that there will be no mistakes regarding data entry. This gives the opportunity to keep workflows tight and eventually be able to supply quality data to researchers.

Guidelines for general use of Progeny have been developed (e.g. how to log in, what security measures have been taken (compliance to US Dept. of Health and Human Services and to European guidelines), rights of the users (admin vs. researcher), etc.).

In the Progeny system, each partner/center can assemble core clinical data per patient. In addition project-specific data and biological sample IDs (EuroMOTOR, SOPHIA, Project MinE, future other projects) can be collected in dedicated folders and per partner/center, but still linked to that patient through an individual ID, facilitating biological sample tracking. Within each project folder (e.g. SOPHIA) different datasheets are set-up for different subprojects/workpackages (e.g. a datasheet for imaging biomarker data (WP3a), neurophysiologic biomarker data (WP3b), neuropathological data, etc.). SOPs are provided if required. The database structure is detailed in Figure 2. Figure 3 shows the set-up of the datasheet(s) for collection of core clinical data.

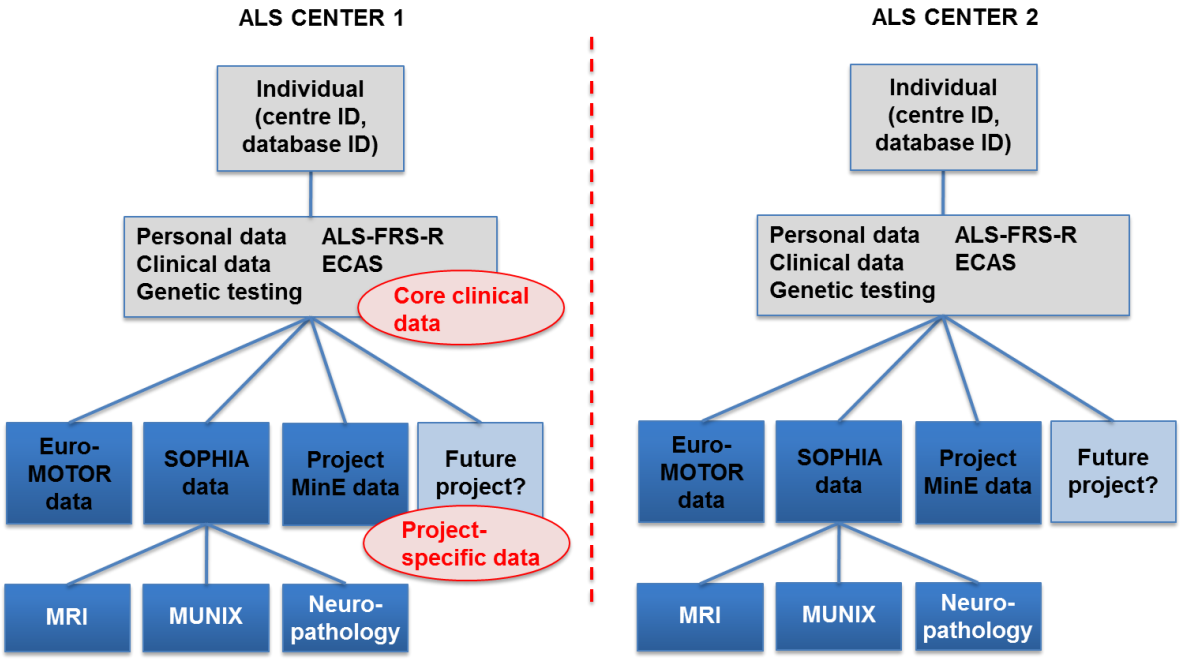

Figure 2 Set-up of the web-based data repository for SOPHIA and other (future) European projects

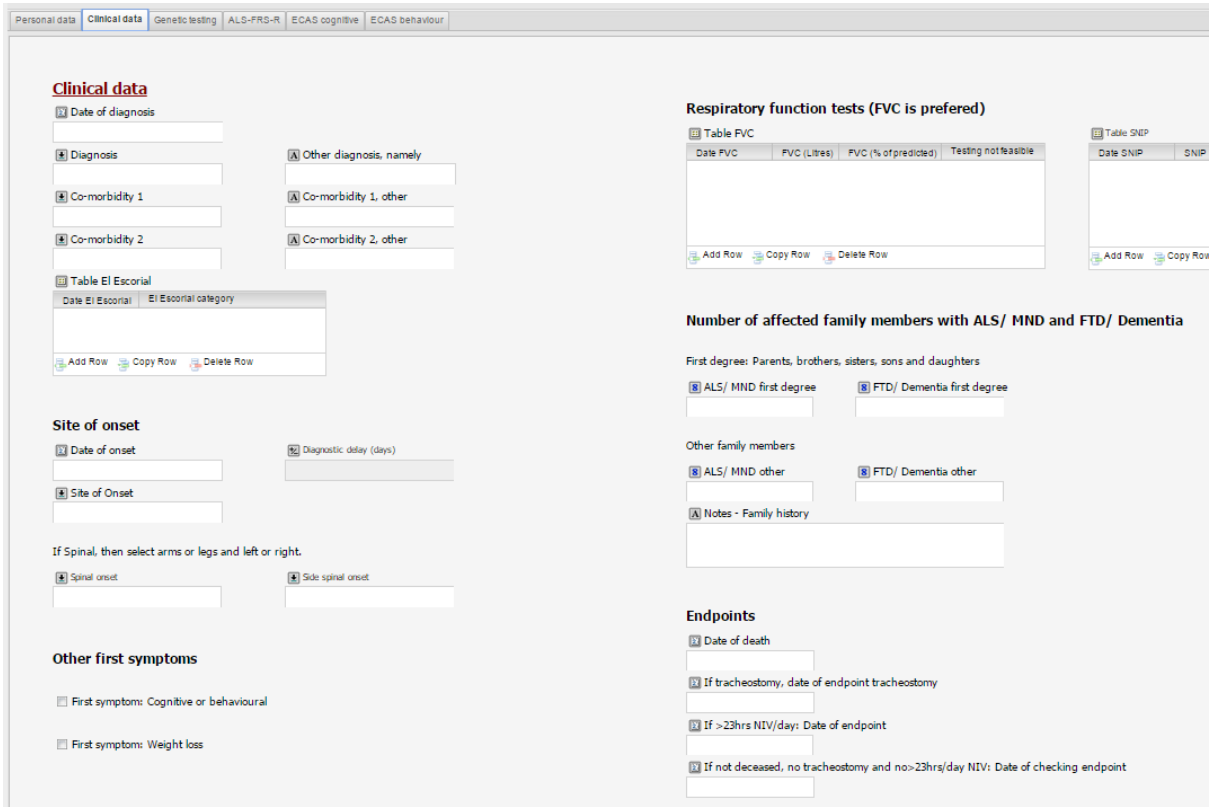

Figure 3 Database set-up for collection of core clinical patient data (Progeny)

All patient data is only visible per centre/partner, but folders can be opened up for data sharing for specific study projects. SOPHIA has developed a data sharing agreement for this purpose. Any researcher can apply for patient data from his own and other institutions. Each application will be reviewed by the data owning partners and they make the decision to share their data or not. Any publications following on from this follow the publications policy set up by SOPHIA.

The web-based data repository is accessible online to all SOPHIA partners. The data repository system has been tested by workpackage leaders and has now been opened up to all SOPHIA partners to start data collection.

Deliverable 1.6, 1.7 and 1.8 regarding dissemination of SOPs and results through publications and presentations has been ongoing since project start. SOPs for the collection and storage of biological samples and clinical have been developed and shared with all SOPHIA partners. This concerns SOPs for

- Blood and urine sample collection and short-term storage
- DNA purification
- Brain dissection
- Core clinical data collection
- ECAS data collection
- MUNIX

The SOPs are also available on the SOPHIA website. Once tested properly by all partners, these protocols will be published more widely by the consortium and project results will be presented to the ALS/MND community (e.g. via Brain Net, European Confederation of Neuropathological Societies, UK Brain Bank Network, etc.).

2.2 WP1 Neuropathology

In WP1 project partners are looking to combine the clinical data of ALS patients with genetic and neuropathological data in order to perform analyses and develop disease sub-classifications. A consolidated database of existing brain donor cohorts is key for such analysis (deliverable 1.3)) and an optimized protocol for the collection and storage of CNS and other tissues is required (deliverable 1.4). The brain dissection protocols have all been optimised and disseminated as well as posted on the SOPHIA website. These have been largely based on existing BrainNet Europe protocols, with updating given recent developments in the field of ALS neuropathology and with the options to obtain fresh samples for RNA, protein, DNA and CSF studies. A template for collection of the neuropathological data has been set up in the Progeny database and has been opened up for data upload by all partners. This also has the facility for an anonymised autopsy report to be uploaded. Genetic screening of existing donor cohorts is ongoing and GWAS data on a number of cases is already available.

Early results with respect to early bio-informatic analysis of already collected neuropathology data with clinical and genetic data (deliverable 1.5) have been reported; Various bioinformatics projects have been published e.g. Highley et al. 2014 and see previous progress reports.

Ongoing projects for which data is being collected:
1. A detailed study of glial pathology in sporadic ALS and C9ORF72-ALS.
2. qPCR validation of microRNA microarray data in sporadic ALS and C9ORF72-ALS.
3. Characterisation of p62 pathology in neurones to relate to microarray data.

A number of pathology projects that are being written up:
1. Pathology of ALS associated with mutation of OPTN
2. Pathology of ALS associated with mutation of ATXN2
3. Pathology of ALS associated with truncation mutation of TARDBP
4. Pathology of FTLD-TDP case with two hexanucleotide expansions of C9ORF72

2.3 WP1 ECAS

In WP1, an additional new validation and harmonisation project was started using the SOPHIA framework. The Edinburgh Cognitive and Behavioural ALS Screen has been developed by Dr. Sharon Abrahams in Scotland (University of Edinburgh) and adopted as an ALS screening tool to be harmonized by SOPHIA.

In 2014, the validation and harmonisation of cognitive screening of ALS patients through SOPHIA has continued.

An ECAS SOP was created and administration and guidance notes (including guidance for translation) were distributed (available on the SOPHIA website). An ECAS data template has been set up in the Progeny database. Currently a certification system for ECAS usage is being set up, supported by ENCALS, the European Network for the Cure of ALS. This is a network of European ALS centers, of which several are member of the SOPHIA consortium as well. Certification will include a nominated individual within each center being trained and responsible for ECAS usage within that center.

The ECAS was successfully translated into Dutch, German, Swiss German, Portuguese, Spanish, French and Italian. Other translations are underway including Polish and Turkish. Data is being collected in centers in the Netherlands, Ireland, Germany, Switzerland, Italy, Portugal, Spain, UK, France and Belgium. The screen has now been validated against extensive neuropsychology in the UK, and against other screening instruments in Italy and Germany, the latter resulting in publication (Lule et al. 2014). Data from 529 ALS patients has been submitted to Prof. Abrahams for analysis in addition to country and language specific normative data. The results have demonstrated that the screen is sensitive to the types of cognitive and behaviour change prominent in ALS across centers and the findings have been related to disease and genetic variables. This data is currently being prepared for publication.

2.4 WP2 Molecular Biomarkers

WP2 interacts directly with WP1, ensuring collection of a common clinical dataset from patients for whom optimally processed CSF and blood samples are also available, with the ultimate goal of generating well defined patient cohorts for scientific interrogation of the biological data.

The first task of this WP was to come to a consensus regarding which protocols to use for biosampling of CSF, blood, blood products (lympocytes), DNA, RNA and which biomarkers to measure and to define a first common basic neurochemical data set with quality control system (deliverable 2.1.1 and 2.1.2). Extensive work in this area had been done beforehand already (Otto et al. 2011).

Within the first months of the SOPHIA project, blood, DNA and CSF of 50 patients with ALS were sampled as high quality reference material. Additionally 100 samples of reference material (CSF and serum samples) of control patients were collected. The most recent SOPs were to be applied (Otto et al. 2012). Sampling protocols for stem cells and for hair cells are still under investigation.

CSF biomarkers

The next step was to implement a first round-robin system, an interlaboratory quality control program which involves sending the predefined standardized CSF samples (the established neurochemical dataset) out as an external quality control of local test performance. First results of this round-robin system have been obtained and were accepted for publication (Lehnert et al. 2014). Abeta and Tau appeared quite variable in both ALS cases and controls and across centers. It is thought that a review of clinical data by different clinicians may be the issue. It turned out that measurement of neurofilaments seem to be most promising with regard to differential diagnostic use, whereas markers like MCP-1, progranulin, and sAPP only have a minimal differential diagnostic use, see Table 1.

Table 1 Results of the first interlaboratory quality control round

	Lab 1		**Lab 2**		**Lab 3**		**Lab 4**		**Lab 5**		**Lab 6**	
	CON	ALS	CON	ALS	CON	ALS	CON	ALS	CON	ALS	CON	ALS
Tau [pg/ml)	223.6 ±178.7	223.4 ±230.1	99.8 ±24.0	228.4 ±73.4	89.9 ±33.4	143.8 ±44.1	78.2 ±75.8	54.0 ±26.2	114.4 ±41.4	180.2 ±99.8	118.2 ±22.3	166.0 ±99.9
Abeta [pg/ml]	700.0 ±375.5	705.0 ±222.3	844.0 ±607.5	880.2 ±317.0	845.0 ±184.2	825.8 ±136.5	492.0 ±121.7	488.0 ±345.4	519.2 ±107.9	582.8 ±375.5	992.6 ±679.9	980.6 ±607.5
Cystatin C [ng/ml]	3234 ±607	3553 ±997	2028 ±1133	2223 ±376	1724 ±680	1928 ±608	2462 ±3170	2152 ±1842	1820 ±1365	2006 ±1217	1794 ±345	2038 ±1079
NFH [pg/ml]	832 ±779	3503 ±1601	287 ±249	5130 ±4603	176 ±74	4876 ±7043	238 ±281	1372 ±1248	565 ±442	1972 ±748	146 ±44	3288 ±3866

S100 [ng/ml]	2.28 ±2.62	1.14 ±0.37	1.22 ±0.56	1.72 ±0.64	0.44 ±0.25	0.30 ±0.10	0.60 ±0.64	0.54 ±0.50	1.22 ±0.70	1.16 ±0.31	1.18 ±0.49	1.12 ±0.38
Progranulin [ng/ml]	2.12 ±0.78	4.7 ±2.86	2.62 ±1.17	2.66 ±1,76	2.18 ±1.09	1.84 ±0.64	1.78 ±1.48	1.74 ±0.86	1.36 ±0.17	1.50 ±0.27	1.74 ±0.50	1.64 ±0.11
MCP-1 [pg/ml]	3691 ±2547	2923 ±763	2153 ±996	3127 ±605	2667 ±948	2286 ±595	4276 ±649	2223 ±865	2017 ±337	3171 ±1406	2079 ±966	1940 ±644

The workgroup for WP2 gathered at the University of Ulm (Germany) on 25 & 26 September 2013. The meeting was organised by the WP-leader and attended by representatives of most partners involved in this work package. Discussions about the first results of the round-robin showed that implementation of the same SOPs for all countries/laboratories is not possible or useful. Instead, the differences between the applied procedures should be investigated: The Ulm-SOP has been distributed among the partners and comments have been collected and distributed for further discussion. This way project partners provide each other with insight in their way of sampling.

Meeting participants agreed to start a two round-robin process for the potential biomarkers neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH) in CSF, consisting of a 'research' round-robin and a 'reverse' round-robin system, with the aim to evaluate inter-laboratory variations and in the longer-term potentially improve the industry-assays. The neurochemical labs in Ulm and Paris were chosen as reference labs. So far 16 different centers joined this round-robin for the measurement of neurofilaments in CSF. For the neurofilament light chain a quality control issue was raised. Together with the company this problem was reduced to a problem in one batch. Experiments with a new batch are now under evaluation. A common basic neurochemical data set with an internal and external quality control system is now nearly set-up (deliverable 2.1.3).

The results of the round-robins were discussed with the company Instand e.V. The WP2-leader applied to perform these round-robins under this society as legal person. This request is currently under review, but WP2 already received the information that Instand will possibly integrate this neurofilament measurement in their system (deliverable 2.2.2). Work is ongoing on deliverable 2.2.3, establishment of a common "round-robin biobank" for internal and external survey.

A key task of WP2 is setting up a monitoring and teaching (educational program) system for biosampling (deliverable 2.2.1). The SOPHIA group itself received their first teaching including hands-on courses during the WP2-meeting in Ulm. It was agreed that an educational program is necessary which should be available in different languages and that it should persist beyond the timeframe of the SOPHIA project. Courses can be held nationally or internationally for SOPHIA, but joint courses with the JPND-BiomarkerAPD group and other interested parties including MS researchers are an option as well. It was discussed how to implement the teaching system in general, focussing on target audience, content, location, accreditation, provision of information (webpage, webinars, hands-on courses), etc. The webpage www.neurochem.info was created, the start of a common IT-structure for a decentralized biomaterialbank (virtual biobank) according to NEALS, Euro-HD, FTLDc and the German ALS registry (deliverable 2.2.4). The existing protocol of the German Society of Neurochemistry and CSF diagnostics is used after harmonization within the SOPHIA group and with the BiomarkAPD group. Two courses were held in 2014 and the formation of an European CSF society has been announced for 2015. The by-laws for this society are currently under review.

Blood biomarkers (deliverable 2.3.1-2.3.6)
Blood and isolated RNA have been collected from 8 patients and 7 aged matched controls under 5 different set conditions: LeukoLOCK, PaxGene, PaxGene + GlobinClear, Tempus and Tempus + GlobinClear. The RNA was assessed for QC and then used for microarray analysis on Human U133 IVT arrays. The resulting data was analysed to establish the most robust method of collection and isolation. It was established that the addition of the GlobinClear step greatly depleted the number of globin transcripts that otherwise masked the gene expression profile, although the PaxGene + GlobinClear gave a reduced yield and quality to that of the Tempus + GlobinClear and LeukoLOCK. Thus, of these two robust methods, which identified comparable biological pathways to be differentially expressed, LeukoLOCK was the preferred method due to be more cost effective and time efficient. This has led to the production of an SOP for blood collection for transcriptomics, which is to be used in future projects.

2.5 WP3a Imaging Biomarkers (MRI)

WP3 extends the clinical data in WP1 with standardized quantification of brain (MRI; upper motor neuron) and spinal cord (MUNIX; lower motor neuron) motor neuron involvement, the hallmarks of ALS. Computational MRI analyses are inherently investigator-independent but contain numerous potential error sources (i.e. protocol, scanner type and analysis tool). Thus, to implement optimization and harmonization, MRI requires continuous multicenter comparative analyses feeding identified sources of quality loss back to ALS centers. Validated SOPs for MUNIX measurements exist, but investigator training is essential to establish cross-center reliability. MUNIX demands analysis of factors influencing test-retest reliability across centers.

Utilizing available resources from the Neuroimaging Society in ALS (NISALS - http://nisals.org) a Jena-based MRI repository has been set up through a secure root repository server (sftp://nedigs03.med.uni-jena.de) (deliverable 3a.1). Access is enabled in a center specific way through an OpenSSH sftp protocol only without shell access. All participating SOPHIA centers have been registered to this MRI database and have been uploading initial MRI datasets. The upload of initial datasets to the repository was somewhat delayed in some centers because of first time ever establishment of research MRI acquisition in ALS.

The members of WP3a have established the bidirectional interface to the core clinical data management in WP1 to obtain harmonization of the MRI acquisition with the clinical dataset (WP1) and other sample procedures (WP2) (deliverable 3a.2): The interface is being defined as an API and its content tested to ensure the subjects identity is preserved in a pseudomized fashion across databases. A core translation table in the MRI repository and the central clinical database (Progeny) has been achieved: the generation of SOPHIA-specific proband IDs allows crosslinking to the MRI repository. This allows the connection of clinical, wet biomarker and MRI datasets for future interrogation and exploration of the additional value of a multimodal profile. Live data exchange will not be feasible due to the excessive amount of space required for MRI data; exchange of information on existing datasets provides a sufficient mechanism to provide SOPHIA members and stakeholders with a starting point for future data interrogation.

Early 2013 a T1 quality control toolbox was available in an alpha version and tested in a subgroup of ten centers. This resulted in a positive automated identification of grossly aberrant datasets. An extreme variability in data formats, as existing datasets were provided in pre-processed form, required the development of a chain of MRI data conversion tools to ensure secure data de-identification, source data harmonization, rights management and query management for interrogation of datasets (preparation of datasets for analysis). The tools identify data outliers within and across centers. In total, development of the conversion tools caused a delay of four months. The result was that all centers were asked to upload DICOM data only, which could now be handled from every center. This included a thorough analysis for remnants of header information with identity data: DTI and T1 datasets have been converted through the NISALS repository to remove any remaining identity information from the DICOM format datasets.

The final deadline for DICOM data upload was adjusted to 31 December 2013 to allow a significant number of centers to participate in the first overall study on acquisition parameters driving variability of T1 and DTI datasets. Uploads total 250 T1 and 250 DTI datasets from ALS patients and controls from in total 11 SOPHIA centers.

The alpha version of the T1 quality control toolbox has developed to a beta version. The toolbox is being used in the overall analysis of the first cohort of data (quantification of the amount of noise, geometric distortion, contrast variability and overall quality). Analyses tasks are divided as follows: Jena (Germany) for T1 data, Ulm (Germany) for DTI data using in house developed software, and Oxford (UK) for DTI data using FMRIB developed software. The available data has been collected from the repository data conversion tool chain for T1 datasets and fed in to quality control analyses. Publication of results and an optimised protocol for MRI (VBM and DTI analysis) in ALS followed after these analyses. For comparison of group-level results from different analysis platforms (SPM versus FSL versus TIFT), the T1 and DTI data sets are being analysed at group level.

SOPHIA funds were used for a post-doctoral MRI analyst (Dr. Menke) to analyse and curate the Oxford MRI database, and upload to the Jena repository; in Jena, the core repository has been set up and quality control approaches for T1 datasets were developed by Robert Dahnke (Christian Gaser Neuroimaging Group).

The T1 and DTI analysis progress and results were presented from the platform at the Neuroimaging Society in ALS (NISALS) meetings in Milano (2013) and Leuven (2014) (deliverable 3a.3). An abstract based on the results of

the DTI analysis was presented as a poster at the ALS/MND International Symposium in Brussels (December 2014). Presentations on the variability of T1 datasets using novel quality parameters have been given at the International Congress for Clinical Neurophysiology and Imaging in Berlin (2014).

Current recommendations for optimisation of the MRI protocol are continuously fed to the community through the NISALS network and at NISALS meetings (deliverable 3a.4). Highlighting specific factors of disturbance, Ricarda Menke and Bradley Foerster have in depth detailed error sources during the NISALS meeting 2014 in Leuven covering T1, DTI, tractography and functional measurements within multimodal and multicenter approaches.

The challenge to test the MRI-protocol against non-ALS neurodegenerative disease cases has gone out to the NISALS community which is the immediate stakeholder for implementation of standardization and harmonization in MRI in ALS. ALS centers with imaging capability from Canada, the USA, Australia and non-SOPHIA members within the EU have responded to the challenge and are willing to add their data for a large international cohort of ALS patients.

With respect to deliverable 3a.5 - Publication of results of multimodal biomarker analyses (WP1, WP2, WP3): A manuscript on the DTI analysis is in circulation with all co-authors ahead of planned submission to The Lancet Neurology in February 2015 (deliverable 3a-5). The manuscript “Turner MR, Benatar M. Ensuring continued progress in biomarkers for amyotrophic lateral sclerosis. Muscle Nerve. 2015 PMID: 25288265” included specific discussion of the SOPHIA initiative and current progress in the wider field. Further manuscripts have utilized the SOPHIA quality controls mechanisms which are being implemented within the NISALS repository (Hartung et al. PlosOne 2014).

Regarding publication of results of monitoring of implemented MRI harmonisation (deliverable 3a.6): The monitoring of the QC implementation was successful when ALS research centers in Lisbon (Portugal), St Gallen (Switzerland) and Sheffield (UK) newly established high resolution imaging in their ALS cohorts. Currently, the NISALS repository undergoes a transition into a Web based system using the LORIS platform of the McGill University Neuroimaging group (Canada). With the system established it will be possible to feed back QC directly to the uploading centers for each single MRI dataset. The T1 and DTI QC processes developed in SOPHIA will report back through the web interface of the Jena MRI repository.

With the establishment of the NISALS repository, the Neuroimaging society in ALS has established governance rules for the sharing and use of MRI data in ALS and the necessary minimum quality requirements as developed in SOPHIA. This repository can be utilized by all ALS researchers worldwide, and has recruited more than 1.000 datasets of patients and controls from 40 centers in 30 countries (http://nisals.org), completing deliverable 3a.7 of WP3a (EU-accessible self-supporting ALS MRI biomarker quality control system).

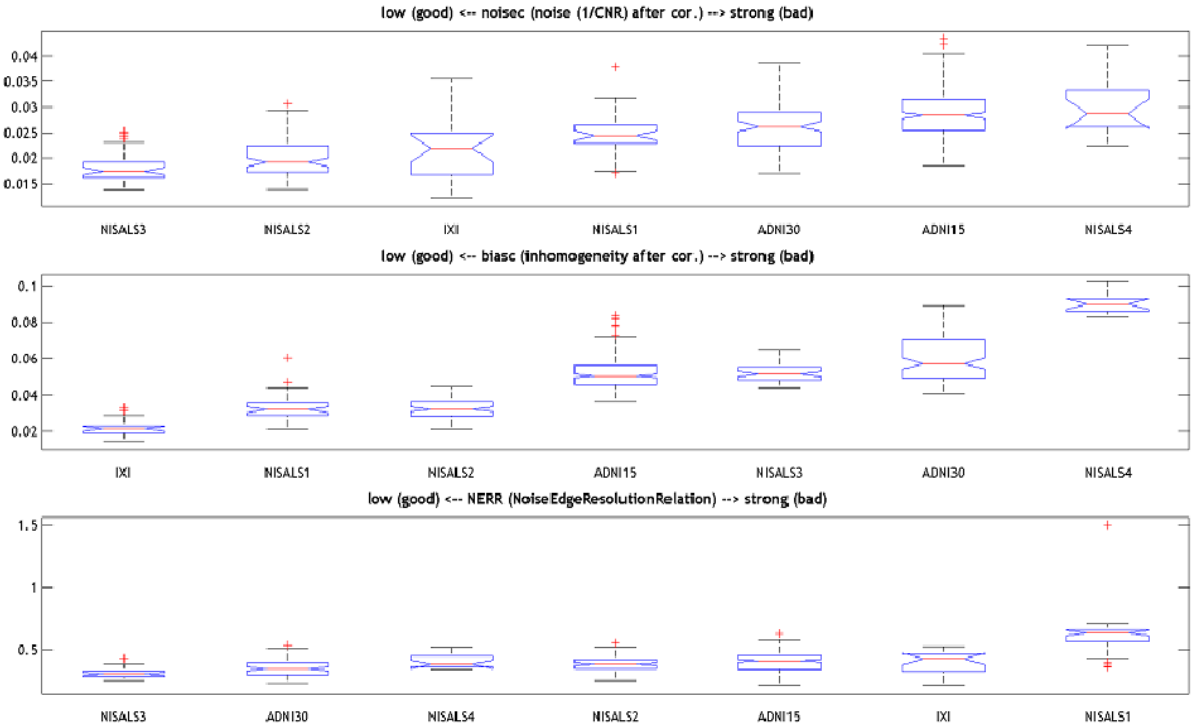

Figure 3 Comparison of quality control parameters of 5 representative NISALS centers which partook in the SOPHIA harmonization initiative. In all categories, NISALS/SOPHIA centers were as good as or better than T1 MRI data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) 1.5 or 3.0 Tesla data sets when appropriate correction for typical artefacts were applied to all datasets.

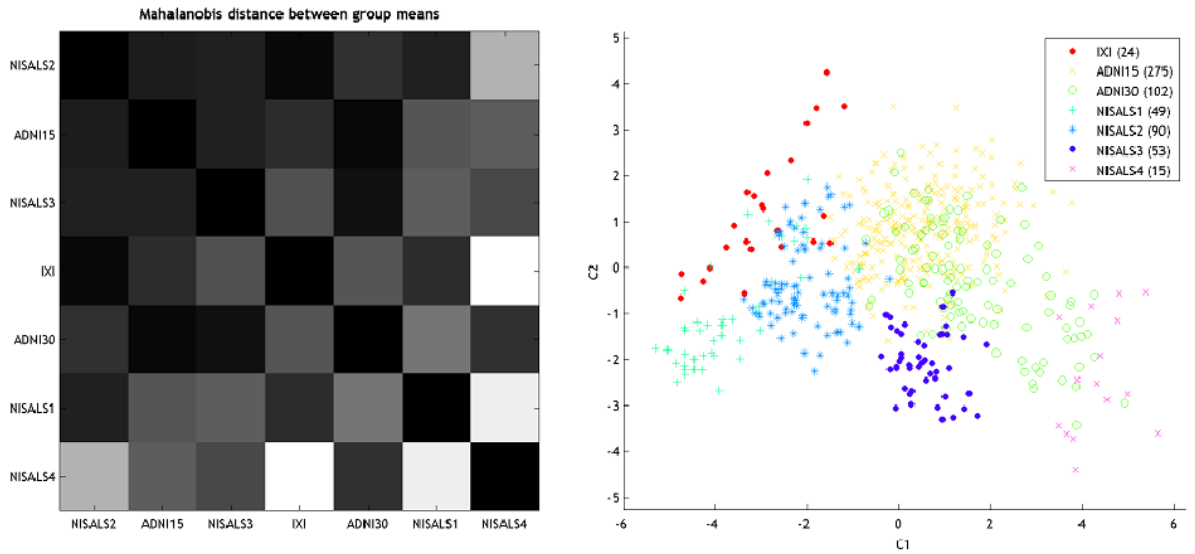

Figure 4 Mahalanobis distance plot integrating all SOPHIA developed QC measures shows that except for NISALS center 4 (which contributed less than 1/3 of the other centers) all NISALS/SOPHIA data harmonizes with the ADNI datasets. This implies that NISALS/SOPHIA QC enables ALS centers to contribute high resolution T1 data with sufficient quality to function as multicenter biomarker in a clinical trial, provided that central preprocessing and artefact correction is applied through an internationally accepted stakeholder like the Neuroimaging Society in ALS (NISALS).

2.6 WP3b Neurophysiological Biomarkers

Investigator training in using MUNIX is one of the key tasks within WP3b in order to establish cross-center reliability in those measurements. A detailed MUNIX protocol including SOPs has been disseminated to all participating centers (deliverable 3b.1). A total of four training courses for participating centers have been organised within SOPHIA. The first course took place in St. Gallen on 23 March 2012, the second course took place during the ENCALS meeting in Dublin, on 25 May 2012. Two more training sessions were held in 2013 after

it became clear from preliminary test-retest results that there were some recurrent pitfalls that could be easily reduced or avoided with appropriate practical training.

During the training courses theoretical lectures were given by Professor E. Stalberg, S. Nandedkar and C. Neuwirth. After the presentations participants were instructed and could familiarize themselves with the MUNIX technique during hands-on sessions. Participants were instructed and supervised by a team of experienced MUNIXers from St.Gallen. The most crucial pitfalls and sources of errors were demonstrated. At the end of the course participants were able to independently perform MUNIX measurements on various muscles relevant for the SOPHIA project.

All centers have been trained now and they all received ethical approval for longitudinal data collection from their local ethics committees. All trained centers have now passed the test-retest variability test where they receive feedback on their individual MUNIX performance. They are in the process of collecting longitudinal data. A test-retest variability (inter- and intra-rater) check across centers and intra- and inter-rater is performed every six months and results are analysed.

Analysis of test-retest variability of MUNIX measurements (deliverable 3b.2) is almost completed: Analysis of inter-rater reliability (center-specific) (deliverable 3b.2.1) and analysis of intra-rater reliability (center-specific) (deliverable 3b.2.2) have been completed; Analysis of inter-rater and intra-rater reliability across centers (deliverable 3b.2.3) and identification of factors influencing reliability of measurements (deliverable 3b.2.4) are ongoing and almost finalised. High ICC values for intra- and inter-rater tests have been found, and reliability appears to increase with experience. BB and AH muscles are less reliable compared to other muscles. Publication of the detailed analysis of the reliability studies (deliverable 3b.2.5) is ongoing.

To avoid collection of bad data and regarding experience from a previous study it was decided that a MUNIX training is mandatory for the data-collecting-investigators of all centers who participate in WP3b, especially if they wish to participate in the longitudinal data collection. This meant training of the lab staff that effectively performs MUNIX as well, as it is unlikely that performance will be up to standards if for example a participant of a previous MUNIX courses teaches their own lab staff.

In the Progeny data repository a datasheet has been created for entering longitudinal MUNIX data. The database is open to the participating centers and MUNIX data uploading has commenced along with the core clinical data collection.

All other deliverables in WP3b are ongoing as planned:
Deliverable 3b.3 (Analysis of multicenter longitudinal data on the rate of motor unit loss in individual patients) is ongoing; Deliverable 3b.3.1 (Evaluation of muscle-specific rate of motor unit loss) and deliverable 3b.3.2 (Evaluation of variability of measurements as a function surviving motor units): Bulbar onset patients also show MU loss in peripheral muscles. The rate of decline is almost identical to the ALS-FRS decline. APB and ADM show the greatest rates of decline.

Deliverable 3b.4 (Publication of multicenter longitudinal data) and deliverable 3b.5 (Publication of results of multimodal biomarker analyses (WP1, WP2, WP3)) are planned for the end of the project.

2.7 WP4 Open Innovation Platform

Interactive European ALS biomarker website (deliverable 4.1)
WP4 aims to set up an interactive European ALS biomarker website with an open innovation platform accessible to the general public, patient organisations, industry and policy makers, etc., and a restricted section containing a virtual biobank and data retrieval and deposition features for the scientific ALS/ND community. WP4 is setting up the basis for this together with the project managers of SOPHIA (WP5). Design and implementation of the virtual biobank will be performed in close cooperation with WP1-members.

As per December 2013 the SOPHIA website is up and running (www.sophiaproject.eu) to serve as a first vehicle to support implementation of the pan-European methodology on ALS biomarker optimization and harmonization. Integration with the data repository (WP1) will be done, however, the data repository is accessible to all SOPHIA partners already through a secure link.

Annual consensus workshops (deliverable 4.2)
After the initial kick-off meeting in September 2012, a short gathering of the SOPHIA group during the ENCALS meeting in June 2013 (www.encals.eu), and the first official annual meeting in September 2013, in 2014 three more project meetings were held: An interim meeting in Leuven (Belgium) during the ENCALS symposium in May, the second annual meeting in London (UK) in October and a short update meeting in Brussels (Belgium) in December during the International MND Symposium and ENCALS interim meeting.

As several new JPND-sponsored ALS projects started early 2014 (*ALS-CarE* led by Prof. Orla Hardiman, *STRENGTH* led by Prof. Ammar Al-Chalabi and *NEEDS in ALS* led by Dorothée Lulé), with many SOPHIA members being partners in these projects as well, it was decided to combine the annual meetings for these projects in one larger JPND ALS projects meeting in London in October, of which SOPHIA was a part. This enhanced collaboration and provided for better tuning of goals and tasks for each of the projects as results from the projects could be shared. For example, the Progeny database will also be used for data collection by STRENGTH and ALS-CarE. Also, with combined JPND meetings more multidisciplinary professionals can be invited and better dedicated workshops can be planned. Partners not involved in all projects were brought up to speed easily and were provided the opportunity to collaborate on other projects as well, despite not officially being a partner. Combined meetings help the SOPHIA project to disseminate its results to the rest of the ALS/MND and wider ND community efficiently.

At the project meetings representatives of most of the participating centers were present, especially at the second annual SOPHIA meeting in London. Progress of the work packages was presented, project results were presented and several project issues were discussed, such as how to make sure SOPHIA efforts continue after the project officially finishes, funding of online databases in the long term, etc.

Open innovation platform (deliverable 4.3)
As reported in the scientific report over 2013, discussions about the continuation of the SOPHIA effort beyond its timeframe have led to the development of a new consortium of ALS centers for clinical ALS research (trials) and biomarkers, which will should be ready for new calls in the future as part of Horizon 2020. All participating (European) centers use European standards for EMG, MRI, clinical data, staging in ALS, etc. (as developed by SOPHIA and EuroMOTOR) and a structure for continuous quality control as developed by SOPHIA, which can be attractive to companies developing new drugs. This should be communicated to the pharmaceutical industry: The consortium offers its experience and several (paid) services in executing more efficient and enhanced ALS clinical research, and this way maintains itself. The effort is called TRICALS (Treatment Research Institute for the Cure of ALS) and has been further developed in 2014, following an initial business plan developed by UMC Utrecht with input of PWC consultants. An online platform has been created (with pro bono help from ICT consultant Accenture) and TRICALS has been launched in The Netherlands for a test phase in May 2014 (www.tricals.org). Already over 100 patients have registered themselves. ALS centers worldwide can register as well, as can pharmaceutical and biotech companies. A TRICALS newsletter is distributed frequently. In 2015 TRICALS hopes to start its first clinical trial in The Netherlands together with a Dutch biotech company. (A Horizon 2020 grant application has been submitted mid-2014 to partly fund this trial as well.) Also, TRICALS will extend to include more countries in the course of the year, e.g. Italy, UK, Ireland, etc.

Dissemination (deliverable 4.4 and 4.5)
Thus far the SOPHIA coordinator has distributed 4 newsletters to the SOPHIA group. These newsletters have also been published on the SOPHIA website, as are the SOPs created within SOPHIA. In the next months until project finish two more newsletters will be distributed. Furthermore papers are published regularly by SOPHIA members acknowledging SOPHIA.

SOP implementation teaching (deliverable 4.6)
At the annual meetings and in between the meetings several SOP implementation teaching programs have organised by the project coordinator and by the workpackage leaders for SOPHIA (see the specific workpackage reports).

2.8 WP5 Management, Clinical Coordination & Ethics

WP5 entails overall management of the project and the consortium, focussing on communication among members but also externally to JPND and the wider ND research community, arrangement of meetings, management of budget, scheduling and project objectives, etc.

The following actions have been taken since January 2014, fulfilling several WP5 tasks and taking care of the WP deliverable regarding annual progress reporting to JPND and national funding agencies:

- With input of all WP leaders, the Project Coordinator (PC) delivered the Scientific Report over 2013 to JPND by 31 January 2014 (deliverable 5.1). The current Scientific Report over 2014 is the final annual progress report of SOPHIA, completing the deliverable. The final report of the SOPHIA project is due after termination of the project on 1 June 2015, to be delivered by the PC (deliverable 5.2).
- The PC organised a short SOPHIA interim meeting on 23 May 2014, during the ENCALS meeting in Leuven (Belgium). The minutes of this meeting were afterwards provided to all members by the coordinator and published on the SOPHIA website.
- The PC organised a teleconference for WP leaders on 23 September 2014 to discuss project progress prior to the annual SOPHIA meeting. The minutes of this meeting were afterwards provided to all members by the coordinator and published on the SOPHIA website.
- The PC, together with the coordinators of the JPND projects ALS-CarE, STRENGTH and Needs in ALS, organised a two-day annual meeting for the SOPHIA consortium and the other projects on 6 & 7 October 2014 in London (UK). The agenda and minutes of this meeting were afterwards provided to all members by the PC and published on the SOPHIA website.
- The PC organised a short update meeting for SOPHIA members during the the International MND Symposium and ENCALS interim meeting on 4 December 2014.
- Fulfilling its communication task within the consortium, the PC released four SOPHIA newsletters so far to its members. At the same time, the PC checked up on project progress, looking at schedules, deliverables and milestones for each WP.

In 2015 there will not be much time for another annual SOPHIA meeting before project finish in June. But, as a second JPND ALS projects meeting will be held for ALS-CarE, STRENGTH and Needs in ALS in September/October in any case, it is envisioned most SOPHIA members will be present anyway for a last wrap-up. The SOPHIA PC will organise time for this for the SOPHIA project.

3. Amendments to the original work plan (if applicable) and its rationale

Currently not applicable

4. Problems and their solutions (if applicable)

Currently not applicable

5. Publications (please state only direct outcome of the funded project)

Multicenter quality control evaluation of different biomarker candidates for amyotrophic lateral sclerosis; Lehnert et al; Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2014; DOI:10.3109/21678421.2014.884592

The Edinburgh Cognitive and Behavioural Amyotrophic Lateral Sclerosis Screen: A cross-sectional comparison of established screening tools in a German-Swiss population; Lule et al.; Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration; 2014; DOI: 10.3109/21678421.2014.959451

Ensuring continued progress in biomarkers for amyotrophic lateral sclerosis; Turner MR, Benatar M; Muscle Nerve 2015 PMID: 25288265

Voxel-Based MRI Intensitometry Reveals Extent of Cerebral White Matter Pathology in Amyotrophic Lateral Sclerosis; Hartung et al; PlosOne 2014

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Highley JR, Kirby J, Jansweijer JA, Webb PS, Hewamadduma CA, Heath PR, Higginbottom A, Raman R, Ferraiuolo L, Cooper-Knock J, McDermott CJ, Wharton SB, Shaw PJ, Ince PG. 2014. Loss of nuclear TDP-43 in amyotrophic lateral sclerosis (ALS) causes altered expression of splicing machinery and widespread dysregulation of RNA splicing in motor neurones. Neuropathol Appl Neurobiol 40: 670-85

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6. Patents, PhD thesis and other outcomes (if applicable)

Currently not applicable

7. Exchange of researchers, students etc. (if applicable)

Currently not applicable